bacillus thuringiensis

Evaluation
of Commercial
Preparations
of Bacillus thuringiensis
with and without
Chitinase against
Spruce Budworm

Information Report CC-X-59
February, 1974

Chemical Control
Research Institute
Ottawa, Ont.

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FOREWORD

Philosophically, the manipulation of pest populations should be achieved through the introduction of natural agents. These agents, whether pathogens or other should seek to influence the pest populations to oscillate at a density which can be tolerated by the host. To this end the Canadian Forestry Service has fostered long range research over a period of several decades.

The most serious pest of eastern Canadian pulp species is the spruce budworm (Choristoneura fumiferana Clem.) and the most promising pathogen emerging from the research program has been Bacillus thuringiensis (B.t.). Numerous field tests by a variety of investigators yielded degrees of promising results. A diversity of conclusions attributed to differences in formulation, concentration, weather and timing prompted the Canadian Forestry Service to consolidate the efforts of several agencies in a well-supported series of tests in 1973. The Chemical Control Research Institute agreed to take the lead role. The object of the exercise was to test the most successful B.t. formulations in a concerted attempt to resolve the inconsistencies which had developed between field evaluations by different agencies and, hopefully, to be in a position to advise clients on the feasibility of using a biological control agent where the spruce budworm threatens valuable spruce - balsam stands. The inter-establishment project was successfully completed and recommendations are being prepared. Follow-up experiments to modify and improve techniques are being planned for 1974 and beyond.

James J. Fettes,
Director,
Chemical Control Research Institute,
February, 1974

ABSTRACT

Two commercially available formulations of Bacillus thuringiensis Berliner, Thuricide 16B® and Dipel WP®, were applied to experimental forest blocks in Algonquin Park, Ontario and Spruce Woods, Manitoba. The materials, with and without the addition of chitinase, were tested against the spruce budworm (Choristoneura fumiferana Clem.). A separate test with chitinase alone was also carried out. The materials were assessed in terms of control of the target insect, protection to host trees, and effects on non-target organisms such as domestic honey bees, birds, small mammals, and aquatic and terrestrial invertebrates. The tests indicated that the Bacillus thuringiensis formulations gave limited protection to the trees and did not have any deleterious effects on other forms of life in the sprayed areas.

RESUME

Deux préparations commerciales de Bacillus thuringiensis Berliner, le Thuricide 16B® et le Dipel WP®, ont été appliquées sur des blocs expérimentaux du parc Algonquin, en Ontario, et de Spruce Woods, au Manitoba. Les deux produits, avec et sans chitinase, ont été mix à l'essai contre la tordeuse des bourgeons de l'épinette (Choristoneura fumiferana Clem.). La chitinase, employée seule, a elle aussi fait l'objet d'un essai. Les insecticides ont été évalués en fonction des trois points suivants: l'efficacité contre l'insecte cible, la protection offerte aux arbres hôtes et les effets sur les organismes non visés comme les abeilles domestiques, les oiseaux, les petits mammifères et les invertébrés aquatiques et terrestres. Les essais ont montré que les préparations de Bacillus thuringiensis offraient aux arbres un certain degré de protection et qu'ils n'avaient aucun effet nuisible sur les autres formes de vie des zones traitées.

Evaluation of Commercial Preparations of *Bacillus thuringiensis* with and without Chitinase Against Spruce Budworm

A. Laboratory Study of Comparative Toxicity Against Fifth-Instar Larvae

by P.C. Nigam

Chemical Control Research Institute
Canadian Forestry Service
Ottawa, Ontario

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A. Laboratory Study of Comparative Toxicity Against Fifth-Instar Larvae

by

P.C. Nigam

INTRODUCTION

A study for comparative effectiveness of commercial formulations of Bacillus thuringiensis (B.t.) and fenitrothion field formulations against spruce budworm Choristoneura fumiferana (Clem.) was carried out at the request of the Canadian Forestry Service by the Insect Toxicology Section of the Chemical Control Research Institute during 1973.

The bacterial spores and protein crystals become pathogenic when they are ingested by the larvae of certain lepidopterous insects. In order to determine the pathogenicity of the B.t. formulations under laboratory conditions, foliage of the host species of the insect is sprayed with or without insects present. Foliage treated with the known dosage is then fed to a predetermined number of insects. The mortality due to treatment after a certain time period is recorded. When chemical insecticides are sprayed on the infested host plants, the mortality of the pest results from the combined effect of contact and stomach toxicity.

This preliminary report describes a laboratory study of Dipel® and Thuricide 16B®, with and without chitinase for stomach toxicity and fenitrothion formulations for contact toxicity against laboratory reared fifth-instar spruce budworm larvae using larch foliage, Larix laricinia (Du Roi) K. Koch, as diet.

METHODS AND MATERIALS

Biological and Chemical Insecticides and Their Formulations:

Three insecticides were used in this study. The details of their formulations and sources are as follows:

(i) Dipel® - Provided by Abbott Laboratories, Abbott Park, North Chicago, Illinois 60064. The concentrations of active ingredient (B.t.) in the wettable powder (WP) was 3.2% or 16,000 International Units of Potency (IU) per mg or at least 25 billion viable spores per gram. List No. 5188.

(ii) Thuricide HP 16B® - Provided by International Minerals and Chemical Corporation, Libertyville, Illinois 60048. The concentration of active ingredient (B.t.) in the aqueous concentrate (AC) was 0.65% or 3,300 International Units of Potency per mg or at least 5 million viable spores per mg.

(iii) Premium Grade Sumithion® (Fenitrothion) - Provided by Sumitomo Chemical Co., Ltd., Osaka, Japan. The concentration of active fenitrothion was 98.7%. Lot No. 993.

The Dipel and Thuricide concentrate were diluted to 0.016, 0.031, 0.063, 0.125 and 0.250% concentrations of active B.t. Brilliant Sulfa Flavine (BSF) at 0.25% concentration was used as a tracer dye.

The Thuricide aqueous concentrate was diluted to various concentrations with 0.25% BSF dyed distilled water.

The 0.25% stock solution of B.t. from Dipel WP was prepared using 7.8 grams Dipel WP, 44.5 ml CIB* molasses, 0.18 ml Chevron sticker

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* Cargill Insecticides Base, supplied by Cargill, Minneapolis, Minn.

and 0.25 gms of BSE, with distilled water being used to bring the volume up to 100 cc. The stock solution (0.25% B.t.) of Dipel was further diluted to the lower concentrations with diluting solution (containing CIB molasses 44.5%, 0.18% Chevron sticker, 0.25% BSF, and distilled water). Chitinase obtained from Nutritional Biochemicals, Cleveland, Ohio, was mixed in the stock solution of B.t. and in the diluting solutions. The B.t. concentrations were formulated fresh every day and were used within 3 to 4 hours.

The premium grade Sumithion was diluted to 0.5% active fenitrothion concentration with solvent mixture containing 2% Arotex 3470 in No. 2 refined fuel oil. The formulations of biological and chemical insecticides used in the laboratory study were different to those used in the field. The field formulations cannot be used in laboratory studies as they contain very high concentrations of the active ingredients.

Spruce Budworm Larvae:

Second-instar hybernated larvae of spruce budworm were received from the Insect Pathology Research Institute, Sault Ste. Marie. The larvae were reared on artificial diet to the fifth-instar at C.C.R.I. The fifth-instar larvae used for the experiments were sorted in batches of 10 larvae in creamer cups at least 2-3 hours before exposure to insecticide (Fig. A-1). The larvae were supplied with a diet of larch foliage during the experiments. The infestation of microsporidia in the fifth-instar was approximately 85.95%.

Larch Foliage:

Larch foliage was used in these experiments as host material for spruce budworm larvae. These experiments were conducted in the month of October when spruce and balsam fir foliage were not suitable for feeding the insects. Native and European larch were used in the experiments depending upon the availability of green foliage (Fig. A-2). Sufficient suitable foliage was collected from natural stands and plantations in the Ottawa area to carry out the experiments.

Insecticide Treatment:

The spraying procedure was similar to that described by Nigam (1970). A modified Potter's tower (Fig. A-3) was calibrated to deposit volumes of dyed fenitrothion equivalent to 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 gallons per acre (gal/ac) and volumes of dyed B.t. solutions equivalent to 0.5, 1.0 and 2.0 gallons per acre. The calibration of the tower was carried out in time units by using a micro-syringe or micro-pipette for the standard deposit on the required surface area (9 cm No. 1 Whatman filter paper circles). The deposits of insecticides were determined by the colorimetric method as described by Rayner (1956).

Thirty larvae per dosage in replicated groups of ten were sprayed to determine the contact toxicity of fenitrothion. Three two-inch twigs of larch foliage were sprayed with B.t. formulations per replications for stomach toxicity studies. Thirty budworm larvae were released on the treated foliage for each concentration, i.e. ten larvae in each replication of the concentration.

The deposit of active ingredient (AI) was calculated in $\mu g/cm^2$, oz/acre and International Units of Potency (IU) and is presented in Tables A-XIV to A-XVII. Two types of controls (i.e. non-insecticide sprays) were used: (1) controls treated with dyed diluting solutions or dyed diluting solution + chitinase and (2) controls without any treatments.

Observations and Analysis of Data:

There were slight differences in observation techniques for contact and stomach toxicity experiments, the details are presented under separate headings.

(i) Contact Toxicity: The larvae were held at 21°C and 55-60% RH in plastic dixie cups (Fig. A-4) after treatment and fresh larch foliage was provided. Mortality counts were made at 24, 48, and 72 hours after treatment and corrected for control mortality according to Abbott's formula (1925).

(ii) Stomach Toxicity: The larvae were released on the treated larch foliage in plastic dixie cups (Fig. A-4) and were held in 21°C and 55-60% RH for observations. Mortality counts were made at 3, 5, and 7 days after treatment. As the treated foliage dried up it was replaced by untreated fresh foliage. The control mortalities of the same day experiments were pooled and used for calculating the corrected % mortality by Abbott's formula.

The data was not analysed for probit analysis as several experiments will be repeated for critical evaluations.

RESULTS AND DISCUSSION

The details of Bacillus thuringiensis tests are presented in tabulated form as Experiment 1-12 (see appendices). The data for seven days after treatment of B.t. formulations applied at the rate of 0.5, 1.0, and 2.0 gpa are summarized in Tables A-XIV to A-XV and A-XVI, respectively. The concentrations of active B.t. sprayed and dosages deposited are given in $\mu g/cm^2$, oz/acre and IU/cm^2. The corrected percentage mortalities for 24, 48, and 72 hours after treatment of 0.5% fenitrothion applied at the rate of 0.1 to 0.6 gpa are summarized in Table A-XVII and details are presented in Experiment 13.

It is clear from Tables A-XIV to A-XVI that Thuricide alone has the lowest toxicity at all dosages. The addition of chitinase to Thuricide enhances its toxicity at all dosage levels. Dipel appears to be better than Thuricide + chitinase at all dosage levels except at 0.125% concentration (Table A-XIV). The toxicity of Dipel and Dipel + chitinase appears to be the same at the dosage rates applied. In order to analyse the effect of chitinase on the toxicity of Dipel further experiments at lower dosages are needed. The results of B.t. treatments on the basis of present data can be summarized tentatively in the following descending order of toxicity.

Dipel = Dipel + chitinase > Thuricide + chitinase > Thuricide

The toxicity of Thuricide and Thuricide + chitinase is ascending with increasing gpa or deposit. The same is true for Dipel and Dipel + chitinase but the effects are not so dramatic as in the case of

Thuricide due to initial high mortality at the dosages used in the experiments.

It can be safely interpreted that addition of chitinase increases the toxicity of Thuricide approximately 1.5 to 3.0 times depending upon mortality level and dosage at which it is compared. When a comparison of Dipel and Thuricide + chitinase is carried out, the toxicity varies 1.5 to 2.0 times depending upon the mortality level and dosage at which it is compared.

It is difficult to compare fenitrothion with B.t. formulations under the present plan of experiments as fenitrothion is tested only for contact toxicity and B.t. for stomach toxicity. Several more critical experiments are required, where both insect and host foliage will be sprayed together with fenitrothion for investigating its complete effectiveness. On the basis of present data, Dipel appears to be more effective than fenitrothion (Tables A-XIV to A-XII). However, the critical evaluation of efficacy of B.t. formulations and comparisons with fenitrothion can be done only after further experimentation.

SUMMARY AND CONCLUSIONS

It can be concluded from these experiments that Dipel alone and Dipel + chitinase are more effective than Thuricide + chitinase or fenitrothion. Applications of Dipel could cause higher insect mortality of spruce budworm larvae in the field and eventually better protection to foliage, if it is made available in an active form to the insect at the feeding site. These studies will continue further, for precise evaluation of comparative efficacy of Dipel, Dipel + chitinase and fenitrothion.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

Mort.	Mortality (%)
Corr. Mort.	Corrected mortality by Abbott's formula
D/T	Dead/Total
gal/ac	gallons per acre
SBW	Spruce budworm
$\mu g/cm^2$	microgram per square centimeter
B.t.	Bacillus thuringiensis

APPENDICES

EXPERIMENT NO. 1

Objective: To determine the stomach toxicity of Thuricide to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	0.5 gal/ac
Treatment:	Seven (five concentrations of B.t., one dye check, one untreated check)
Concentration(s) of Bacillus thuringiensis (B.t.):	0.016%; 0.031%, 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	10 x 3 = 30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	Ninety (ST 14, 15 and 16 pooled)
Experimental Code:	ST-14
Date:	October 12, 1973

TABLE NO. A-I

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0088	0/29	0	0	0/29	0	0	0/29	0	0
.031	.0175	1/30	3	0	4/30	13	4	4/30	13	2
.063	.0350	4/30	13	10	9/30	30	22	10/30	33	25
.125	.0701	3/30	10	8	11/30	37	30	15/30	50	44
.250	.1401	5/29	17	14	17/29	59	54	17/30	59	54
Dye		0/30	0	0	0/30	0	0	0/30	0	0
Check		3/30	10		6/30	20		6/30	20	
Pooled Check		3/90	3		9/90	10		10/90	11	

EXPERIMENT NO. 2

Objective: To determine the stomach toxicity of Thuricide and chitinase to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	0.5 gal/ac
Treatment:	Seven (five concentrations of B.t. and chitinase, one dye and chitinase check, one untreated check)
Concentration(s) of Bacillus thuringiensis (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	10 x 3 = 30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	Ninety (ST 17, 18 and 19 pooled)
Experimental Code:	ST-17
Date:	October 15, 1973

TABLE NO. A-II

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0088	1/30	3	3	6/29	21	21	6/29	21	20
.031	.0175	6/30	20	20	9/30	30	30	17/30	57	57
.063	.0350	10/30	33	33	18/30	60	60	22/30	73	73
.125	.0701	8/29	28	28	18/29	62	62	24/29	83	83
.250	.1401	6/29	21	21	21/29	72	72	28/29	97	97
Dye + chitinase		0/30	0	0	0/30	0	0	0/30	0	0
Check		0/30	0		0/30	0		1/30	3	
Pooled Check		0/90	0		0/90	0		1/90	1	

EXPERIMENT NO. 3

Objective: To determine the stomach toxicity of Dipel to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	0.5 gal/ac
Treatment:	Seven (five concentrations of B.t., one dye check, one untreated check)
Concentration(s) of Bacillus thuringiensis (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	10 x 3 = 30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	One hundred (ST 22, 23 and 24 pooled)
Experimental Code:	ST-22
Date:	October 24, 1973

TABLE NO. A-III

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0088	4/30	13	13	7/30	23	20	14/30	47	44
.031	.0175	7/30	23	23	14/30	47	45	24/30	80	79
.063	.0350	7/30	23	23	23/30	77	76	27/30	90	89
.125	.0701	8/30	27	27	19/30	63	61	23/30	77	76
.250	.1401	10/30	33	33	23/30	77	76	29/30	97	97
Dye		0/30	0	0	1/30	3	0	1/30	3	0
Check		0/40	0		0/40	0		0/40	0	
Pooled Check		1/100	1		4/100	4		6/100	6	

EXPERIMENT NO. 4

Objective: To determine the stomach toxicity of Dipel and chitinase to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	0.5 gal/ac
Treatment:	Seven (five concentrations of B.t. and chitinase, one dye and chitinase check, one untreated check)
Concentration(s) of *Bacillus thuringiensis* (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	10 x 3
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	One hundred (ST 25, 26 and 27 pooled)
Experimental Code:	ST-25
Date:	October 25, 1973

TABLE NO. A-IV

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage $\mu g/cm^2$	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0088	1/30	3	3	9/30	30	26	18/30	60	56
.031	.0175	2/30	7	7	13/30	43	39	20/30	67	64
.063	.0350	5/30	17	17	17/30	57	54	23/30	77	75
.125	.0701	9/29	31	31	26/29	90	89	28/29	97	97
.250	.1401	9/31	28	28	27/31	87	86	31/31	100	100
Dye + chitinase		0/30	0	0	3/30	10	4	4/30	13	4
Check		0/40	0		2/40	8		4/40	10	
Pooled Check		0/100	0		6/100	6		9/100	9	

EXPERIMENT NO. 5

Objective: To determine the stomach toxicity of Thuricide to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	1.0 gal/ac
Treatment:	Seven (five concentrations of B.t., one dye check, one untreated check)
Concentration(s) of *Bacillus thuringiensis* (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.25
Replications:	3
No. of larvae per treatment:	30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	Ninety (ST 14, 15 and 16 pooled)
Experimental Code:	ST-15
Date:	October 12, 1973

TABLE NO. A-V

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0175	0/30	0	0	2/29	7	0	5/29	17	7
.031	.0350	7/30	23	23	10/30	33	26	12/30	40	33
.063	.0701	12/30	40	40	16/30	53	48	18/30	60	55
.125	.1401	8/30	27	27	21/30	70	67	24/30	80	78
.250	.2803	17/30	57	57	27/30	90	89	29/30	97	97
Dye		1/30	3	0	1/30	3	0	1/30	3	0
Check		0/30	0		0/30	0		0/30	0	
Pooled Check		3/90	3		9/90	10		10/90	11	

EXPERIMENT NO. 6

Objective: To determine the stomach toxicity of Thuricide and chitinase to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	1.0 gal/ac
Treatment:	Seven (five concentrations of B.t. and chitinase, one dye and chitinase check, one untreated check)
Concentration(s) of *Bacillus thuringiensis* (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	Ninety (ST 17, 18 and 19 pooled)
Experimental Code:	ST-18
Date:	October 15, 1973

TABLE NO. A-VI

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0175	4/30	13	13	8/30	27	27	12/30	40	39
.031	.0350	3/28	11	11	14/28	50	50	16/28	57	57
.063	.0701	8/30	27	27	20/30	67	67	24/30	80	80
.125	.1401	13/30	43	43	22/30	73	73	29/30	97	97
.250	.2803	21/29	72	72	28/29	97	97	29/29	100	100
Dye + chitinase		1/30	3	3	1/30	3	3	1/30	3	2
Check		0/30	0		0/30	0		0/30	0	
Pooled Check		0/90	0		0/90	0		1/90	1	

EXPERIMENT NO. 7

Objective: To determine the stomach toxicity of Dipel to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	1.0 gal/ac
Treatment:	Seven (five concentrations of B.t., one dye check, one untreated check)
Concentration(s) of Bacillus thuringiensis (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	One hundred (ST 22, 23 and 24 pooled)
Experimental Code:	ST-23
Date:	October 24, 1973

TABLE NO. A-VII

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0175	5/30	17	16	17/30	57	55	26/30	87	86
.031	.0350	9/29	31	30	21/29	72	71	22/29	76	74
.063	.0701	8/30	27	26	24/30	80	79	28/30	93	93
.125	.1401	14/30	47	46	27/30	90	90	29/30	97	97
.250	.2803	19/30	63	63	30/30	100	100	30/30	100	100
Dye		0/29	0	0	1/29	3	0	3/29	10	4
Check		1/30	3		3/30	10		5/30	17	
Pooled Check		1/100	1		4/100	4		6/100	6	

EXPERIMENT NO. 8

Objective: To determine the stomach toxicity of Dipel and chitinase to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	1.0 gal/ac
Treatment:	Seven (five concentrations of B.t. and chitinase, one dye and chitinase check, one untreated check)
Concentration(s) of Bacillus thuringiensis (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	One hundred (ST 25, 26 and 27 pooled)
Experimental Code:	ST-26
Date:	October 25, 1973

TABLE NO. A-VIII

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0175	8/30	27	27	17/30	57	54	21/30	70	67
.031	.0350	9/30	30	30	20/30	67	65	27/30	90	89
.063	.0701	7/30	23	23	24/30	80	77	26/30	87	86
.125	.1401	11/30	37	37	26/30	87	86	29/30	97	97
.250	.2803	18/30	60	60	28/30	93	93	30/30	100	100
Dye + chitinase		0/30	0	0	0/30	0	0	1/30	3	
Check		0/30	0		0/30	0		1/30	3	
Pooled Check		0/100	0		6/100	6		9/100	9	

EXPERIMENT NO. 9

Objective: To determine the stomach toxicity of Thuricide to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	2.0 gal/ac
Treatment:	Seven (five concentrations of B.t., one dye check, one untreated check)
Concentration(s) of Bacillus thuringiensis (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	Ninety (ST 14, 15 and 16 pooled)
Experimental Code:	ST-16
Date:	October 12, 1973

TABLE NO. A-IX

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0350	2/30	7	4	6/30	20	11	9/30	30	21
.031	.0701	2/30	7	4	13/30	43	37	14/30	47	40
.063	.1401	8/29	28	26	18/29	62	58	21/29	72	69
.125	.2803	14/29	48	46	27/29	93	92	28/29	97	97
.250	.5605	23/30	77	76	29/30	97	97	29/30	97	97
Dye		1/30	3	0	2/30	7	0	2/30	7	0
Check		0/30	0		3/30	10		4/30	13	
Pooled Check		3/90	3		9/90	10		10/90	11	

EXPERIMENT NO. 10

Objective: To determine the stomach toxicity of Thuricide and chitinase to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	2.0 gal/ac
Treatment:	Seven (five concentrations of B.t. and chitinase, one dye and chitinase check, one untreated check)
Concentration(s) of Bacillus thuringiensis (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	Ninety (ST 17, 18 and 19 pooled)
Experimental Code:	ST-19
Date:	October 15, 1973

TABLE NO. A-X

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0350	6/30	20	20	15/30	50	50	20/30	67	67
.031	.0701	9/30	30	30	21/30	70	70	24/30	80	80
.063	.1401	12/29	41	41	24/29	83	83	27/29	93	93
.125	.2803	21/30	70	70	30/30	100	100	30/30	100	100
.250	.5605	28/30	93	93	30/30	100	100	30/30	100	100
Dye + chitinase		2/30	7	7	2/30	7	7	2/30	7	6
Check		0/30	0		0/30	0		0/30	0	
Pooled Check		0/90	0		0/90	0		1/90	1	

EXPERIMENT NO. 11

Objective: To determine the stomach toxicity of Dipel to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	2.0 gal/ac
Treatment:	Seven (five concentrations of B.t., one dye check, one untreated check)
Concentration(s) of Bacillus thuringiensis (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	One hundred (ST 22, 23 and 24 pooled)
Experimental Code:	ST-24
Date:	October 24, 1973

TABLE NO. A-XI

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0350	18/29	62	62	25/29	86	85	28/29	97	97
.031	.0701	19/30	63	63	28/30	93	93	30/30	100	100
.063	.1401	18/30	60	60	28/30	93	93	30/30	100	100
.125	.2803	14/30	47	46	27/30	90	90	29/30	97	97
.250	.5605	16/31	52	52	29/31	94	94	31/31	100	100
Dye		0/30		0	2/30	7	3	4/30	13	7
Check		0/30		0	1/30	3		1/30	3	
Pooled Check		1/100	1		4/100	4		6/100	6	

EXPERIMENT NO. 12

Objective: To determine the stomach toxicity of Dipel and chitinase to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	2.0 gal/ac
Treatment:	Seven (five concentrations of B.t. and chitinase, one dye and chitinase check, one untreated check)
Concentration(s) of *Bacillus thuringiensis* (B.t.):	0.016%; 0.031%; 0.063%; 0.125%; 0.250%
Replications:	3
No. of larvae per treatment:	30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	One hundred (ST 25, 26 and 27 pooled)
Experimental Code:	ST-27
Date:	October 25, 1973

TABLE NO. A-XII

B.t.		Mortality Counts After								
		3 Days			5 Days			7 Days		
Conc. % B.t.	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.016	0.0350	9/30	30	30	25/30	83	82	27/30	90	89
.031	.0701	17/30	57	57	24/30	80	79	27/30	90	89
.063	.1401	17/30	57	57	30/30	100	100	30/30	100	100
.125	.2803	27/30	90	90	29/30	97	97	30/30	100	100
.250	.5605	17/30	57	57	30/30	100	100	30/30	100	100
Dye + chitinase		0/30	0	0	1/30	3	0	2/30	7	0
Check		0/30	0		3/30	10		4/30	13	
Pooled Check		0/100	0		6/100	6		9/100	9	

EXPERIMENT NO. 13

Objective: To determine the contact toxicity of fenitrothion to fifth-instar laboratory reared spruce budworm.

Plan of Experiment:

Rate of Application:	0.1, 0.2, 0.3, 0.4, 0.5, 0.6 gal/ac
Treatment:	Seven (six rates of application, plus check)
Concentration(s) of insecticides:	0.5%
Replications:	3
No. of larvae per treatment:	30
Total No. of larvae utilized:	30 x 7 = 210
No. of larvae for untreated check:	Thirty
Experimental Code:	Exp-SBL 73
Date:	October 29, 1973

TABLE NO. A-XIII

Insecticide Fenitrothion 0.5%		Mortality Counts After								
		3 Days			5 Days			7 Days		
gal/ac	Dosage µg/cm²	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.	D/T	% Mort.	Corr. Mort.
0.1	0.056	0/29	0	0	0/29	0	0	0/29	0	0
0.2	.112	7/28	25	25	7/28	25	25	10/28	36	36
0.3	.168	10/27	37	37	10/27	37	37	12/27	44	44
0.4	.224	21/30	70	70	21/30	70	70	22/30	73	73
0.5	.280	21/29	72	72	24/30	80	80	24/30	80	80
0.6	.336	30/30	100	100	30/30	100	100	30/30	100	100
Check		0/30	0			0			0	

TABLE NO. A-XIV

Toxicity of B.t. formulations at 0.5 gal/ac against fifth-instar laboratory reared spruce budworm - seven days after treatment

% Conc. of active B.t.	Dosage (AI)			Corrected Percentage Mortality After Seven Days			
	$\mu g/cm^2$	IU/cm^2	oz/acre	Thuricide	Thuricide & chitinase	Dipel	Dipel & chitinase
0.016	0.0088	4.38	0.013	0	20	44	56
.031	.0175	8.76	.025	2	57	79	64
.063	.0350	17.53	.050	25	73	89	54
.125	.0701	35.06	.100	44	83	76	97
.250	.1401	70.12	.200	54	97	97	100
Untreated check mortality (%)				11	1	6	9

TABLE NO. A-XV

Toxicity of B.t. formulations at 1.0 gal/ac against fifth-instar laboratory reared spruce budworm - seven days after treatment

% Conc. of active B.t.	Dosage (AI)			Corrected Percentage Mortality After Seven Days			
	µg/cm^2	IU/cm^2	oz/acre	Thuricide	Thuricide & chitinase	Dipel	Dipel & chitinase
0.016	0.0175	8.76	0.025	7	39	86	67
.031	.0350	17.53	.050	33	57	74	89
.063	.0701	35.06	.100	55	80	93	86
.125	.1401	70.12	.200	78	97	97	97
.250	.2802	140.23	.400	97	100	100	100
Untreated check mortality (%)				11	1	6	9

TABLE NO. A-XVI

Toxicity of B.t. formulations at 2.0 gal/ac against fifth-instar laboratory reared spruce budworm - seven days after treatment

% Conc. of active B.t.	Dosage (AI)			Corrected Percentage Mortality After Seven Days			
	µg/cm²	IU/cm²	oz/acre	Thuricide	Thuricide & chitinase	Dipel	Dipel & chitinase
0.016	0.0350	17.53	0.050	21	67	97	89
.031	.0701	35.06	.100	40	80	100	89
.063	.1401	70.12	.200	69	93	100	100
.125	.2802	140.23	.400	97	100	97	100
.250	.5604	280.46	.800	97	100	100	100
Untreated check mortality (%)				11	1	6	9

TABLE NO. A-XVII

Toxicity of fenitrothion (0.5% active ingredient) against fifth-instar laboratory reared spruce budworm

gal/ac	Dosage (AI)		Corrected Percentage Mortality		
	µg/cm²	oz/acre	24 hours	48 hours	72 hours
0.1	.056	.080	0	0	0
0.2	.112	.160	25	25	36
0.3	.168	.240	37	37	44
0.4	.224	.320	70	70	73
0.5	.280	.400	72	80	80
0.6	.336	.480	100	100	100
Untreated check mortality			0	0	0

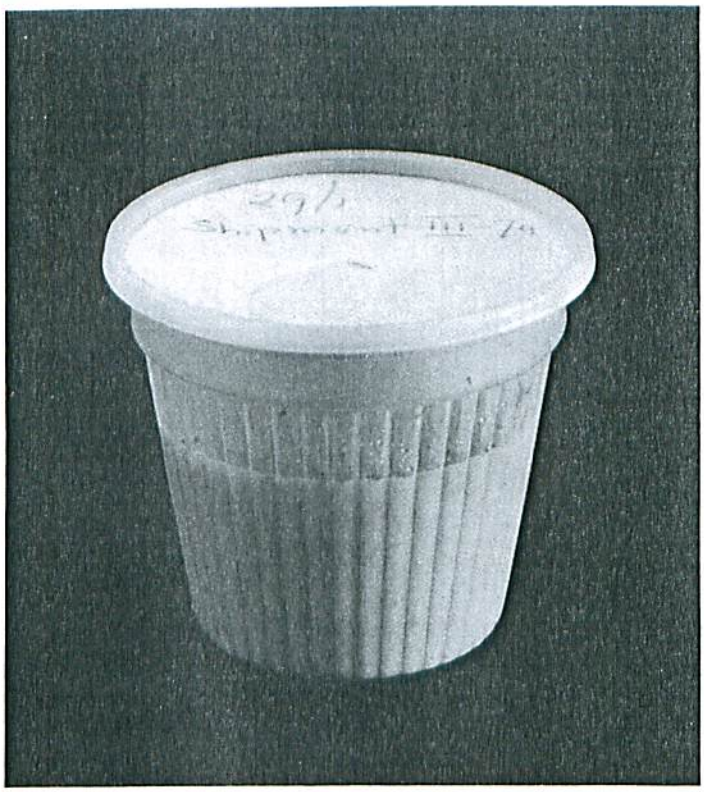

Fig. A-1 Creame Cups

Fig. A-2 Larch Foliage

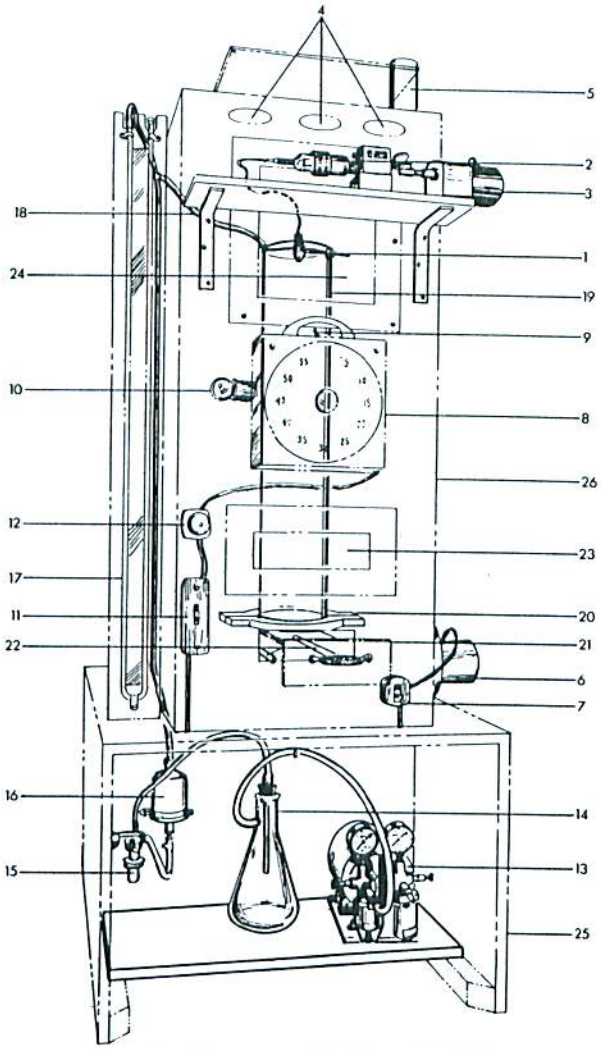

Fig. A-3

CONTACT TOXICITY TOWER 1. Potter's nozzle intermediate design 2. Manostat micropipet buret 3. Apcor multi-ratio gear motor 4. Air intake ports and hinged cover 5. Exhaust pipe 6. Exhaust fan - Electrohome model 2882-44-05-088 7. Exhaust fan switch 8. Gralab Universal Timer - electric 9. Timer switch 10. Timer light 11. Power switch 12. Power light 13. Gast airpump - pressure vacuum senior model 14. Flask filtering pyrex 1000 ml. 15. Air pressure auto expansion valve model 240C 16. Koby air purifier 17. Mercury manometer - Pyrex U - tube 90 cm. 18. Airtube to nozzle - Tygon (I.D. 1/4" O.D. 3/8") 19. Glass cylinder 20. Cylinder clamp 21. Sliding tray 22. Sliding tray guide rods 23. Window-glass 24. Window - glass 25. Plywood stand on casters 3/4" plywood - W. 27" H. 19" D. 19" 26. Plywood tower 1/2" plywood W. 18" H. 42" D. 16"

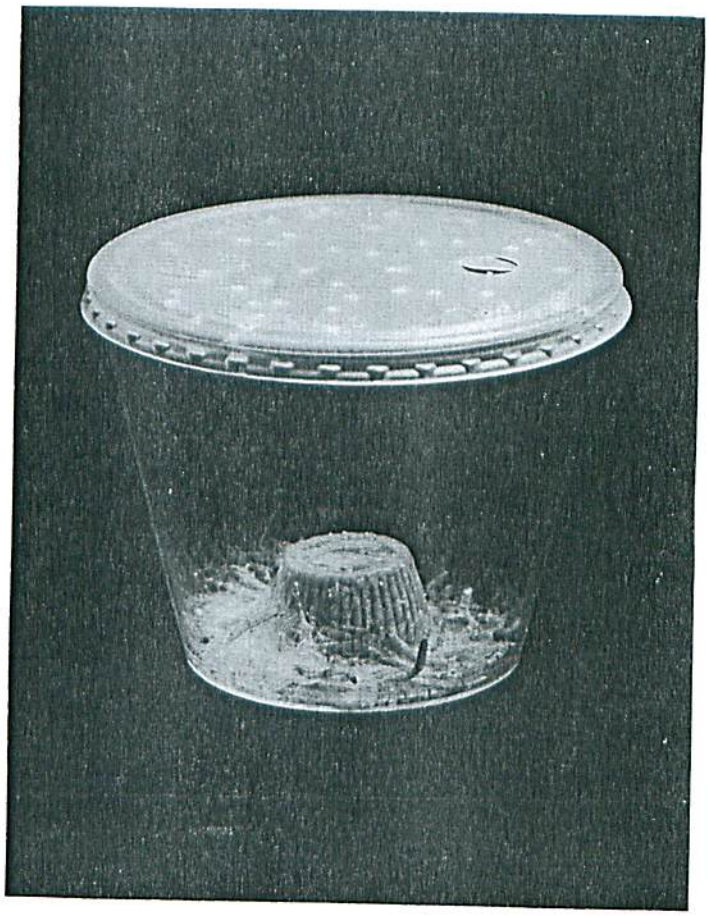

Fig. A-4 Plastic Dixie Cups

Evaluation of Commercial Preparations of *Bacillus thuringiensis* with and without Chitinase Against Spruce Budworm

B. Simulated Aerial Sprays in a Young White Spruce Plantation, Shawville, Quebec

by W.W. Hopewell

Chemical Control Research Institute
Canadian Forestry Service
Ottawa, Ontario

Information Report CC-X-59

February, 1974

TABLE OF CONTENTS

B. Simulated Aerial Sprays in a Young White Spruce Plantation, Shawville, Quebec

by

W.W. Hopewell

INTRODUCTION

The tests reported here were supplemental to the experiments carried out in Algonquin Park, 1973, by the Chemical Control Research Institute on efficacy of some commercial preparations of Bacillus thuringiensis (B.t.) for control of spruce budworm, Choristoneura fumiferana (Clem.). The opportunity to contribute to the investigation of the efficacy of certain B.t. formulations was taken while field testing an experimental device and procedure (Hopewell 1974[1]) for applying simulated aircraft spray in measured doses to individual small trees. The spray formulations were identical to those used in the Algonquin Park experiments as described by Morris and Hildebrand (Part E of this report), plus two additional B.t. treatments containing a very small amount of fenitrothion. It should be noted that in these tests all trees were white spruce, Picea glauca (Moench) Voss, while in the Algonquin Park experiments, most were balsam fir, Abies balsamea (L.) Mill.

The main objectives were:

1. To field test the device and technique for applying measured

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[1] W.W. Hopewell, 1974. A device and technique for applying measured amounts of simulated aircraft spray to individual small trees. (Manuscript in preparation).

doses of pesticide in the form of an aerial spray deposit to individual small trees.

2. To determine the activity, under natural weathering conditions, of the two commercial preparations of B.t. when applied as aerial sprays to small trees infested with spruce budworm.

3. To determine the effect of small amounts of fenitrothion added to the B.t. preparations and applied in the same way.

4. To contribute to the CCRI study on evaluation of commercial B.t. preparations and their possible registration for use against spruce budworm.

MATERIALS AND METHODS

Selection of Study Trees

The tests were carried out on a privately-owned tree farm near Shawville, Que., in an area comprised of white spruce 3 to 20 feet in height, from plantings over the past 20 years. The 8-acre test area was marked off in 5 sections 150 feet wide and up to 500 feet deep. Trees 8 to 10 feet in height and with at least moderate budworm infestation, as indicated by abundance of mined needles, were selected and numbered. Four branches on each test tree were selected and tagged with plastic ribbon 18 inches back from terminal buds, one branch in each quadrant at 4 to 5 feet above ground level. A count of mined needles and number of new shoots on each branch from the tag outward to the tips was made and recorded.

Spray Application

Prior to application, a portable shelter 8 feet in height and surrounding a ground surface area 7 x 7 feet was erected around the tree to be treated. A measured 2.2 ml of the test mix (for a nominal application rate of 66 fl. oz. per acre) was emitted from a spinning disc spray device being moved systematically over the enclosed area. The device was fixed to the end of a 7 ft. horizontal arm on an 8 ft. vertical shaft (Fig. B-1). Power to the spinning disc and feed mechanism was supplied from a variable voltage source (2 to 6 volts). One in every five test trees was left as an untreated check for determination of normal population levels. To eliminate the possibility of bias, trees were identified by number only so that treatment to each tree was not known to the person making post-spray observations.

Spray treatment was carried out during the period May 23 to 30. At the beginning of this period the new shoots were generally compact with bud caps on; by the end of the period shoots were extended 1 to 3 inches.

Spray Solutions

All the spray formulations were made up in 100 ml volumes. The required amounts of Dipel WP®, Thuricide 16B®, technical grade fenitrothion (Sumithion®), and Brilliant Sulfo Flavine dye (BSF) were transferred to 125 ml erlenmeyer flasks at the laboratory and the remaining ingredients (water, Cargill Insecticide Base molasses (CIB), Chevron sticker, Arotex 3470, chitinase) added just before use in the field. The chitinase was made up as an aqueous solution containing 1 mg/ml for ease of measuring. All components were part of those used in the

Algonquin Park experiments and described in Section E of this report.

The composition of each spray mix is given in Table No. B-I.

TABLE NO. B-I

Composition of Formulations, Shawville, 1973.

Treatment	Components	
A. Dipel	Dipel WP	11.9 g
	Chevron sticker	0.2 ml
	BSF dye	0.1 g
	Water:molasses 1:1 to make	100.0 ml
B. Dipel + chitinase	As for A + 0.5 mg chitinase	
C. Thuricide	Thuricide 16B	50.0 ml
	BSF dye	0.1 g
	Water	50.0 ml
D. Thuricide + chitinase	As for C + 0.5 mg chitinase	
E. Chitinase	Chitinase	0.5 mg
	BSF dye	0.1 g
	Chevron sticker	0.2 ml
	Water:molasses 1:1 to make	100.0 ml
F. Thuricide + fenitrothion	Thuricide 16B	50.0 ml
	Fenitrothion (tech.)	0.3 ml (0.37 g)
	BSF dye	0.1 g
	Water	50.0 ml
G. Dipel + fenitrothion	Dipel WP	11.9 g
	Fenitrothion (tech.)	0.3 ml
	Chevron sticker	0.2 ml
	BSF dye	0.1 g
	Water:molasses 1:1 to make	100.0 ml
H. Fenitrothion	Fenitrothion (tech. Sumithion®)	11.3 ml (15.0 g)
	Arotex 3470	29.2 ml
	Fuel oil	58.6 ml
	Automate red dye	0.9 ml

Spray Deposit Sampling

Samples of spray deposit were taken at two positions (A & B) during application, on opposite sides of the tree and approximately two feet from the stem. The sample units, a pertri dish for colorimetric assessment of deposit density, and a Kromekote card* for droplet count, were mounted on staked holders about 4 feet above ground level and clear of overhanging foliage (Fig. B-3).

Post-spray Observations

The final count of living budworm larvae and pupae was done on June 8, 11, 12 and 13, i.e., 2 weeks ± 2 days after treatment. Each 18-inch branch was collected and processed using the technique and apparatus (Fig. B-2) described by DeBoo et al. (1973) and Martineau and Benoit (1973). Number of larvae (by instar) and pupae were counted and recorded.

RESULTS AND DISCUSSION

The effect of the various treatments was evaluated by comparison of the mean infestation levels of the branch samples from trees in each treated group against levels from untreated check trees. The infestation level on each tree was the number of living budworm per 100 shoots based on totals of shoots and budworm on the 4 sample branches of each tree. The mean infestation and standard deviation for each treatment group was determined and the results are given in Table No. B-II.

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* Kruger Paper Co., Montreal, Que.

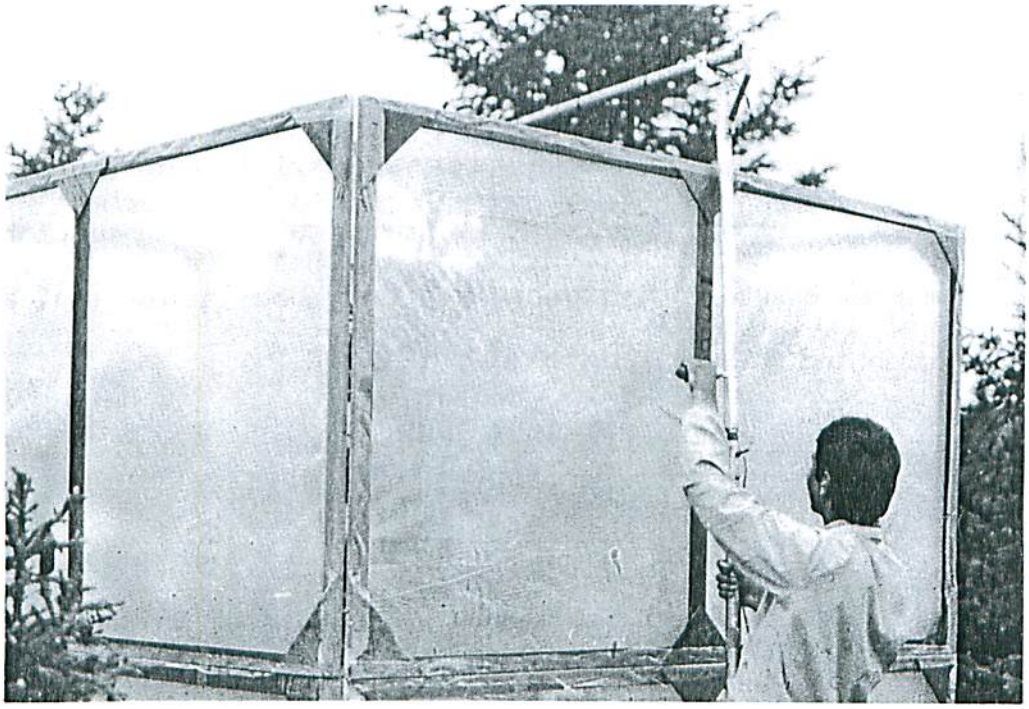

Fig. B-1 Spray devise in use at Shawville

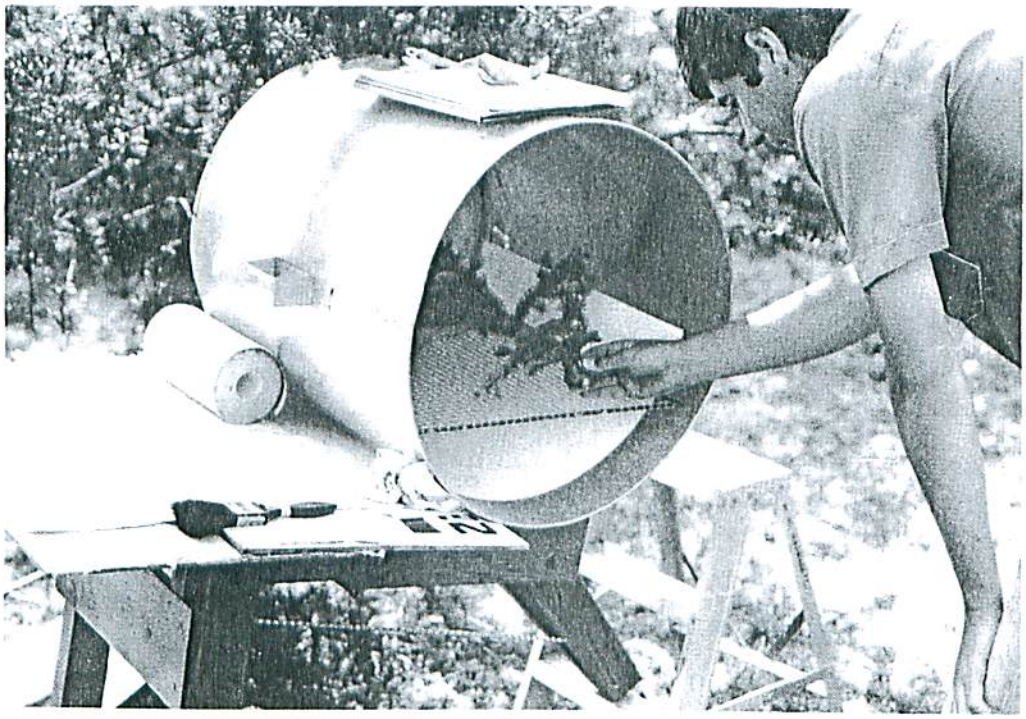

Fig. B-2 Modified use of the sampling apparatus for determining larval densities on branch samples

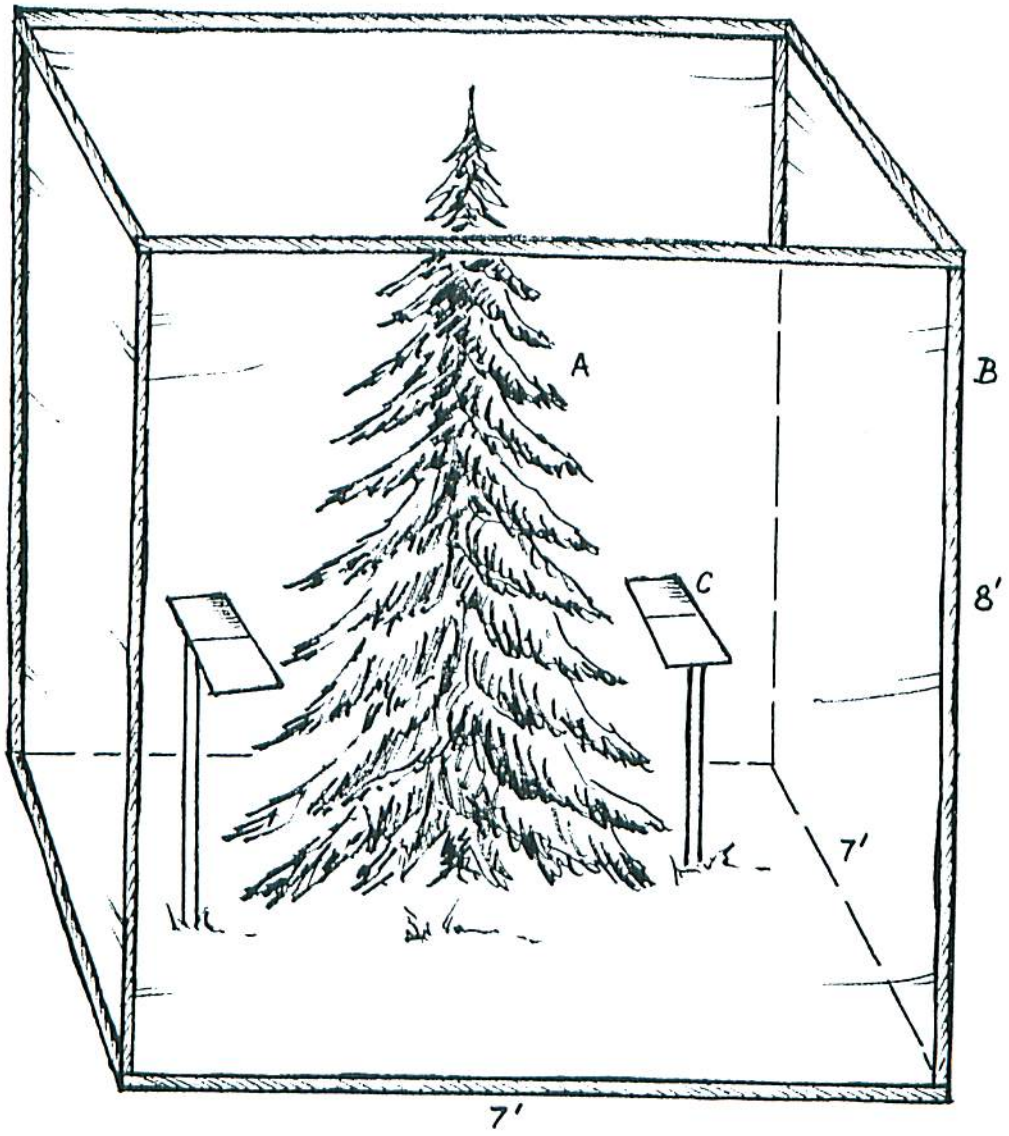

Fig. B-3 Arrangement for treatment test trees and sampling spray deposit.
Test tree (A) surrounded by portable shelter (B) and samples of deposit collected on staked holders (C)

The results indicated that:

1. None of the B.t. treatments reduced mean infestation levels compared with check trees.

2. Chitinase did not improve effectiveness of either Dipel or Thuricide sprays.

3. A small amount of fenitrothion (0.4%) combined with Dipel and Thuricide formulations resulted in a reduction in budworm infestation levels (a) compared with check and (b) compared with either Dipel or Thuricide treatments.

4. Fenitrothion at an average deposit of 5.9 oz. AI/acre (applied at a nominal 10 oz. AI in 69 fl. oz/acre) gave greatest reduction of infestation, i.e., 31% below check trees.

5. Fenitrothion applied at an aerial operational dosage rate of 3.3 oz. AI in 22 fl. oz/acre and giving an average measured deposit of 2.2 oz. AI in 14.5 fl. oz/acre resulted in a 14% decrease in population density compared with the mean check population.

The data on budworm development distribution at 14 days post-spray have been summarized for each treatment and are given in Table No. B-III. The data show the percent of surviving budworm in the 4th and 5th instar ($L_4 + L_5$) combined, L_6 and pupae. There are two cases where distribution varies significantly from the checks, i.e., Thuricide alone and Dipel + fenitrothion, where development seems to have been retarded to the extent that few had pupated. However, in the latter case, there were only 2 trees in the test and results could be attributed to natural variation.

The actual deposits as determined by sampling were summarized and the averages for each treatment given in Table No. B-IV. The deposit

data give some clues to the physical characteristics of the formulations when applied as sprays. The Dipel treatments showed higher percent recovery of emitted spray over Thuricide. This appears to be a result of much larger drop size from Dipel formulations than was obtained from Thuricide sprays; even with a much lower average deposit of Thuricide than Dipel (ca 30 vs 46 fl. oz/acre, respectively) there were more drops/cm^2 (ca 28 vs 20). The Thuricide spray formulations appear to be more readily dispersed into fine drops than Dipel sprays.

In general, the spray coverage was good with averages ranging from 23 to 55 fl. oz/acre and 15 to 40 drops/cm^2, with the nominal output rate of 66 fl. oz/acre. The diameter of drops of average volume was calculated from the deposit data on fl. oz/acre and drops/cm^2 (Table No. B-IV), and ranged from 110 μ to 150 μ. It is likely that this wide range of average drop diameters reflects differences in the formulations and meteorological conditions at time of application.

SUMMARY AND CONCLUSIONS

Applications of the two commercial preparations of Bacillus thuringiensis, Dipel WP and Thuricide 16B, did not result in a significant decrease in the mean budworm infestation levels on small spruce trees 2 weeks after treatment. Also, the addition of chitinase to the formulations did not increase their activity against budworm within this same time period. It is possible that with this slow acting organism (compared with chemical pesticides) more time is required for its total effect to become evident.

A small fenitrothion content (0.4%) in the B.t. spray mixtures

gave mean population reductions as great as obtained with 10 times the amount of fenitrothion alone. This suggests synergistic action and significant potentiation over either active ingredient used alone, and should be further studied.

Two treatments (Thuricide, Dipel + fenitrothion) resulted in apparent retardation of budworm development (very little pupation) during the observation period. This would require further study to clarify or determine if significant.

Fenitrothion applied at a rate used in operational aerial sprays (3.3 oz. AI in 22 fl. oz/acre) and giving a measured deposit dose of 2.2 oz. AI/acre in this treatment, gave only slight reduction of infestation levels on white spruce trees. When applied at a rate of 10 oz. AI in 69 fl. oz/acre and giving an average measured deposit of 5.9 oz. AI/acre, the mean population reduction due to treatment was about 30%.

The device and technique for applying simulated aerial spray deposit on small trees gave generally good results. Spray coverage in some cases was more variable than expected, apparently a result of air turbulence. However, the average number of drops/cm^2 (15 to 40) is in the range expected from aircraft emission. The diameter of drops of average volume (110 to 150 μ) was also within the range encountered with aerial spray deposits.

ACKNOWLEDGEMENTS

Thanks are extended to Mr. Geo. Eades who kindly allowed full use of facilities on his tree farm near Shawville, Que., under ideal conditions for this experiment. Invaluable assistance in all phases of

work was provided by Mr. M. Hobbs, while Mr. Herman Emerson made a substantial contribution to the field phase. The interest and helpful suggestions of Dr. R.F. DeBoo are gratefully acknowledged. The B.t. preparations were supplied by International Minerals and Chemicals Corp., Libertyville, Ill., and Abbott Laboratories, North Chicago, Ill.

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Martineau, R. and P. Benoit. 1973. A sampling technique for estimating numerical trends in larval populations of insect defoliators on conifers. II. Modification and operational use of the technique for extensive sampling of spruce budworm populations in Quebec. Ibid. 23-31.

TABLE NO. B-II

Budworm Infestation Levels on Each Group of Test Trees

(Means and Standard Deviations, Differences from Check Trees)

Treatment	No. of Trees	No. Budworm/ 100 Shoots (Mean ± S.D.)	Difference from checks (%)
A. Dipel	8	10.2 ± 6.1	-2
B. Dipel + chitinase	4	10.4 ± 5.2	0
C. Thuricide	4	13.3 ± 8.3	28
D. Thuricide + chitinase	4	13.8 ± 6.5	33
E. Chitinase	4	12.5 ± 2.6	20
F. Thuricide + fenitrothion	6	8.6 ± 2.5	-17
G. Dipel + fenitrothion	2	9.4 ± 7.2	-10
H_1. Fenitrothion	4	7.2 ± 2.3	-31
H_2. Fenitrothion	4	8.9 ± 4.6	-14
Untreated Checks	22	10.4 ± 9.4	0

TABLE NO. B-III

Budworm Development Distribution in Each Treatment Group at Final Count

Treatment	No. of Trees	Total No. budworm	Per Cent in Stage		
			L_4+L_5	L_6	Pupae
A. Dipel	8	192	21	59	20
B. Dipel + chitinase	4	91	11	47	42
C. Thuricide	4	109	29	70	1
D. Thuricide + chitinase	4	120	14	56	30
E. Chitinase	4	101	12	62	26
F. Thuricide + fenitrothion	6	151	9	60	31
G. Dipel + fenitrothion	2	39	28	69	3
H_1. Fenitrothion	4	79	6	47	47
H_2. Fenitrothion	4	96	24	53	23
Untreated Checks	22	512	18	53	29

TABLE NO. B-IV

Summary of Deposit Data for Each Treatment

	Treatment	Avg. Vol. (ml) emitted tree*	Avg. deposit found (fl. oz./acre)	Fenitrothion deposited (oz. AI/ac)	% Recovery	Avg. No. drops/cm^2	Diam. of drop of avg. volume (μ)
A.	Dipel	2.1	40.9	-	65	20.1	140
B.	Dipel + chitinase	2.4	51.8	-	72	21.0	150
C.	Thuricide	1.8	30.4	-	56	28.2	114
D.	Thuricide + chitinase	2.4	27.2	-	38	27.6	110
E.	Chitinase	2.2	29.6	-	45	19.4	128
F.	Thuricide + fenitrothion	2.0	23.3	0.09	39	15.3	128
G.	Dipel + fenitrothion	2.3	55.0	0.22	80	24.5	148
H_1.	Fenitrothion	2.2	39.2	5.9	59	25.2	128
H_2.	Fenitrothion	0.7	14.5	2.2	69	12.2	117

* Intended volume per tree was 2.2 ml for all except H_2, but technical difficulties resulted in some variation

Evaluation of Commercial Preparations of *Bacillus thuringiensis* with and without Chitinase Against Spruce Budworm

C. Assessment of Effectiveness by Mistblower and Aerial Application, Spruce Woods, Manitoba

by R.F. DeBoo and L.M. Campbell

Chemical Control Research Institute
Canadian Forestry Service
Ottawa, Ontario

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C. Assessment of Effectiveness by Mistblower and Aerial Application, Spruce Woods, Manitoba

by

R.F. DeBoo and L.M. Campbell

INTRODUCTION

Recent field evaluations in Canada and the United States have shown that larvae of the spruce budworm, Choristoneura fumiferana (Clem.) are highly susceptible to infection by improved commercial preparations of the bacterial insecticide Bacillus thuringiensis Berliner (B.t.) currently available (Dulmage 1973, Tripp 1972, Yamvrias and Angus 1970). Also, recent studies have indicated that spray efficacy (i.e. increased stress on larval feeding habits) may be enhanced in terms of foliage protection by the addition of small quantities of the enzyme chitinase to spray mixtures (Dimond 1972, Smirnoff 1971, Smirnoff et al 1973).

As part of the 1973 field program of the Chemical Control Research Institute (C.C.R.I.) to evaluate B.t. and B.t. + chitinase sprays, a study was undertaken at the Spruce Woods Provincial Park and Forest area in Manitoba where serious infestations of the spruce budworm had occurred during the past few years. The project also was designed to satisfy the research requirement in the license granted to the Parks Branch of the Manitoba Department of Tourism, Recreation and Cultural Affairs for application of fenitrothion to approximately 9,000 acres of the most seriously threatened stands. Major objectives of the study were:

(1) To compliment the main C.C.R.I. study at Algonquin Park in Ontario through geographical replication of certain B.t. spray treatements.

(2) To determine the efficacy of B.t. sprays applied at 4 billion International Units (IU)/acre on budworm infesting pure stands of white spruce, Picea glauca (Moench) Voss.

(3) To determine the effectiveness of the same rate of application (i.e. 4 billion IU) selected for aerial experimentation at Algonquin Park, but in larger volumes of water-molasses spray mixture base.

(4) To determine the effectiveness of dilute B.t. sprays (also containing 4 billion IU/acre) using ground application equipment.

(5) To contribute data on the efficacy of B.t. sprays for possible federal registration, particularly for use in parks and other amenity areas where treatment of trees by synthetic organic chemicals is not feasible.

MATERIALS AND METHODS

The Study Area

Average stocking of white spruce in the Spruce Woods area is low compared to densities in eastern forests - about 300 stems/acre. Individual trees, or groups of trees ranging upwards to a few hundred in number, occur randomly throughout the rolling grassland countryside (Fig. C-1). Since many trees are open-growing, foliage and branch retention is great, and the crowns may extend to ground level. The forest-

grassland complex is characterized by these tall, mature (and majestic) spruces which commonly reach 70 ft. in height and 12-18 in. in trunk diameter.

Other major tree species include trembling aspen (Populus tremuloides Michx.), balsam poplar (P. balsamifera L.) and bur oak (Quercus macrocaspa Michx.). In addition, several thousand acres of coniferous plantations (mostly jack pine, Pinus banksiana L., and Scots pine, P. sylvestris L.) have been established in the Spruce Woods during the past 50 years. The predominance of white spruce in the grassland environment, however, is the single most important aesthetic quality of this unique recreational resource in central Canada.

Populations of the spruce budworm have been reported in the Spruce Woods since the inception of the Forest Insect and Disease Survey of the Canadian Forestry Service in 1937. Populations of the insect ranging from 20-30 per 18 in. branch tip were recorded by the authors in 1968 and 1969 during the course of other studies. At that time, the heavily foliated trees had sustained only minimal injury due to budworm feeding; in fact, the visual appearance of most trees was excellent. By 1972, staff of the Northern Forest Research Centre (N.F.R.C.) at Winnipeg had recorded dramatic increases in budworm population densities (to 50 or more/18 in. branch), and crown top-killing due to serious foliage depletion was observed for the first time in many areas. Subsequently, the Manitoba Parks Branch proposed aerial spray treatment for the protection of the spruce trees during 1973, and two areas were reserved for experimental applications of B.t.

Aerial spray blocks were located near the northern boundary of

the Spruce Woods Provincial Forest, whereas the area selected for treatment by mistblower was located within the southern half of the Spruce Woods Provincial Park (Fig. C-2). Stand composition of these selected treatment areas was characteristic of the Spruce Woods, although trees in both areas had sustained more serious budworm injury during 1972 than trees at most other locations.

Experimental Design

Two 100-acre blocks were established for (non-replicated) aerial spray treatment. Another 100-acre area was reserved for untreated check-tree sampling. The spray treatment blocks were separated by a buffer strip approximately 0.5 miles wide.

An area of approximately 30 acres was delineated for ground-spray applications. Naturally-segregated groups of 25 trees were utilized for establishment of treatment replicates. Two groups (replicates) were sprayed for each treatment. Again, two similar groups of trees were selected as the untreated check. Groups of treated and untreated trees were at least 1,000 feet apart.

Spray Treatments

Aerial spray treatments were applied during optimal meteorological conditions which occurred during the period June 8-10 (Table C-I) against mostly fourth (L_4) - and fifth (L_5) - instar larvae (Fig. C-3). Treatments were approximately one week late due primarily to a prolonged period of high wind. Spray ingredients were mixed sequentially as for the treatments at Algonquin Park (Section D, this report), but did not include chitinase due to supply shortages. Also, sugar beet molasses

was used in place of Cargill's Insecticide Base (Table C-II).

Applications were made by Cessna Agwagon (of similar basic design to the Agtruck used at Algonquin Park) equipped with four Micronair AU2000 emission units (Fig. C-4). The aircraft was calibrated to deliver the prescribed volumes of spray mixtures (e.g. 2 and 4 U.S. gal/ac) as clouds of uniformly small droplets approaching 100 microns in size (mass median diameter). The aircraft was guided over each 50 ft. swath by flagmen located along the northern and southern boundaries of each 100-acre block. Flying height was 10-20 ft. over treetops and about 60-100 ft. above ground level depending upon tree size. All sprays were applied immediately after mixing, as carefully as possible to each block of trees.

Ground spray treatments were applied by trailer-mounted John Bean model 51 Rotomist equipped with an optional air cone attachment (Fig. C-5). Maximum delivery of this mistblower was approximately 12,000 cu. ft./min., at a velocity (100 mph) sufficient to allow spray droplets to reach the tops of the tallest trees. Applications were made during the night of June 9 and early morning of June 10 (2200 - 0230 hrs.) using spotlights for careful delivery of sprays to peripheral trees of the selected clumps. Applications of B.t. treatments were mixed as for aerial sprays, but at higher dilution rates (Tables C-I, C-II). Fenitrothion, at approximately 6 oz. AI/acre, was applied as a standard treatment for comparative purposes.

Spray Deposit Analysis

Aluminum support stands containing Kromekote cards and glass

slides were located along two transects at right angles to swath direction in each of the aerial spray blocks (20 sampling stations/ block). Droplet collections on the cards and plates were used for visual estimates of droplet density (no./cm^2) and size characteristics, and for colorimetric analyses of spray volume deposits (oz./acre) at ground level.

Droplet deposit collections were not made in the ground treatment sprays. Instead, volume delivery estimates on a per acre basis were calculated assuming tree stocking at 300 fifty-foot trees/ acre and uniform distribution of spray coverage. Emitted volumes of spray mixtures and areas of treatment in each plot were then used as bases for calculations of actual spray volumes emitted/acre.

Larval Infection and Mortality

The sampling technique developed by DeBoo et al. (1973) and the apparatus as modified by Martineau and Benoit (1973) were employed in the study for obtaining indices of budworm densities. In both of the aerial spray blocks, samples of two 18 in. branch tips were taken from the mid-crown region of each of 20 randomly selected trees. Collections were made three times before treatment and three times after. Curves were fitted to the average numbers obtained, and Abbott's formula (1925) was used to correct for natural population decline. Extrapolations from the curves were then made to determine density differences between treated and untreated populations of the budworm at five and ten-day intervals after treatment.

A similar procedure was followed for determining larval

densities after mistblower treatments. Fewer trees (10/treatment) were sampled, however.

Representative collections of dead larvae collected from B.t. treated trees were analysed for the presence of B.t., virus and microsporidia by staff of the Integrated Control Section of C.C.R.I. These collections were made five and ten days after treatment.

Foliage Protection Assessments

A modification of the method developed by Fettes (1951) was used to determine defoliation levels on both treated and untreated check trees. With the assistance of staff of the Northern Forest Research Centre, a total of 40 branches from the mid-crown region of selected trees in each of the two aerial spray blocks and the companion untreated area were collected for detailed examination of current-year foliage condition. The average percent defoliation was then calculated in each case.

Defoliation was not systematically evaluated in the groundspray plots. An arbitrary visual assessment, satisfactory (less than 50% defoliation), unsatisfactory (50-100% defoliation) was made 12 days after treatments to estimate foliage protection levels and crown condition.

Post-Treatment Effects

Notations were made of the general condition (i.e. healthy, moribund, dead) of larvae and purpae after treatment with B.t. sprays. Also, branch samples were used for obtaining indices of both pupal and egg mass densities in both treated and untreated populations of the spruce budworms.

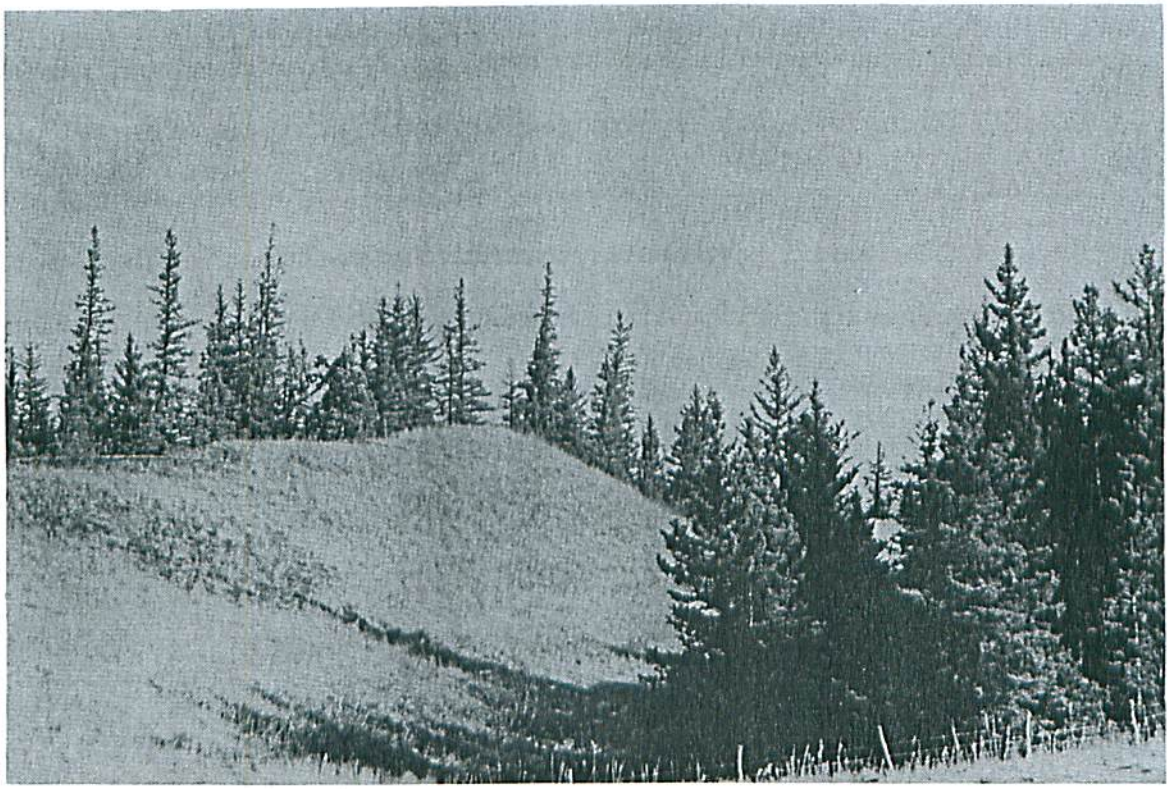

Fig. C-1 An example of distribution patterns of white spruce in the Spruce Woods area of Manitoba

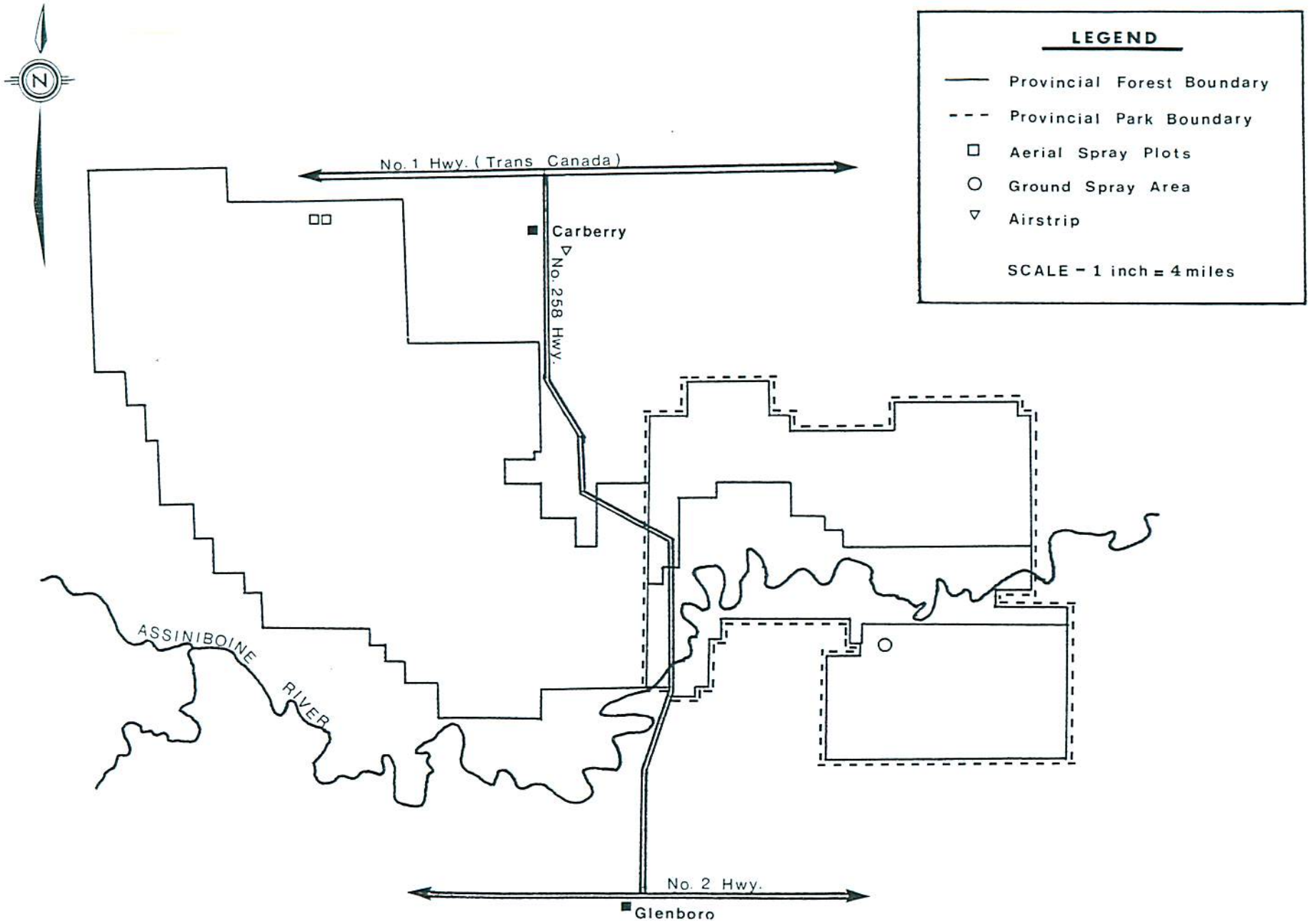

Fig. C-2 Location of B.t. treatment plots at the Spruce Woods area, Manitoba

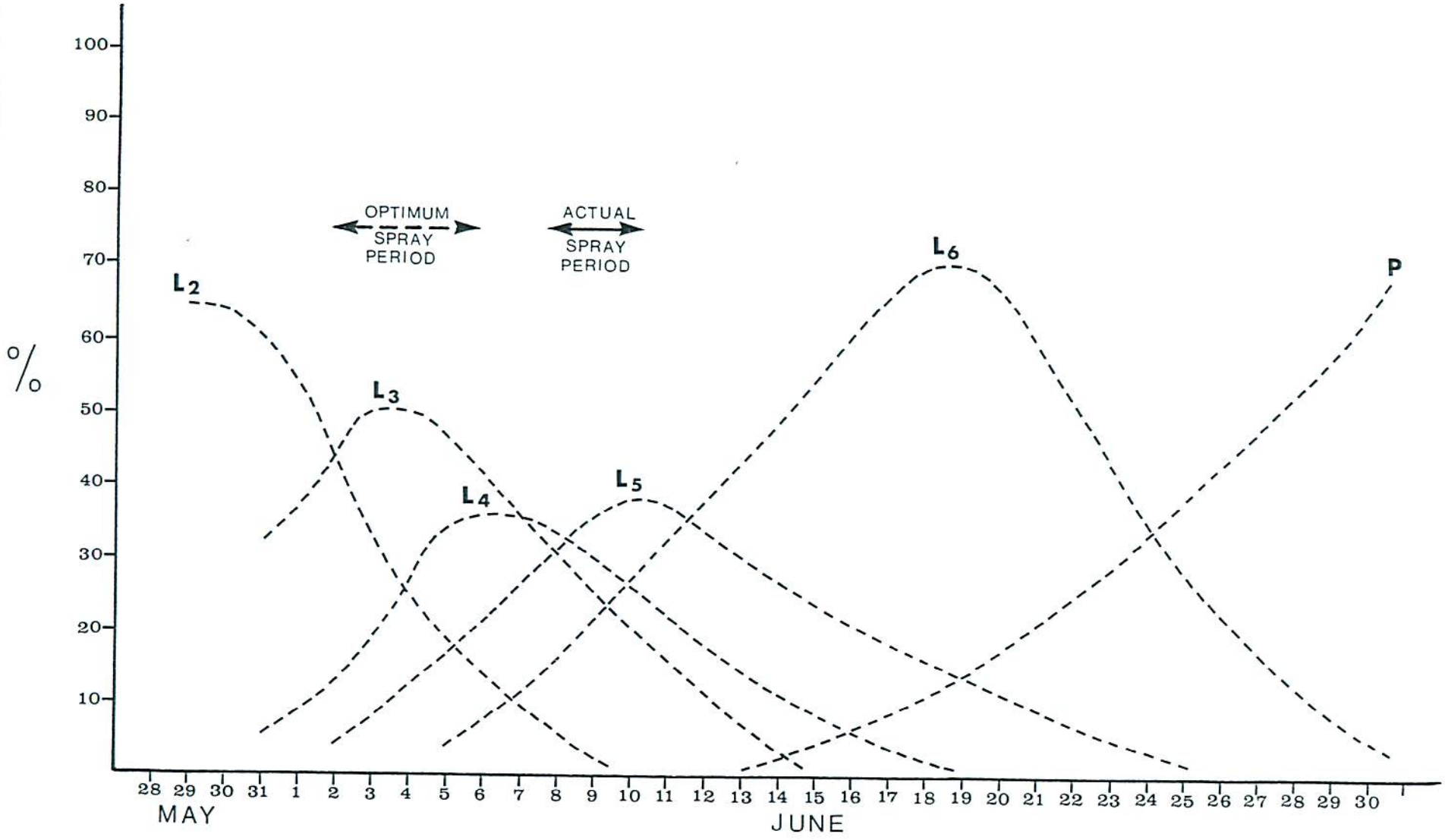

Fig. C-3 Larval (L) and pupal (P) development patterns for the spruce budworm at the Spruce Woods, Manitoba, during 1973

Fig. C-4 The Cessna Agwagon spray aircraft fitted with Micronair AU 2000 units

Fig. C-5 The model 51 John Bean Rotamist fitted with an optional 35° angle discharge head permitting spray delivery to 65° vertical pattern

TABLE NO. C-I

Aerial and Ground Spray Treatments and Meteorological Conditions
During Applications at the Spruce Woods, Manitoba, 1973

Treatment	Application Rate (AI/acre)	Vol. (gal) spray mix/acre	Area treated (Acres)	Meteorological Conditions: Spray date (June) and time	Wind (mph)	Temp (^{o}F)	R.H. (%)	Sky Condition
		A. Aerial Applications of Dipel						
Dipel	4 billion IU	2	100	10 (0610-0835)	2-6	57	72	Scat. cloud
Dipel	4 billion IU	4	100	8 (1950-2145)	2-5	68	43	Clear
				9 (0800-0835)	2-5	61	55	Clear
Untreated Check	-	-	100	-	-	-	-	-
		B. Mistblower Applications of Dipel, Thuricide and fenitrothion						
Dipel	4 billion IU	14	0.5	10 (0100-0230)	0-2			Overcast[1]
Thuricide	4 billion IU	18	0.5	9 (2310-2350)	2-4			Overcast
Fenitrothion	6 oz.	29	0.5	9 (2100-2235)	2-5			Overcast
Untreated Check	-	-	1.0	-	-			-

[1] Approximately 0.2 in. rain immediately after application

TABLE NO. C-II

Ingredients and Mixing Sequence for Aerial and Ground Spray Treatments, Spruce Woods, Manitoba, 1973

Ingredient (in order of mixing)	Quantity/ 100 U.S. gal. total mixture[1]
A. Aerial Spray Treatments	
1. Dipel treatment at 4 billion IU in 2 gal. total mix/acre	
Water	50 gal. (approx.)
BSF dye	190 gm
Dipel WP	25 lb.
Chevron sticker	16 oz.
Molasses	50 gal. (approx.)
2. Dipel treatment at 4 billion IU in 4 gal. total mix/acre	
Water	50 gal. (approx.)
BSF dye	190 gm
Dipel WP	12.5 lb.
Chevron sticker	16 oz.
Molasses	50 gal. (approx.)
B. Ground Spray Treatments	
3. Dipel treatment at 4 billion IU in approx. 20 gal. total mix/acre	
Water	50 gal. (approx.)
Dipel WP	2.5 lb.
Chevron sticker	16 oz.
Molasses	50 gal. (approx.)
4. Thuricide treatment at 4 billion IU in approx. 20 gal. total mix/acre	
Water	98.75 gal. (approx.)
Thuricide 16B	1.25 gal.
Chevron sticker	16 oz.
5. Fenitrothion treatment at 6 oz. AI in approx. 20 gal. total mix/acre	
Water	99.5 gal. (approx.)
Fenitrothion (operational spray mixture containing 77.5% of 97% technical fenitrothion, 11.6% of Atlox 3409 emulsifier, 10.9% Texaco Arotex 3470 oil)	39 oz.
Chevron sticker	16 oz.

[1] Volume of water and molasses adjusted to allow for adjustment of other ingredients

RESULTS AND DISCUSSION

Aerial Spray Deposits

Aerial spray deposit measurements indicated that droplet collections at ground level were about average under the conditions of application (Table C-III). Droplet size was small (ca. 100-130 μ) and uniform. Due primarily to low relative humidity (43-72%), spray volumes at ground level averaged less than 20% of the emitted quantities. Droplet densities (ca. 31/cm^2 for sprays at 2 gal/ac, and 67/cm^2 at 4 gal/ac), however, were considered adequate for control of budworm larvae on the heavily-branched spruce.

Larval Infection and Mortality

The aerial applications of Dipel induced high levels of B.t. infection in budworm larvae (Table C-IV). Mortality rates in treatment blocks, however, were not dramatically different from the natural population decline rate in the untreated area (Fig. C-6). Reduction levels 10 days after treatment at 2 gal/ac were 43% and 61% at 4 gal/ac. Populations of larvae in both treatment blocks, however, were significantly lower than in the untreated area 10 days after the spray applications. The mortality rates were unsatisfactory primarily due to the excessively high population densities on the trees initially (Table C-V) and the lateness of spray applications (Fig. C-3) due to inclement weather. The very high naturally-occurring microsporidia infection levels (Table C-IV) apparently did not improve the efficacy of the B.t. treatments. Although many sickly larvae and blackened cadavers could be readily detected on

branches of trees a few days after treatment, as many (or more) healthy-appearing larvae were present and feeding on the new shoots.

Results of groundspray applications by mistblower (Table C-V) were basically similar to those results of the aerial treatments. At 10 days after treatment, the highest mortality of larvae occurred on trees treated with Thuracide 16B. The Dipel treatment by mistblower was greatly influenced by rainfall after application, and most of the insecticide was probably washed off immediately. The 20% reduction level attained, then, should not be construed as a failure of the Dipel formulation by mistblower application. Also, the criteria cited as reasons for poor population reduction in the aerial spray blocks (i.e. high population densities, late spray applications) apply equally as well to the inferior results in the groundspray areas (Table C-IV, Fig. C-7).

Foliage Protection

The visual appearance of trees (with reference to foliage retention) was indistinguishable between aerial treatment blocks and the untreated check area. In all cases defoliation of new shoots approached 50% or more (Table C-VI). Top-killing of trees by the 1973 budworm infestation was equally apparent in all three blocks. Similarly, defoliation of trees treated with B.t. by mistblower was severe, and differences between sprayed and unsprayed trees could not be detected. Basically, then, none of the B.t. sprays provided satisfactory overall protection of the foliage on new shoots (Fig. C-8), but many small shoots which apparently received good spray coverage were

protected by the sprays (Fig. C-9)[1].

Post-Larval Effects

Assessments of pupal densities 10 and 15 days after spray treatment indicated that fewer pupae occurred after B.t. treatment than after chemical spray treatment (i.e. fenitrothion). Also, 50% (or more) fewer live pupae were found in the B.t. blocks than in companion untreated areas (Table C-VII). Surveys of egg mass densities indicated fewer in the B.t.-treated blocks, but differences between these and the density in the untreated area were statistically not significant (Table C-VIII). These observations, therefore, support the probability of effects of the spray on budworm populations after the larvae stage and, subsequently, also indicate that it is possible that some protection of the forest may carry over for a second year after B.t. treatment.

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[1] It should be noted that the application of fenitrothion at 6 oz. AI/ac by mistblower also gave unsatisfactory results. Aerial treatments of fenitrothion and carbaryl, when applied under good meteorological conditions in the Spruce Woods, did provide acceptable levels of foliage protection, however (V. Hildahl, C.F.S., Winnipeg, Personal Communication).

TABLE NO. C-III

Summary of Dipel Aerial Spray Deposits, Spruce Woods, Manitoba, 1973

Treatment and emitted volumes	Sampling transect	Droplet Characteristics[1]				Spray Deposit Volume[2]		
		Diam. of droplet of avg. volume (μ)	Size class range (μ)[3]	Max. diam (μ)[4]	Avg. no/ cm^2	% of volume collected	Approx. avg. vol. collected (oz/ac)	Range vol. collected (oz/ac)
Dipel at 2 gal. mix/ac (256 oz/ac)	1	125	30-250	600	34	19	48	20-80
	2	125	30-250	500	28	15	38	10-135
	($\bar{X}$)	125	-	-	31	17	43	-
Dipel at 4 gal. mix/ac (512 oz/ac)	1	130	30-200	800	75	24	123	24-294
	2	100	30-250	750	58	9	44	16-92
	($\bar{X}$)	115	-	-	67	15	79	-

Note: All information on droplet sizes and spray volumes is approximate

1 Data based on droplet stains on Kromekote cards as read visually by Microcard Reader

2 Data based on droplet collections from glass plates; fluorimetric analysis

3 For > 90% of all droplets

4 Upper size class mean of largest droplet(s) collected

TABLE NO. C-IV

Incidence of Disease Microorganisms in Spruce Budworm Larvae Collected 10 and 15 Days After Treatment With *Bacillus thuringiensis*, Spruce Woods, Manitoba, 1973

Disease Microorganisms	Incidence of Infection[1]	
	Approx. 10 Days postspray	Approx. 15 Days postspray
Bacillus thuringiensis	89.1%	93.4%
Microsporidia	81.8%	93.4%
Virus	3.6%	7.0%

[1] Based on random collections of larval cadavers (L_4 - L_6) from branch samples of trees in aerial spray block treated with Dipel (4 billion IU in 2 gal. spray mixture/acre). Numbers of larvae examined at 10 and 15 days intervals after spray were 55 and 91, respectively.

TABLE NO. C-V

Summary of Larval Population Trends of Spruce Budworm After Spray Treatments, Spruce Woods, Manitoba, 1973

Treatment	Average number of live budworm larvae/18 in. branch tip (May, June)																									5 days Post - spray[1]	10 days Post - spray[1]	Corrected percent reduction 5 days Post - spray[2]	Corrected percent reduction 10 days Post - spray[2]
	Prespray									Postspray																			
	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24				
A. Aerial Applications																													
Dipel (2 gal mix/ac)	78					88				87						20						19				4	25	9	21	43**
Dipel (4 gal mix/ac)			80			75				115						22						11				3	18	7	18	61**
Untreated Check																30						26				9	33	19	-	-
B. Mistblower Applications																													
Dipel									89[a]							18						13				4	28	12	32	20
Thuricide									89[a]							8						4				3	8	4	80**	73**
Fenitrothion									89[a]							18						8				3	20	8	51**	43**
Untreated Check									89[a]							22						9				4	39	15	-	-

[1] Extrapolated from curves, Figures 6, 7

[2] Corrected by Abbott's formula (1925); differences in population levels one day before spray and at 5 and 10 days after spray

** Significantly different from untreated check at 1% confidence level

[a] Total area selected for mistblower sprays presampled once as a single block

TABLE NO. C-VII

Densities of Pupae of the Spruce Budworm After Spray Treatments,
Spruce Woods, Manitoba, 1973

Treatment	Average number of budworm pupae/18-in. branch tip					
	Approx. 10 days after spray			Approx. 15 days after spray		
	Live	Dead	% Living[1]	Live	Dead	% Living
A. Aerial Applications						
Dipel (at 2 gal. mix/ac)	0.1	0	100	0.7	0	100
Dipel (at 4 gal. mix/ac)	0.4	0.1	80	0.8	0	100
Untreated Check	1.8	0.05	97	1.6	0	100
B. Mistblower Applications						
Dipel	0.6	0.6	50	0.4	0.05	88
Thuricide	0.1	0.2	29	0.2	0.2	44
Fenitrothion	1.0	0.2	87	0.8	0.2	83
Untreated Check	0.4	0	100	0.8	0.05	94

[1] % living of total number collected

TABLE NO. C-VIII

1973 Spruce Budworm Egg Mass Density Surveys at the Spruce Woods, Manitoba[1]

Treatment	Average no. egg masses/ 100 sq. ft. of foliage	Average length of egg masses (mm)
A. Aerial Application		
Dipel (2 gal. mix/ac)	670	4.4
Dipel (4 gal. mix/ac)	599	4.6
Untreated Check	809	4.8
B. Mistblower Application		
- not sampled due to small size of spray plots		

[1] Sampled July, 1973, approximately 6 weeks after treatment

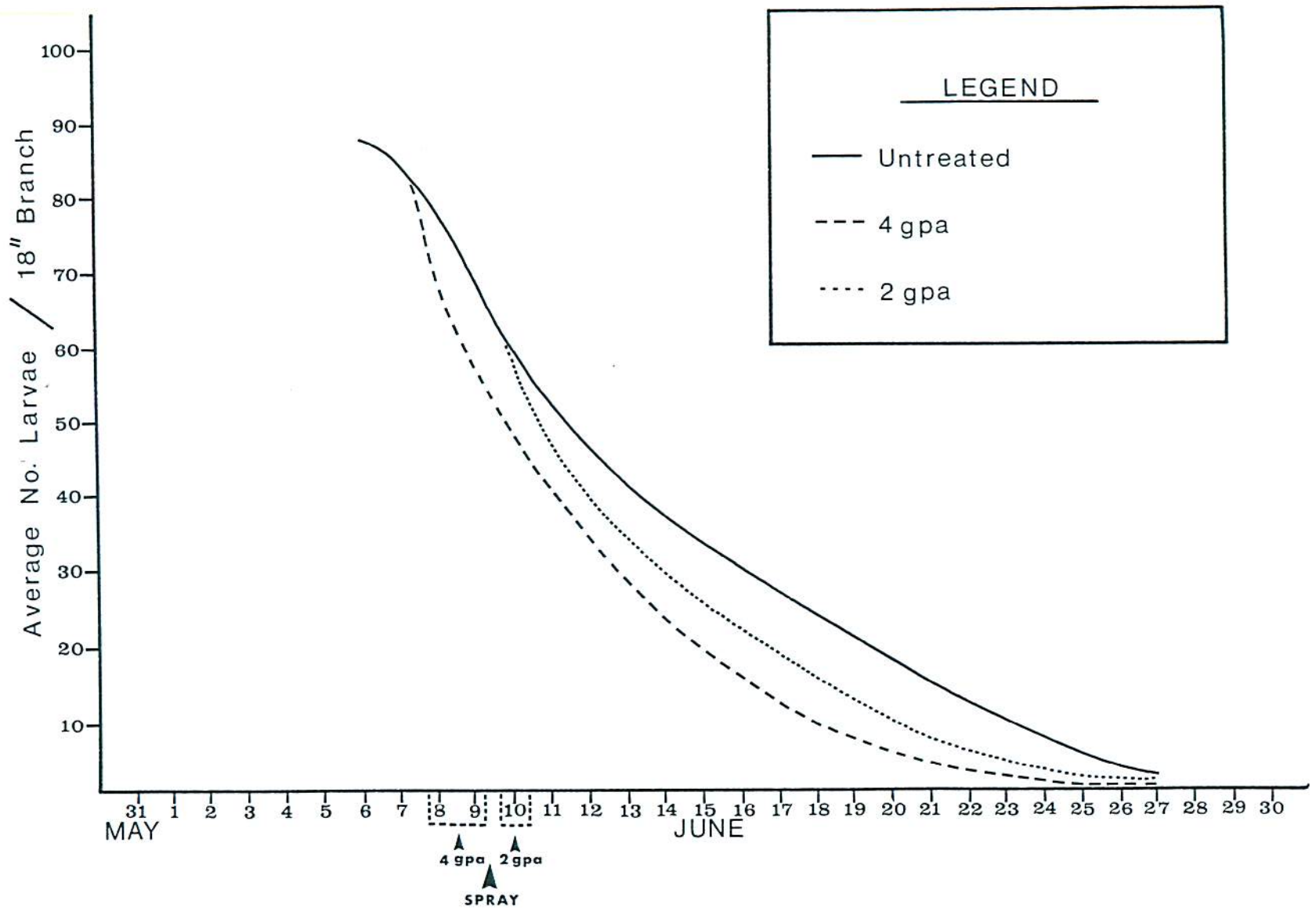

Fig. C-6. Trends in population levels of larvae of the spruce budworm on trees treated with Dipel by aircraft and on untreated check trees, Spruce Woods, Manitoba, 1973

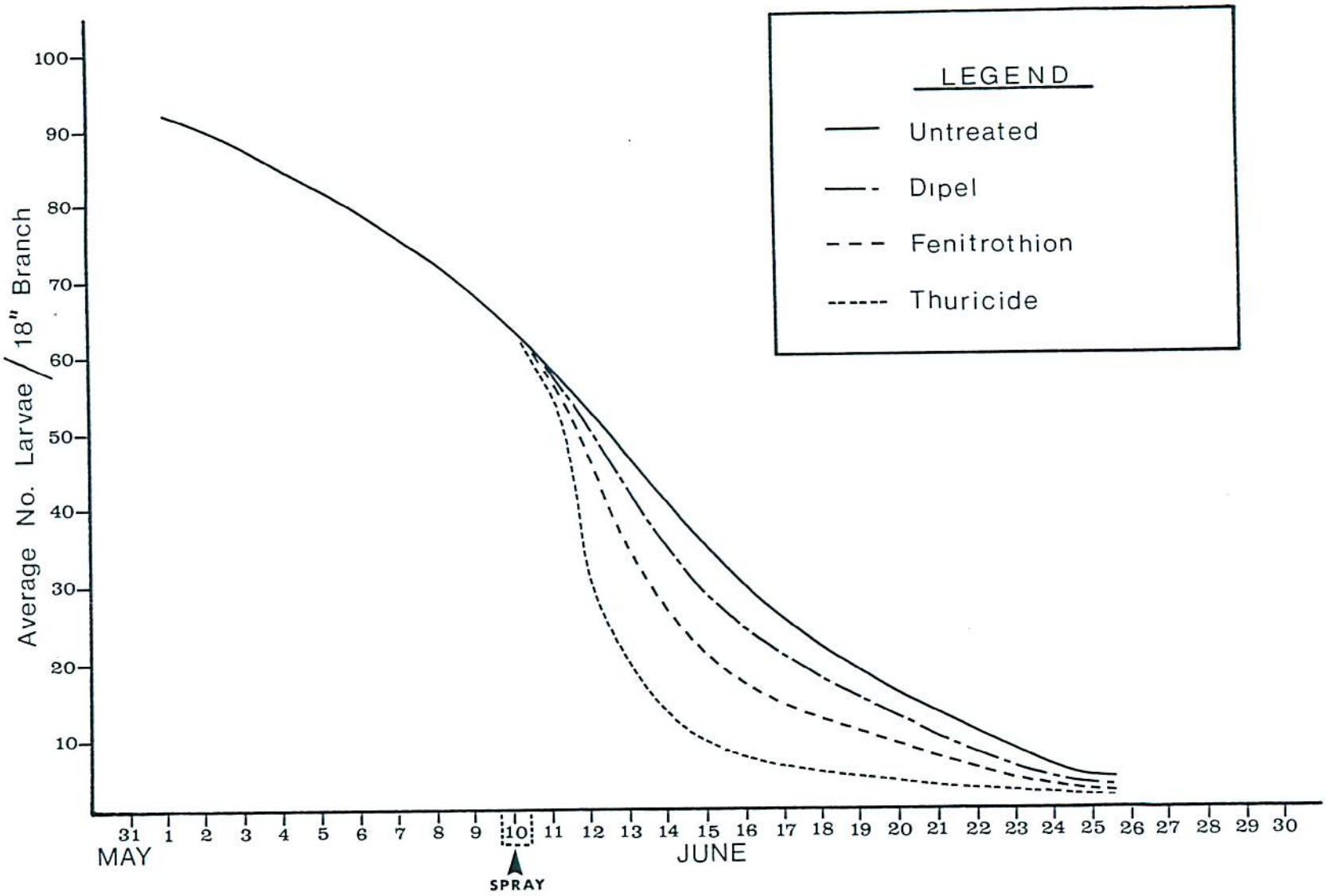

Fig. C-7. Trends in population levels of larvae of the spruce budworm on trees treated with Dipel, fenitrothion or Thuricide by mist-blower and on untreated check trees, Spruce Woods, Manitoba, 1973

Fig. C-8 Severe defoliation of branches of a tree treated with Dipel at 2 gal/ac

Fig. C-9 Protection of foliage of a new shoot from tree in Dipel plot treated at 2 gal/ac

SUMMARY AND CONCLUSIONS

The results of applications of commercial preparations of B.t. by aircraft and by mistblower in the Spruce Woods area of Manitoba clearly indicated that this biological insecticide can induce a significant mortality of spruce budworm larvae on white spruce hosts. The degree of foliage protection, at the concentration used (4 billion IU/acre) at least, did not provide acceptable protection of foliage, however. Major obstacles hindering direct comparison of these results with those obtained at Algonquin Park (Section E of this report) were (1) forest composition, (2) extremely high population densities and advanced morphological development of budworm larvae, (3) a period of extreme wind and low relative humidity during the optimal spray period, and therefore, (4) the spray treatment of a highly unusual spruce budworm infestation.

It may be concluded, however, that the potential for the use of Bacillus thuringiensis for control of spruce budworm is good providing some or all of the following stipulations can be met:

(1) Larvae population densities should not exceed 20-30 (L_3)/18-in. branch tip as infection of all larvae is not possible by either aerial or ground spraying techniques. Larval densities in excess of this range are too great for control levels in the vicinity of 80% population reduction to be meaningful in terms of obtaining satisfactory foliage protection.

(2) Timing of sprays is critical, especially in Manitoba where larval development appears to be faster than in eastern forests. Applications of B.t. against L_4 to L_6 larvae feeding voraceously

and in large numbers appears to be a useless exercise. In Manitoba, or at other locations where B.t. must be applied under such conditions, two heavy applications (ca. 4 billion IU in 2-4 gal/ac) during the period L_2 to L_4 should be considered.

(3) A heavy coverage (50 + droplets/cm^2) of small droplets (ca. 100 μ or smaller) may be required for adequate tree crown penetration and distribution to the maximum number of budworm feeding sites. It is apparent, at least from the Manitoba experience, that large volumes (e.g. 2-10X those for chemical sprays) may be required to obtain desirable levels of foliage protection. Accordingly, areas for treatment may be restricted due to financial and physical limitations of a spray program.

(4) The surprisingly high incidence of a naturally-occurring microsporidial disease organism did not have additional significant impact on budworm larvae sprayed with B.t. (based at least on the observations made) and considering the severe defoliation in both treated and untreated areas. An adjuvant such as chitinase (not available for the Manitoba experiments) or any other ingredient or adjuvant to improve spray deposit and efficacy should be considered as the added stress of naturally occurring agents of control may not be sufficiently effective in reducing budworm damage in the year of treatment.

It is strongly recommended, therefore, that research of B.t. spray efficacy continue. Experiments using new formulations, spray

dosages, and adjuvants should receive prime consideration. The importance of immediate and satisfactory control levels of spruce budworm using new and highly potent B.t. sprays cannot be ignored - it now appears to be within the realm of possibility.

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Evaluation of Commercial Preparations of *Bacillus thuringiensis* with and without Chitinase Against Spruce Budworm

D. Logistics of Aerial Application, Algonquin Park, Ontario

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Chemical Control Research Institute
Canadian Forestry Service
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D. Logistics of Aerial Application, Algonquin Park, Ontario

by

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INTRODUCTION

Formulations of commercial preparations of Bacillus thuringiensis Berliner (B.t.) have been applied by air in many studies to perfect the use of biological organisms for the control of the spruce budworm (Choristoneura fumiferana Clem.) in forested areas in Canada. These studies have been carried out in Ontario, Quebec and New Brunswick (Angus et al., 1970, Smirnoff et al., 1972, Morris and Hildebrand, Section E of this report). In 1973 the Chemical Control Research Institute was requested to carry out a series of spray applications to compare the efficacy of two commercially available formulations of B.t., Dipel® and Thuricide 16B®. Smirnoff et al. (1973) have reported that the biological activity of B.t. is enhanced by the addition of the enzyme chitinase to the spray mix. For a description of the mode of action and references to the work on chitinase again see Morris and Hildebrand, Section E. It was therefore decided that in the proposed trials not only would the effectiveness of the two materials be measured, but the effect on each of the addition of chitinase would be determined. A separate trial, to measure the effectiveness of chitinase alone was also planned. Thus a total of five applications were planned: Dipel, Dipel + chitinase, Thuricide, Thuricide + chitinase, and chitinase alone.

In order to provide good data for comparison it was necessary that the applications be identical in all aspects and where there were uncontrollable factors these should be measured. In selection of the plots care was taken to ensure that they were as uniform as possible with respect to tree type, tree condition and budworm population density. The plot selection is described in Section E (Morris and Hildebrand). Spray aircraft, with the most efficient spray dispersal equipment available were to be selected, the mixing and loading facilities were to be such that batches of material could be prepared with accuracy, and the system could be washed between mixes to prevent any cross contamination. The uncontrollable factor in a spray application is the weather. Armstrong (1973) has described the effect of meteorological conditions on spray drift and deposit. Once the spray is released from the aircraft the spray cloud moves according to wind speed and direction, the rate of fall is affected by temperature conditions and during the time of droplet settling the spray drops are affected by the relative humidity. Temperature and relative humidity are very important when an aqueous spray is used.

A check on the information describing the previous aerial applications of B.t. reveals that, in many cases, only partial information was collected to describe the meteorological conditions at the time of spray application. Thus, there is the possibility that interpretation of the results did not take in to account the affect of meteorological conditions on the spray cloud and the test may have been a test of the effect of these parameters on droplet deposition and not on the efficacy of the formulation on the budworm. With this knowledge, it was decided that for the 1973 trials it was absolutely

essential that every care be taken to measure the weather conditions at the time of spray application.

In this publication is described the calibration of the spray aircraft, the equipment used to measure the weather conditions at the time of spray application, the control of the operation in terms of communications and the equipment used to mix the insecticide formulations. Comments are also given on the effect of weather on spray deposition and the efficacy of the spray.

CALIBRATION OF SPRAY AIRCRAFT

Prior to the time of spray application sufficient Dipel and Thuricide were obtained to permit single lots of the completed formulations to be made. A rate of application of 0.5 gal/ac (US) was accepted as the treatment. It was agreed that the material should be applied using Micronair AU 3000 units with a fan blade setting to produce a spray cloud with the droplets in the 50-100 micron size range. Arrangements were made to hire commercial spray aircraft for the application (see Acknowledgements section) and before the date of application the aircraft, a Cessna Agtruck and a Piper Pawnee, were checked to ensure that they would deliver the required 0.5 gal/ac using the fully formulated material, i.e. the required amount of active ingredient plus any additives, stickers, dye and solvent. To facilitate a quick check on drop size characteristics of the spray cloud a series of sample cards were prepared in the laboratory sprayed with the formulation at the equivalent of 0.5 gal/ac and with the correct drop size range. With the delivery rate determined adjustments were then made to

the Micronair fan blades to alter the speed of rotation in order to ascertain the appropriate setting required to produce the correct drop size range. A line of Kromekote[1] cards was laid out across the grass runway at the Lake of Two Rivers airstrip such that the aircraft could fly an upwind flight at right angles to the line of cards. A strip of 4" wide calculator paper tape was laid out parallel to the line of cards and just beside it. The cards and tape extended for a distance of about 400 feet. The aircraft then made an upwind pass emitting the dyed spray at the required rate; the spray was started about 400 yards before the line of cards and emission was continued for about 400 yards after flying over the cards. Flights were made about 50 feet above ground level. These runs were repeated using fresh cards and tape for each run with different Micronair blade settings until the desired drop size was achieved. This final setting was used for all spray applications. From available information in the Micronair AU 3000 Handbook[2], a swath width of 200 feet at flying height of 50-100 feet above the tree tops was accepted and used for determination of swath spacing and flight pattern of the spray blocks.

LOCATION OF METEOROLOGICAL EQUIPMENT

The precise location and description of the spray blocks is given by Morris and Hildebrand (Section E). Since it was essential that the sprays be carried out under known optimum meteorological

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[1] Kromekote cards available from Kruger Pulp and Paper, Montreal, Canada.

[2] Micronair AU 3000 Handbook, Micronair (Aerial) Ltd. Bembridge Fort, Isle of Wight, U.K.

conditions it was necessary to have a set of sensors in the spray blocks at the time of spray application. Ideally the weather conditions should be measured in each spray block at the time of its own spray treatment. However, the plan of the experiment dictated that the treatments be applied as quickly as possible to ensure a uniformity in development of the target insect and the host trees. The spray blocks were set out with a minimum one mile separation to prevent cross-contamination. It was accepted that, considering the topography of the area provided (Annie Bay, Lake Opeongo, Algonquin Park), and the necessity to complete all sprays in as short a time span as possible, it would be impossible to move the meteorological tower and equipment to each individual block for its own particular application. It was therefore decided to place the tower in an area most suited to provide data for all tests.

The plan of operation was to treat the large block of 2,500 acres first and then treat the four 100 acre blocks. With the restricted load capacity of the aircraft working off a grass strip (load limit about 125 gallons) and the volume of material to be sprayed, it was estimated that, if the weather was normal, two spray sessions would be required to complete the spray on the large block. The small blocks would be completed in the same, or less, time. The meteorological tower, equipment and trailer were positioned adjacent to the 2,500 acre block and left there for the complete set of applications. Figure D-1 indicates the position of the tower relative to all spray blocks and the distance of the tower from the airstrip. The site of the tower was also checked to ensure that the tree type and topography of the area were generally the same as for the other blocks. The tower and trailer were put in position and checked to ensure that all systems were functioning about one week before the estimated spray date.

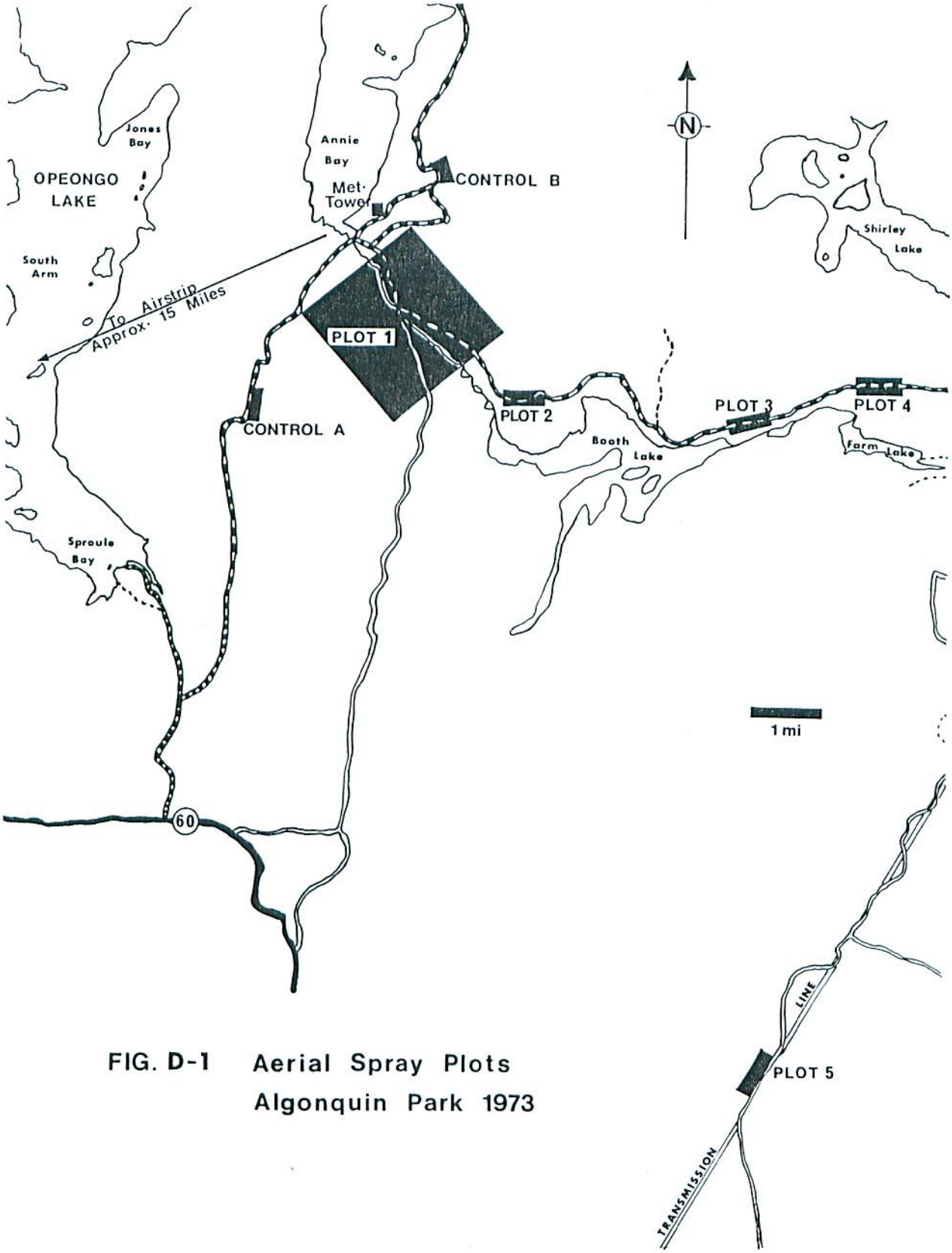

FIG. D-1 Aerial Spray Plots Algonquin Park 1973

METEOROLOGICAL MEASUREMENTS

Sensors to measure wind speed, wind direction, turbulence, wet and dry bulb temperatures, and temperature differentials were mounted on a portable tower (Armstrong 1971). Measurements were recorded at heights of 18 feet and 80 feet above ground level. At ground level the barometric pressure was recorded on a recording microbarograph. Temperature differentials were determined between 6 and 18 feet and between 18 and 80 feet. The information from the wind and temperature differential sensors was recorded on Esterline-Angus[1] strip chart recorders; the wet and dry bulb readings were taken manually at 15 minute intervals using remote sensing equipment. The tree height in the region of the tower was about 30 feet, thus measurements at 18 feet were considered to represent conditions at about mid-crown position and the measurements at 80 feet were considered to represent those at the point of spray emission. The spray aircraft flew at a nominal altitude of 100 feet. Preliminary trials and studies with the meteorological sensors (see Armstrong 1973) have indicated that the weather conditions most important in affecting the drift of the spray cloud are those in the air mass above the trees. Thus, for control of the spray the meteorological conditions indicated by the sensors at the top of the tower and the temperature differential between 18 and 80 feet were used.

With the remoteness of the tower site, the person in charge

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[1] Esterline Angus. Division of Esterline Corp., Box 2,400, Indianapolis, Indiana, 46224, U.S.A.

of the meteorological equipment found it more convenient to live in the trailer at the tower site. When the biological assessment indicated that the budworm were in the right stage for treatment the sensors and recorders were activated. On the particular morning or evening of the spray the system was started about 2 hours before the earliest expected time of spray. Thus, on a spray morning records started at about 0400 hours (before sunrise, earliest expected spray time about 0600 hours) and in the evening the records were started about 1700 hours. Continuous records were taken until one hour after completion of spray application.

Unfortunately no good pictures of the meteorological tower and sensors were available from this particular study. Figure D-2 is a photograph from a different experimental site but showing the tower, prior to extension, with the full set of sensing equipment in position.

COMMUNICATIONS

With the remoteness of the tower and the experimental blocks from the airstrip, it was essential that there be a good radio link between the experimental blocks and the airstrip. A base radio (Motorola) with antenna mounted at the top of the tower was set up at the tower site. A second base radio was set up at the airstrip with its antenna mounted on a 60 foot tower. The crews working in the test plots carried portable radios and were in constant contact with the tower base radio which could relay messages to the airstrip. Thus, for all spray applications, it was possible to relay information about position of aircraft, readiness to spray etc. Good radio contact was maintained

at all times. Unfortunately there was no radio link between the ground and the spray aircraft.

To permit transmission of last minute instructions to the pilot, a visual ground system using cloth marker strips was employed. The strips were bright orange cloth cut to approximately 1.5 feet by 10 feet. The signals employed were: a large square, indicating "hold", i.e. conditions are not good now, or there is a delay in plot preparation but shortly the spray can be applied; a cross, indicating "cancel the spray", return to the airstrip; an arrow pointing in the direction of the wind, indicating "OK" that the spray can be started and also giving final indication of wind. The signals were placed in a pre-determined clearing of sufficient size for easy visibility by the pilots.

Upon receipt of information from the meteorological tower that conditions were good for a spray, the pilot was ordered to proceed to the particular block. The ground crews in the block, and the meteorological tower operator were informed of the time of departure of the spray aircraft. Prior to take-off, the pilot was given last minute instructions as to wind speed and direction and also told on which side of the block he should commence spraying (spraying always started on the down-wind side with the pilot working up-wind flying at right-angles to the wind direction). At the spray block he first checked the ground signals and then proceeded according to directions. Upon completion of the spray, the ground crew informed the meteorological tower operator that the spray was completed and whether or not a satisfactory application had been made. If swaths had been skipped the

pilot was informed as soon at he returned to the airstrip and, if necessary, adjustments to flight plan were made for the next load.

All personnel resided in Whitney for the duration of the spray. A normal telephone existed between the airstrip (a telephone booth near to the strip) and the place of residence of the crews; thus on a potential spray date one person went to the airstrip and made radio contact with the meteorological tower. On confirmation of possible good weather approaching a telephone call to Whitney alerted all ground personnel who could then proceed to their posts where they were in communication via the radio link.

AIRSTRIP, MIXING AND LOADING FACILITIES

The airstrip used was located immediately to the west of Lake of Two Rivers, about 15 air miles from the 2,500 acre plot. The operations control trailer which served as a field office and laboratory, the stock of insecticidal material, mixing and loading facilities and all supplies for the spray aircraft were located at the airstrip.

All sprays were formulated in water. To ensure that there was no contamination between the different spray mixes sufficient water had to be available to prepare the formulations and to wash out the mixing and loading systems between each spray batch. A 500 gallon tank plus two smaller tanks, to give a total water reserve of about 600 gallons were positioned close to the mixing rig. The mixing rig, a trailer mounted unit consisting of a 300 gallon main mixing tank and a secondary 125 gallon tank with its own power source, pump and meter was positioned about 15 feet from the reservoir tank. All insecticides

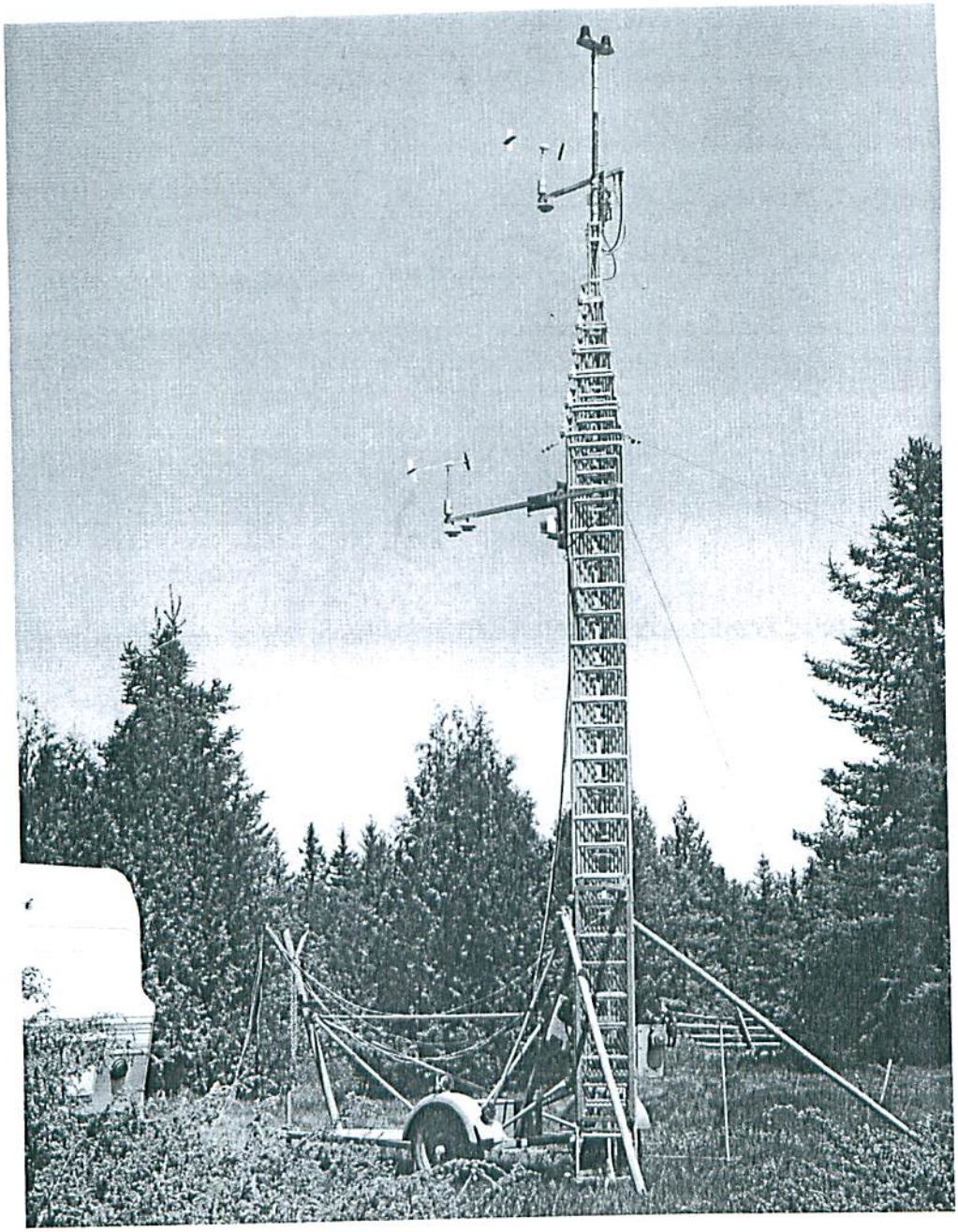

Fig. D-2 The meteorological tower prior to extension with the full set of sensors mounted

and reserve materials (such as additives) were stock-piled in an enclosure. Extra water was brought from a nearby stream using the mixing rig as a tank trailer.

TIMING AND SEQUENCE OF SPRAY APPLICATIONS

The sprays were to be applied with the budworm at peak of the third instar (Morris and Hildebrand, Section E). The sprays were also to be applied over the shortest possible time span so that all tests would be with the insect in the same stage of development and with the trees at the same stage of foliage development. The conditions at the time of spray application were to be: (1) wind speed less than 8 miles per hour, (2) inversion temperature conditions, and (3) minimal conditions of turbulence. Since water sprays were being used relative humidity was an important factor but was not used as a limiting parameter to determine the suitability of spray conditions.

A good comparison of the effectiveness of a spray application can only be made when it is possible to determine the conditions of the weather in such a way as to be able to apply a comparison value. Yates, Akesson and Cheng (1967) have evaluated the use of the stability ratio for comparing the meteorological factors affecting a spray cloud. The stability ratio (S.R.) is derived from a formula using the wind speed and a temperature differential between two heights and yields a number which may be positive (indicating stable weather and good spray conditions) or negative (indicating unstable weather and bad spray conditions). The numerical value obtained indicates the degree of

stability or instability. The formula for calculation of the stability ratio is given in Appendix I.

The sprays to be applied were Thuricide and Dipel alone, each of these materials with chitinase added and a separate chitinase spray. Since care was to be taken to prevent cross contamination, and to have the most efficient sequence of mixing, the sprays were to be applied in the following sequence: (1) Thuricide alone, (2) Thuricide + chitinase, (3) Dipel alone, (4) Dipel + chitinase, (5) chitinase alone. With this order of sequence it was not necessary to wash the system between the applications of Thuricide alone and Thuricide + chitinase or between Dipel alone and Dipel + chitinase. The mixing - tank cleaning regimen was: (1) prepare and apply Thuricide alone, (2) prepare and apply Thuricide + chitinase, (3) wash all tanks, hoses and aircraft, (4) prepare and apply Dipel alone, (5) prepare and apply Dipel + chitinase, (6) wash all tanks, hoses and aircraft, (7) prepare and apply chitinase alone.

The allocation of treatments to particular plots is described by Morris and Hildebrand (Section E). One of the aims of the study was to measure the effects of B.t., B.t. + chitinase mixtures, and chitinase alone on domestic honeybees and other non-target organisms. The 2,500 acre block was needed to meet the requirements of the environmental impact study group. Buckner et al. (Section F) describe the studies done in this large block and the other small blocks.

SPRAY FORMULATION

The formulations of the different sprays are given in Appendix II. The Thuricide arrived in the form of a liquid suspension

and all that was required was to mix it with an equal volume of water and add the required amount of dye. For the Thuricide + chitinase the amount of chitinase needed was first dissolved in water to ensure complete mixing; to this was then added the required amount of Thuricide and dye. The Dipel however, arrived in the form of a wettable powder. In the preparation of this formulation it was necessary to add molasses, Chevron sticker, dye, and water. The mixing protocol was to first measure the required amount of water into the spray tank. Five to ten gallons of this water was then transferred to a 25 gallon container and a slurry with the necessary amount of Dipel powder was prepared. When the slurry was ready the molasses and sticker were added to the water and finally the slurry was mixed in. In preparation of the Dipel + chitinase the same routine was followed except that after the slurry was made, and before addition of the molasses and sticker, the chitinase was mixed in the water. In the preparation of the chitinase alone the chitinase was mixed in the water and then to it was added the required amount of molasses and Chevron sticker. All spray preparations were applied to the experimental plots immediately after mixing.

SPRAY APPLICATION

Thuricide Alone (Plot 1)

This spray was applied in two lifts; the first during the evening of 29 May and the second the morning of 30 May. The spray was applied under very good weather conditions (see Table D-I) with a S.R. = +39.2, moderate to light turbulence (= 0.114) and a wind of 3.0 miles

per hour. The spray on the morning of the 30 May was, as expected for a morning spray applied under better conditions with a S.R. = + 76.1 and a wind speed of 1.2 miles per hour. The weather was very calm, turbulence factor was 0.07 and cool with a mean temperature for the period of spray application of 9.5°C at 80 feet. During the course of the night before the spray on the 30 May, the temperature fell sufficiently to result in frost formation (ca 0°C). At 0745 hours the temperature at 18 feet was 5°C.

Thuricide + Chitinase (Plot 4)

This spray was applied the evening of 30 May. Applications were made under conditions of a very strong inversion, light wind (S.R. = + 174.2, wind speed of 1.6 miles per hour) and little turbulence (factor = 0.102). This spray was applied under the most adverse conditions of relative humidity with values of 38% and 34% at 18 and 80 feet respectively. Again, this data is shown in Table D-I.

Dipel Alone (Plot 2)

The development of the budworm was progressing to the extent that it was necessary that all sprays be completed as quickly as possible. The Dipel alone was applied the morning of 31 May under unstable conditions (S.R. = -7.5) and a turbulence factor of 0.218. On this particular morning there was a heavy ground fog which did not clear off until the sun was high enough to warm the air thus resulting in lapse conditions. The wind speed at the time of application was 2.7 miles per hour and the relative humidity at 80 feet was 69% (Table D-I).

Dipel + Chitinase (Plot 3)

This plot was also treated the morning of 31 May. The meteorological data are shown in Table D-I. At the time of application the wind had dropped slightly (wind speed 2.2 miles per hour) to give slightly better conditions (S.R. = -7.0, and turbulence of 0.187), but still unstable and not ideal for spray application. The relative humidity was the same as for the Dipel alone plot.

Chitinase (Plot 5)

This plot was treated the evening of 31 May under conditions of slight inversion (S.R. = + 1.3). The wind speed was 3.7 miles per hour and there was a moderate degree of turbulence (= 0.207). The relative humidity at the time of application was 50%.

SUMMARY AND CONCLUSIONS

In general, all aspects of this portion of the B.t. project proceeded without problems. The major difficulty occurred in the formulation of the spray materials which was attributable to the type of spray concentrate used and the mixing equipment at hand. There were also slight problems associated with lack of communication with the spray aircraft.

Formulation of Sprays

(i) Thuricide Sprays

The Thuricide concentrate arrived in 55 gallon (US) drums. The original plan was to pump the concentrate into the water in the spray

TABLE NO. D-I

Meteorological Conditions at the Time of *Bacillus thuringiensis* Applications in Algonquin Park

Plot No.	Treatment	Date of Spray	S.R.[1]	Turbulence Factor[2]	Wind Speed (mph)	Wind Dir.	Dry bulb Temp. °C. 18'	Dry bulb Temp. °C. 80'	R.H.% 18'	R.H.% 80'
1.	Thuricide alone	29/V-pm	+ 39.2	0.114	3.0	WNW	11.3	12.8	64	57
1.	Thuricide alone	30/V-am	+ 76.1	0.07	1.2	SSE	8.6	9.5	55	73
2.	Dipel alone	31/V-am	- 7.5	0.218	2.7	WSW	12.5	12.5	63	69
3.	Dipel + chitinase	31/V-am	- 7.0	0.187	2.2	WSW	12.5	12.5	63	69
4.	Thuricide + chitinase	30/V-pm	+174.2	0.102	1.6	SSW	16.1	18.1	38	34
5.	Chitinase alone	31/V-pm	+ 1.3	0.207	3.7	WNW	14.8	15.0	50	50

[1] S.R. = Stability ratio, see Appendix I for derivation of the value

[2] Turbulence factor, a number derived from the frequency and amplitude of the vertical movement of a Bi-vane. See Appendix I.

mixing tank. The Thuricide was a thick, vicous slurry which could not be pumped by the available equipment. The only way the material could be handled was to transfer the concentrate by hand, in 4 gallon lots, to the mixing tank. The required volume of water was first pumped into the mixing tank and a recirculation pump was started. The Thuricide was then added. It mixed readily with the water. When all the Thuricide was in, and to ensure a good mix, a 2" portable centrifugal pump was used as a recirculation pump for about 15-20 minutes. This pump was also used for loading the spray aircraft permitting an aircraft to be loaded in 1-2 minutes.

(ii) Dipel Sprays

The major problem with the Dipel was in the preparation of the slurry. This was a time consuming job and unless one had a powerful paddle mixing system (which was not avilable at the airstrip) it had to be done by hand. The formulation used required that the slurry be mixed with water to which was then added molasses (see Appendix II for formulation ingredients). To ensure complete mixing the slurry-water mix was recirculated using a portable 2" pump; the required amount of molasses was then added slowly. As the density of the mix increased with the increasing proportion of molasses in the mix the efficiency of the mixing system decreased. This problem was resolved by increasing the time of mixing.

Spray Applications

The number of applications, and the necessity to complete all

within a very limited time span dictated the necessity of applying two sprays (Dipel alone and Dipel + chitinase) under less than ideal conditions. This could only have been prevented, under the particular conditions that existed in 1973, by having an extra spray aircraft, more ground equipment and larger crews to permit two blocks to be sprayed at the same time. These costs were beyond the available budget.

Communications With the Spray Aircraft

As stated, there was no radio communication with the spray aircraft and once departed from the airstrip the only communication was the set of visual ground signals. The plot boundaries were delineated by marker balloons to assist the pilots (Morris and Hildebrand, Section E); also, prior to the experiment the pilots were sent on practice runs to familiarize themselves with the route to and from the spray blocks and with the general terrain and layout of the blocks. On two occasions the ground crews in the blocks reported that the pilots had missed runs; with the communication net between the blocks and the airstrip it was possible to notify the pilot after the particular load and corrections were made at the time of the next load.

Meteorological Conditions and Their Effect on Spray Deposit

Data are given in Table D-II showing meteorological conditions at the time of spray application and the percentage of spray deposited. Morris and Hildebrand (Section E, Table E-V) also indicate the deposits in terms of International Units of potency of B.t. and the quantity of viable spores deposited.

TABLE NO. D-II

A Correlation of Meteorological Conditions at the Time of Spray Application With the Percentage of Spray Deposited

Plot	Stability ratio	Turbulence factor	R.H. (80')	Spray Emitted (oz)	Spray Deposited (oz)	% Deposit
Thuricide alone[1]	+ 39.2	0.114	57%	64	21.8	34.1
	+ 76.1	0.07	73%			
Thuricide + chitinase	+174.2	0.102	34%	64	52.1	81.4
Dipel alone	- 7.5	0.218	69%	64	21.1	33.0
Dipel + chitinase	- 7.0	0.187	69%	64	17.1	26.7
Chitinase alone	+ 1.3	0.207	50%	64	11.5	18.0

[1] Two application sessions - evening and morning sprays (See Table D-I)

The block which received the best deposit was Thuricide + chitinase (81.4% deposit) which was sprayed under conditions which gave a S.R. = + 174.2. This strong lapse condition offset the effect of the low R.H. (= 34%). The next best deposit was in the Thuricide alone block (34.1% deposit) which was sprayed under conditions of S.R. = + 39.3 and + 76.1 for the two spray days, and an R.H. which was between 50 and 73%. The Dipel alone and Dipel + chitinase, although sprayed under lapse conditions (S.R. = -7.5 and 7.0) benefitted from the relative humidity which was high enough (69%) to assist in preventing evaporation of the droplets and thus helped the deposit rate. These blocks received 33.0 and 26.7% of the spray emitted, respectively. The chitinase alone block was sprayed under conditions barely suitable for spraying (S.R. = + 1.3) and this combined with the low relative humidity (50%) resulted in an unsatisfactory deposit.

The two Thuricide sprays and the chitinase alone spray show a correlation between stability ratio and percentage of spray deposited. The inconsistancy in the case of the Dipel sprays can possibly be explained by the characteristics of the spray formulation. Dr. O.N. Morris has reported (personal communication) that microscopic examination of the Dipel formulation showed a large amount of inert material (inert ingredient from the Dipel powder), whereas the Thuricide formulation did not have inert material. An analysis of the physical properties of the spray formulations by W. Hopewell (C.C.R.I.) showed that the Thuricide spray formulation had a density of 1.096 gm/ml, the chitinase alone spray formulation had a density of 1.099 gm/ml, and the Dipel spray formulation had a density of 1.159 gm/ml. It therefore appears that the presence of inert ingredients and the greater density of the Dipel formulation provided characteristics which permitted a good deposit even when applied under lapse conditions.

GENERAL COMMENTS AND RECOMMENDATIONS FOR THE APPLICATION OF B.T. SPRAYS

The analysis of factors affecting spray deposit and of the efficacy of the deposit provided information which indicated that certain recommendations for the application of B.t. sprays can be made. The drop size (approximately 100 micron) and droplet density (> 50/cm^2) recommended by the manufacturer were shown to be necessary to achieve satisfactory control. Being water based, the sprays were susceptible to evaporation. The addition of molasses was an advantage in that it acted as an anti-evaporant and permitted a good deposit even when the spray was applied at low relative humidities. The presence of inert material in the Dipel spray formulation was also an apparent advantage in that the spray droplets were heavy enough that a good deposit was achieved even when the spray was applied under lapse conditions.

It is therefore recommended that to ensure a good deposit of a B.t. formulation and attain good effect of the spray the spray be applied using an emission system that will produce a spray such that the majority of droplets are less than 100 microns in size; and that the deposit have at least 50 drops per square centimetre. The addition of an anti-evaporant/anti-drift adjuvant is considered essential to ensure good deposit under less than ideal conditions. The spray must be applied under stable weather conditions.

The physical characteristics of B.t. are such that adequate mixing can only be achieved with a mechanical agitation system. A large capacity (approximately 50 gallons per minute) positive displacement pump is required to pump material of these physical characteristics. This is particularly important under cool weather conditions.

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APPENDIX I

Stability Ratio

The stability ratio is determined from the expression:

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

where T_1 is the temperature in degrees centigrade at the low position

T_2 is the temperature in degrees centigrade at the high position

$\bar{u}$ is the mean wind speed in cm/sec at the position of the high temperature sensor.

Turbulence Factor

The wind sensors used by C.C.R.I. are a Bi-Vane unit. The vertical movement of the vane indicates the amount of turbulence in the air mass. The turbulence factor is determined from a calculation of the frequency and amplitude of the vertical movement of the Bi-Vane multiplied by a factor to give a number less than unity. The number obtained is a relative value only.

APPENDIX II

Bacillus thuringiensis Formulations, Algonquin Park, 1973

(Information Abstracted From Morris and Hildebrand, Section E)

Treatment	Area (acres)	Thuricide (U.S. gallons)	Dipel (lbs)	Chitinase (gm)	Molasses (U.S. gallons)	Chevron sticker (ml)	BSF dye (gm)	Water (U.S. gallons	pH
Dipel	100	-	50	-	25	400	190	25	7.5
Dipel + chitinase	100	-	50	1.8	25	400	190	25	7.5
Thuricide	2,500	625	-	-	-	-	4,750	625	6.5
Thuricide + chitinase	100	25	-	1.8	-	-	190	25	6.5
Chitinase	100	-	-	1.8	25	400	190	25	7.5

Evaluation of Commercial Preparations of *Bacillus thuringiensis* with and without Chitinase Against Spruce Budworm

E. Assessment of Effectiveness of Aerial Application, Algonquin Park, Ontario

by O.N. Morris and M.J. Hildebrand

Chemical Control Research Institute
Canadian Forestry Service
Ottawa, Ontario

Information Report CC-X-59

February, 1974

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E. Assessment of Effectiveness of Aerial Application, Algonquin Park, Ontario

by

O.N. Morris and M.J. Hildebrand

INTRODUCTION

Commercial Bacillus thuringiensis Berliner (B.t.) preparations formulated in North America contain spores and toxic crystals (endotoxin) as active ingredients. Most of the European industrial products contain, in addition, an exotoxin which is toxic to a variety of non-lepidopterous insects. This toxin is not present in the registered American products. The spore is essentially the hardy form of the bacteria and the endotoxin is a toxic protein produced within the bacterial cell. The mode of action of the ingested endotoxin is to modify the gut of the susceptible insect so as to facilitate entry of the bacteria into the hemocoel (Louloudes and Heimpel 1969, Fast and Morrison 1972, Luthy 1973) where lethal septicemia develops.

The spruce budworm, Choristoneura fumiferana (Clem.) is known to be highly susceptible to commercial preparations of B.t. (Yamvrias and Angus 1970, Morris 1973). However, past attempts to control this important forest defoliator by aerial application of B.t. have met with mixed results due mainly to inferior formulation and application technology and the peculiar feeding habits of the budworm (Smirnoff 1973, Klein and Lewis 1966, Angus et al. 1961, 1970). Recently, Smirnoff et al. (1972), Dimond (1972) and Smirnoff et al. (1973) have shown that the addition of a small

amount of the enzyme chitinase to a water suspension of B.t. enhances its effectiveness in terms of both population reduction and foliage protection.

Chitin (N-acetyl-D-glucosamine) is present in the peritrophic membranes of insects in a free state and partly bound in the form of glycoprotein complexes (Jeuniaux 1971) and chitinase, its hydrolysing enzyme occurs naturally in the digestive juices of some larvae (but not in adults), the enzymatic activity being confined to the midgut region (Saxena and Sarin 1972). It is also of interest to note that Beauveria bassiana (Bals.) Vuill., an insect pathogenic fungus of very wide geographical distribution, contains substantial amounts of chitinase as a constitutive enzyme (Leopold and Samsinakova 1970).

There is at present a need to develop alternative means to chemical insecticide control of insect pests in an attempt to avoid or reduce several problems associated with their intensive use, e.g., resistance, target pest flareback, destruction of natural enemies, human health and environmental pollution (Adams 1972). The need is particularly pressing in the national and provincial park systems and in urban areas where chemical sprays are not permitted. During 1973, experiments were designed to determine the efficacy of commercial Bacillus thuringiensis with or without chitinase in reducing defoliation by spruce budworm of balsam fir, Abies balsamea (L.) Mill.

MATERIALS AND METHODS

Plot Description and Preparation

The test plots (Fig. E-1) consisted of balsam fir stands sparsely mixed with white spruce, Picea glauca (Moench) Voss, located in

Algonquin Park, Ontario. The 30 ft. to 50 ft. high trees had been under severe attack by spruce budworm for 3-4 years. The plots located at 1,350 ft. - 1,550 ft. above sea level were 2,500 acres, (Thuricide 16B® alone treatment) or 100 acres (all other treatments and 2 checks) in area. Assessment of efficacy of the Thuricide alone treatment was restricted to 100 of the 2,500 acres. This large plot was established primarily for environmental impact studies (Section F of this report).

Two sampling transects were established across each plot. A total of 48 sampling stations (1 per tree) were chosen in each plot and cleared for spray deposit sample units at ground level (Armstrong and Randall 1969). Unfortunately, there were not enough white spruce trees in any of the plots to provide any suitable information on this species so only balsam fir trees were used in the tests.

Meteorological equipment was set up near the plots for determining weather conditions at time of spray (Section D of this report). In addition, a recording tipping bucket rain gauge, a hygrothermograph and a pyranograph were installed in one of the plots for continuous recording of weather data throughout the sampling period.

Spray Formulation and Applications

The formulations used (Table E-I) were arrived at after consultation with, and on the advice of, International Minerals and Chemicals Corp., Libertyville, Illinois (manufacturer of Thuricide 16B®) and Abbott Laboratories, North Chicago, Illinois (manufacturer of Dipel WP®). The high percentage of molasses used in the Dipel preparations was necessary to equate the two commercial preparations not only in terms of active ingredients but also on an equivalent component content per unit volume

of spray mixture. The Thuricide 16B (Lot #IW 30091) used was a specially formulated slurry for forestry applications with a potency of 4 billion International Units of active ingredient per quart and contained 5 million viable spores per milligram. The recommended dosage rate for spruce budworm on the label is 1-2 quarts per acre of commercial product. The Dipel wettable powder (Lot 24623CD) had a potency of 16,000 International Units per milligram and contained 25 billion viable spores per gram. The potency of two pounds of Dipel is therefore equivalent to 1 gallon Thuricide 16B. Brilliant Sulfo Flavine dye (Chemical Developments of Canada Ltd., Toronto) was used as a fluorescent tracer at the same concentration as used by Dimond (1972). Prior to field use, the dye was tested for compatibility with B. thuringiensis. Chevron Spray Sticker (Chevron Chemical (Canada) Ltd., Oakville, Ontario), containing a mixture of alkyl olefin aromatic polymers as active ingredient was added at the concentration of 400 ml/25 gal. water as recommended by Dr. W.A. Smirnoff (Laurentian Forest Research Centre, Quebec). Cargill Insecticide Base (Cargill, Minneapolis, Minn.) was used as the molasses ingredient. The chitinase (NBC, Ohio and Cal Biochem, California) used was of microbial origin (Streptomyces griseus) and contained over 40 units of activity per mg. Water source was a stream nearby the Lake of Two Rivers airfield in Algonquin Park; pH of the water was 7.0. Mixing sequence of the ingredients was as follows: A. Thuricide: (1) water (in mixing tank); (2) chitinase in solution; (3) dye concentrate; (4) Thuricide; B. Dipel: (1) water (in mixing tank); (2) chitinase in solution; (3) dye concentrate; (4) molasses; (5) Dipel, in slurry; (6) sticker. Tank mixtures of dye and bacteria were never allowed to remain in contact for more than 1 hour. The Cessna Agtruck and a Piper Pawnee used, were both fitted with 4

Micronair AU 3000 emission units which were calibrated to deliver the desired dosage rate with droplet sizes in the 50-100 micron range.

Applications were made during the mornings and evenings of May 29-31 when budworm developments was 58% - 3rd, 40% - 4th and 1% - 5th instar at the rate of 4 billion International Units of active ingredient in 0.5 U.S. gal/ac. Swaths were 200 ft. wide with the planes flying about 100 ft. above tree tops.

Deposit Analysis

Spray sample units (Fig. E-2) consisting of two 7.5 cm x 5 cm glass plates, two 37 mm diameter Millipore filter membranes and one 10 mm x 10 mm Kromekote card were placed in clearings adjacent to each of the 48 sample trees per treatment. In addition, vertical sample units were installed at 4 sample tree locations per plot at 10 ft., 15 ft. and 25 ft. levels of tree crown to measure vertical distribution of droplets.

The units were collected 30 minutes after spray application. Spray card analyses were performed to determine drop size and density (Smirnoff et al., 1973). Deposits on the glass plates were evaluated by fluorometric analysis. Millipore filters were placed directly onto nutrient agar and incubated for 24 hours at 29°C. The number of bacterial colonies developing was used to estimate the rate of viable spore deposit.

Biological Assessment

Duplicate (for population sampling) and quadruplicate (for defoliation and egg mass survey studies) 18-inch branch tips were collected from the upper and middle thirds of each of 48 sample trees per plot at intervals of 5, 15 and 26 days post-spray. Pre-spray samples were

collected from the Thuricide alone, Dipel + chitinase and two untreated check plots on May 12-16 and on other plots on May 22-23. Branch tips were examined at the field laboratory at the Petawawa Forest Experiment Station (DeBoo et al. 1973, Martineau and Benoit 1973) and data collected throughout the test period for the following studies:

1. Spruce budworm larval mortality.
2. Incidence of introduced and naturally occurring pathogens.
3. Budworm population reductions due to treatments.
4. Foliage protection due to treatment.
5. Effect of treatments on larval feeding activity and development.
6. Post-larval effects.

In addition, canvas mats were placed under 4 trees per plot to collect falling non-target insect species in an attempt to determine the effect, if any, of treatments on non-target arthropods.

Pupae collected from each plot were sexed, weighed and the emerging adults mated in 3' x 2' x 2' plastic screen cages housed in an open metal storage shed located at the Petawawa Forest Experiment Station. Data were collected on moth emergence, oviposition, fecundity and egg viability. An egg mass survey was carried out after oviposition in the field was completed and expressed as the number of egg masses per 100 square feet of foliage.

To estimate the relationship between B.t. deposits and population reduction or defoliation, the means of four ranges of deposits per plot were compared with the percent population reductions or percent defoliation at the corresponding sample stations. The data were analysed

using probits and regression lines fitted to determine dosage-mortality relationships.

Studies on the Residual Activity of B.t.

In residual activity studies, branches were collected from a single tree that was well exposed to sunlight for most of the day in the Thuricide 16B sprayed block. Only that portion of the tree receiving the most sun was sampled. Collections were made 5, 15 and 42 days post-spray and the foliage was bioassayed in the field laboratory for residual activity of spray deposit. Two to four replicates of 100 healthy field collected (5 and 15 days post-spray) or laboratory reared (42 days post-spray) 4th- and 5th-instar budworm larvae per sunlight exposure period were used in the assays. Untreated checks consisted of 100-400 larvae on unsprayed foliage.

RESULTS AND DISCUSSION

Formulation, Application and Deposit Analysis

Results of pre-application compatibility tests (Tables E-II and E-III) indicated that the tracer dye used in the field tank mixtures had no detrimental effect on B.t. viability or pathogenicity.

Temperature, relative humidity and solar radiation were about seasonal for the whole sampling period (Table E-IV). The daily rate of solar radiation, however, was highest in the first few days post-application so that an early decline in viability of sunlight-exposed bacteria was expected. Cumulative rainfall throughout the test period and the precipitation rate for each sample period were low.

The stability ratios and turbulence factors corresponded well with rates of microbial deposits (Table E-V). Thuricide + chitinase deposited at ground level with highest efficiency (81%) followed by Thuricide alone, and chitinase alone = Dipel + chitinase. Note that both Dipel treatments were applied while the stability ratios were negative. This indicates a meteorological condition of lapse rather than inversion, and deposits of the spray under these conditions were greatly reduced. Data from Millipore filter membranes indicated that there was no relationship between deposits of viable spores and International Units of active ingredient. The Thuricide alone/Thuricide + chitinase deposit ratios were 0.42 and 0.35 in terms of International Units and viable spores, respectively. Corresponding figures for Dipel alone and Dipel + chitinase were 1.23 and 0.64 indicating that only half as many viable spores were deposited as expected from the latter formulation. In view of Yamvrias and Angus' (1970) finding that the most pathogenic preparation of B. thuringiensis var alesti for C. fumiferana is that containing a mixture of both spores and crystals, it may be concluded that viability counts alone are not reliable indices of spray deposit and coverage.

The drop measurement data (Table E-VI) show that the Thuricide + chitinase plot received considerably better droplet distribution than the Thuricide alone plot but this may simply be a reflection of higher volume deposited. There was no difference in droplet density between the two Dipel treated plots. It was evident that coverage in the Thuricide + chitinase plot was the best of all treatments both in terms of volume and droplet density.

There were little or no differences in deposit rates or drop distribution between 10 ft., 15 ft. and 25 ft. tree height levels indicating that all levels of the trees received essentially similar coverage in each treatment (Tables E-VII and E-VIII). Trees which received Thuricide + chitinase treatment received the best coverage at all levels.

Larval Development

Figures E-3 to E-10 show larval development for each spray plot separately and for all plots combined on pre-spray and post-spray sampling dates and development on two unsprayed check plots. From these data it was evident (Fig. E-11) that in the time period of June 14-17 the two untreated and the chitinase alone treated populations were 75% - 88% 6th instars, compared with 65% for Thuricide and Thuricide + chitinase and 53% - 59% for Dipel and Dipel + chitinase treated populations. It is evident that chitinase did not affect the rate of budworm development but that the bacteria did. A similar retardation in development time of B.t. fed tobacco budworm, Heliothis virescens (F.), has been reported by Dulmage and Martinez (1973) who found development time to be directly related to the amount of the endotoxin consumed by this budworm.

Effect of Weathering on Residual Activity of B. thuringiensis

Results of tests on the residual activity of B.t. (Table E-IX) show a drastic reduction in spore activity 15 days post-spray. The incidence of bacterial septicemia among larvae feeding on test foliage dropped from 41% to 10% between the 5th and 15th day after spray application.

There was still some activity up to 42 days post-spray. From the data it appears that solar radiation has considerable effect on B.t. spore viability. This observation supports Angus et al. (1970) who reported that about 65% reduction in viability of B.t. spores take place after 4 hours exposure to direct sunlight. Frye et al. (1973) and Ahmed et al. (1973) have reported that solar radiation and rainfall caused drastic inactivation of commercial B.t. spores following 24-48 hours of exposure to sunlight. We have observed 93% reduction in the viability of spores of commercial Dipel after 48 hours of direct exposure to sunlight. These observations stress the need for methods of protecting entomopathogens from such rapid decline in activity. Morris (1972), for example, has shown benzyl cinnamate to be potentially effective in this regard.

Effect of Treatments on Population Reduction and Mortality of Target and Non-Target Arthropods

The data (Table E-X) indicate that the Dipel and Dipel + chitinase treatments (but not Thuricide with or without chitinase) were highly effective in reducing budworm populations on balsam fir despite their relatively low deposit rates. Dipel + chitinase treatment was nearly twice as effective (94%) in reducing budworm population as the operational dosage of fenitrothion (55%) at 26 days post-spray. Part of this difference in effectiveness was most probably due to the relatively high pre-spray populations of the budworm in the Dipel plots resulting in some mortality from eventual starvation. This argument is strengthened by the fact that the numerical difference in pre-spray density between Dipel and Dipel + chitinase is almost identical to the percentage difference in population reduction between the same treatments.

These observations suggest that the addition of chitinase to the B.t. spray does not cause significantly higher larval mortality than does B.t. alone. Dimond (1972) reported similar levels of spruce budworm population reduction with B.t. and B.t. + chitinase. The data on larval mortality (Table E-XI) and incidence of pathogens among recovered larvae (Table E-XII) adds further support to these results. Granted that the dead larvae found on the sample branches may not reflect the true total mortality due to larval fall and wind distribution, the data, nevertheless, show that the mortality rate as well as the incidence of septicemia were highest in the Dipel-alone plot for all three sample periods. There was no difference in incidence of bacteria in dead insects between the two Thuricide treatments (Table E-XII).

Regression analyses, however, for Dipel and Dipel + chitinase (Fig. E-12) deposits in relation to population reduction reveal that, for both applications, given the respective pre-spray population densities, a population reduction of 66% (probit 5.4) took place with a deposit of 15.9 fl. oz/acre (Log 1.2). To achieve 72.6% reduction (probit 5.6), 31.6 ft. oz. of Dipel-alone (Log 1.5) and 17.5 fl. oz. Dipel + chitinase (Log 1.24) per acre deposit would be necessary. Thus, only half as much Dipel + chitinase as Dipel-alone was required to produce the same population reduction. A deposit of 30.2 fl. oz. of Dipel + chitinase reduced the population by 93%.

There was no difference in population reduction at upper and middle tree crowns from which samples were taken (Table E-XIII). The incidences of naturally occurring nuclear polyhedrosis virus and microsporidia were extremely low for all plots (Table E-XII).

Data on mat collections (Table E-XIV) suggest that none of the treatments, including fenitrothion at 4 oz. AI/acre, had no substantial detrimental effect on mortality of non-target arthropods. This is of some importance because evaluation of safety of microbial or chemical insecticides should include safety for beneficial and other non-target organisms in the treated ecosystem. Indeed, one of the most important reasons for the current interest in pathogens of pest insects lies in their relative specificity and harmlessness to beneficial organisms. Exotoxin free commercial B.t. is generally considered a safe insecticide (Bailey 1971, Vail et al. 1972).

Effect of Treatments on Current Defoliation and Feeding Activity

Thuricide + chitinase was the only treatment causing substantial protection from defoliation of the balsam fir trees (Table E-XV). The low efficacy of the Dipel treatments is largely due to the fact that meteorological conditions at the time of spray were less than ideal, resulting in low deposit rates. The difference between 41% and 90% defoliation on the Thuricide + chitinase plot and Thuricide-alone or untreated check plot was statistically significant at the 99% level of confidence. Regression lines show the relationships between spores deposited (Fig. E-13) or total fluid deposits (Fig. E-14) and current defoliation.

In order to determine the effect of the addition of chitinase to Thuricide, the mean deposit at 10 sampling stations on the Thuricide-alone plot was calculated as 22.5 fl. oz/acre. The mean percent defoliation at these stations was 72.2. This deposit, with a log equivalent of 1.35 when superimposed on the Thuricide + chitinase regression

line (Fig. E-13), indicated 54% defoliation (probit 5.1).

It is clear, then, that the addition of chitinase to the Thuricide increased its foliage protection efficiency by 18.2%. This was supported by the data on the effects of treatments on feeding activity (Table E-XVI) which showed the population on the Thuricide + chitinase plot with the lowest frass weight/population density ratio. On the frass/density basis, it was shown that the treatments depressed feeding activity with the following decreasing order of efficiency: Thuricide + chitinase > Dipel + chitinase > Dipel alone > chitinase alone > Thuricide alone > untreated check.

An unanswered question at this point is whether differences in pre-spray population densities and in spray deposit rate, if taken into account, would change the general defoliation results. To this end, the ratio of percent defoliation/pre-spray density was calculated for each treatment and expressed in logarithmic form (Table E-XVII). The ratios were then adjusted for deposit differences using the formula:

$$\frac{RL \times PD}{DR}$$

where RL represents log of the ratio of defoliation/pre-spray density, PD = sum of means of all population densities and DR = deposit rate in fl. oz. per acre. The results summarized in Table E-XVII show that with adjustments for variations in population densities and deposits rates between plots, the effectiveness of the treatment in terms of foliage protection decreased in the order: Thuricide + chitinase > Dipel + chitinase > Dipel alone > Thuricide alone > chitinase alone. The data closely approximate the observations on feeding activity (Table E-XVI).

The present observations support the findings of Smirnoff et al. (1973) that chitinase increases the effectiveness of B. thuringiensis in protecting balsam fir trees from excessive defoliation by the spruce budworm.

Post-Larval Effects

The average weight of both male and female pupae, oviposition rate, and egg viability in laboratory and field (egg mass survey) were lower in the Dipel-alone treated plot than in all other plots (Table E-XVIII). The plot treated with fenitrothion (operational dosage) had the highest egg mass density at the end of the season. The weight of females from the Dipel spray plot was about one-half that from the untreated check. Pupae from B.t. treated plots were generally lower in weight than those from the chitinase alone treated plot and from untreated check plots. Male and female pupae from the Thuricide + chitinase treated population showed the lowest percent moth emergence but none of the treatments appeared to have affected sex ratio emergence of moths. Previous evidence of these and other post-larval effects of B.t. have been well documented. Increased development time, appearance of teratologies, increased general debility of survivors, decreased pupal weights and sizes, moth emergence, fecundity, fertility, pupation and adult longevity have been reported by Angus (1965), Burgerjon and Biache (1967), Morris (1969), Soliman et al. (1970), Abdallah and Abul-Nsar (1970), and Dulmage and Martinez (1973). These observations focus attention on the often made suggestion that different criteria be used in judging efficacy of bacterial and chemical insecticides. Microbial agents produce long term effects which are usually reflected in succeeding

generations of treated insect populations. A good example of this long term effect with B. thuringiensis was recently reported by Grison et al. (1969) who found that the efficacy of the bacteria aerially applied against a leaf roller, Zeiraphera diniana GN., was maintained for at least two generations after application. Since the bacteria is not transmitted from one generation to the next, the long term effect of this pathogen is likely a reflection of the general debilitating effect produced in survivors of the generation against which the pathogen was initially applied.

SUMMARY AND CONCLUSIONS

In 1973, spruce budworm infested balsam fir and white spruce trees in Algonquin Park were aerially sprayed with Thuricide 16B (International Minerals and Chemicals Corp., Libertyville, Illinois) or Dipel Wettable Powder (Abbott Laboratories, North Chicago, Illinois). The sprays were formulated with and without chitinase, an enzyme which, when ingested by insects, renders the pest more susceptible to bacterial infection, and with a tracer dye, Brilliant Sulfo Flavine FFA. Test plots were 100 acres in size, except for the Thuricide alone treatment plot which was 2,500 acres.

Applications were made May 29-31 when the budworm larvae were mostly in the 3rd instar at the rate of 4 billion International Units (in 0.5 U.S. gallons of liquid suspension) per acre. A Cessna Agtruck and a Piper Pawnee, both fitted with 4 Micronair AU 3000 emission units, were used for all applications. A variety of meteorological equipment was used to determine the best conditions for time of spray and for recording weather conditions during the entire test period.

Spray deposits collected on glass plates, Millipore membrane surfaces, and Kromekote cards were analysed for volume and viable spore deposit rates and for droplet density and size.

Efficacy of the treatments was assessed by studying larval development, effect of weathering on residual activity of the pathogen, effect of treatments on population reduction, target and non-target insect mortality, feeding activity, current growth defoliation, pupal weights, moth emergence, fecundity and egg viability.

The following conclusions were drawn from the results:

1. The fluorescent tracer dye used in the spray mixtures had no detrimental effect on B.t. viability or pathogenicity.

2. Eighty-one percent of emitted Thuricide + chitinase and 18% - 34% of the other suspensions were deposited at ground level. The low deposit rates on Dipel treated plots was due to inferior meteorological conditions under which application took place. Drop densities (16-98/cm^2) and drop sizes (91-111 µm) were within expected ranges. Deposit rates at three crown levels were approximately equal. No direct relationship exists between viable spore deposit and volume deposit.

3. B.t. treatment with or without chitinase retarded development of spruce budworm.

4. Residual activity of B.t. was drastically reduced after 5 days exposure to weathering.

5. Dipel and Dipel + chitinase (but not Thuricide or Thuricide + chitinase) treatments were highly effective (75% and 94%, respectively) in reducing spruce budworm population densities on balsam fir and, in this regard were superior to fenitrothion at 4 oz. AI/acre. Addition of chitinase to Dipel increased population reduction.

6. The incidence of naturally occurring microsporidia and polyhedrosis virus was extremely low in all treated and untreated populations.

7. None of the treatments, including fenitrothion at 4 oz. AI/acre, had any observable detrimental effect on non-target arthropods.

8. Thuricide + chitinase treatment resulted in significant foliage protection of balsam fir trees when differences in pre-spray populations and spray deposits are disregarded. If these criteria are calculated in the defoliation estimates, Dipel + chitinase was also effective in foliage protection. Fenitrothion at the dosage evaluated was less effective than Thuricide + chitinase in protecting foliage.

9. The treatments inhibited feeding in the following decreasing order of efficiency: Thuricide + chitinase > Dipel + chitinase > Dipel alone > chitinase alone > Thuricide alone.

10. There was no direct relationship between larval mortality and foliage protection in any of the treatments. The former criterion should not be used to judge efficacy in the year of application.

11. Pupal weights, oviposition rates, and egg viability were reduced by B.t. treatments.

12. Both Thuricide 16B and Dipel WP were considered effective for control of the spruce budworm.

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TABLE NO. E-I

Summary of B. thuringiensis Formulations - Algonquin Park 1973

Treatments	Area[1] (acres)	Thuricide (U.S. gallons)	Dipel (lbs)	Chitinase (gm)	Molasses (U.S. gallons)	Chevron sticker (ml)	BSF dye (gm)	Water (U.S. gallons	Spray mixture pH
Dipel	100	-	50	-	25	400	190	25	7.5
Dipel chitinase	100	-	50	1.8	25	400	190	25	7.5
Thuricide	2,500	625	-	-	-	-	4,750	625	6.5
Thuricide chitinase	100	25	-	1.8	-	-	190	25	6.5
Chitinase	100	-	-	1.8	25	400	190	25	7.5
Fenitrothion (operational dosage)[2]	100	- 4 oz. AI/acre (applied at 20 oz. spray mixture/acre)							

[1] Only the middle 100 acres of the 2,500 acre block were sampled for post-treatment biological assessment.

[2] Test conducted at Petawawa Forest Experiment Station, Chalk River, Ontario.

TABLE NO. E-II

Effect of Brilliant Sulfo Flavine Dye[1] On Viability of *Bacillus thuringiensis* Spores[2]

Contact Time (hrs.)	Spray Tower Deposits of Viable Spores/cm^2	
	Treated	Check
0	6.9	-
½	7.4	4.7
1	7.0	5.0
2	7.8	4.7
4	9.1	4.8
6	6.9	4.9
24	5.9	4.6
48	6.3	5.3

1 The dye was added to the Thuricide suspension at the concentration used in field trials (Table E-I).

2 Thuricide 16B diluted 10^5 and sprayed onto 10 replicates of Millipore filter membranes. Incubation on nutrient agar for 24 hours at 29°C.

TABLE NO. E-III

Compatibility of Brilliant Sulfo Flavine Dye

With Commercial *Bacillus thuringiensis*[1]

for Spruce Budworm Mortality

Treatment	Corrected Percent[2] Mortality - 5 Days Post-Treatment
Thuricide 16B alone	98.9
Thuricide 16B + BSF	94.6
BSF alone	3.0

[1] B.t. and BSF dye were allowed to stand for 2 hours before contaminating foliage

[2] Five replicates of 20 each, 4th-instar larvae. Corrected by Abbott's formula (1925). Mortality refers to insects which died from bacterial septicemia.

TABLE NO. E-IV

Meteorological Conditions Following Aerial Sprays, Algonquin Park - 1973

Inclusive Dates	Temperature (^{o}C) Mean Min.	Mean Max.	Relative Humidity (%) Mean Min.	Mean Max.	Cumulative Solar Radiation (Cal/cm^2)	Average Solar Radiation (Cal/cm^2/Day)	Cumulative Rainfall (Inches)	Average Daily Rainfall (Inches)
May 29 - June 4	6	22	23	97	3042	608	0.5	0.09
May 29 - June 11	7	23	33	98	8420	359	3.0	0.20
May 29 - June 18	6	22	34	98	20495	503	4.5	0.17

TABLE NO. E-V

Deposit Rates on Plots Treated With Commercial *Bacillus thuringiensis* (and/or Chitinase) of Fenitrothion

Treatments	Stability[1] Ratio (SR)	Turbulence Factor	Relative[1] Humidity (%) High/Low	Deposits per Acre			Percent Deposited
				Fl. oz.[2]	IU	No. Viable[3] Spores	
Thuricide alone	+ 39.2	0.114	57/64	21.8	13.6×10^8	1.3×10^9	34.1
Thuricide + chitinase	+174.2	0.102	34/38	52.1	32.6×10^8	3.7×10^9	81.4
Dipel alone	- 7.5	0.218	69/63	21.1	13.2×10^8	0.9×10^9	33.0
Dipel + chitinase	- 7.0	0.187	69/63	17.1	10.7×10^8	1.4×10^9	26.7
Chitinase alone	+ 1.3	0.207	50/50	11.5	-	-	18.0
Fenitrothion alone	+ 0.3	-	-	4.3	-	-	13.4

[1] Best spray conditions indicated by large SR accompanied by small turbulence factor and high relative humidity. High and low refer to tower positions (See Section D for details).

[2] As determined by fluorometric analysis.

[3] As determined by agar plate counts.

TABLE NO. E-VI

Summary of Drop Measurements On

Kromekote Cards

Treatments	Mean Droplet Density (N/cm^2)	Mean oz. AI/cm^2	Dia. Drop of Av. Vol. (µm)
Thuricide 16B alone	36.7	0.632	96
Thuricide 16B + chitinase	98.1	0.542	91
Dipel alone	15.0	1.36	124
Dipel + chitinase	17.1	1.0	111
Chitinase alone	15.9	0.685	98
Fenitrothion (operational)	22.7	-	-

TABLE NO. E-VII

Deposit Rates (Fluid Oz. U.S.) at Three Levels of Balsam Fir Tree Canopy Following Application of Commercial *Bacillus thuringiensis* and/or Chitinase at 0.5 Gallons Per Acre[1]

Treatment	Deposits (at elevations above ground level)								
	10'			15'			25'		
	Oz./Ac.	Percent[2]	N/cm^2	Oz./Ac.	Percent[2]	N/cm^2	Oz./Ac.	Percent[2]	N/cm^2
Thuricide alone	26	40	39.5	27	42	37.1	25	39	37.6
Thuricide + chitinase	42	65	110.2	48	72	86.8	50	77	85.4
Dipel alone	18	28	13.3	20	31	14.0	-	-	-
Dipel + chitinase	16	25	9.0	17	27	13.7	-	-	-
Chitinase alone	8	13	13.9	8	13	13.4	8	13	12.8

1 Four sample stations per treatment with one sample unit at each tree level.

2 Percent of emitted rate deposited as determined by fluorometric analysis.

TABLE NO. E-VIII

Deposit Rates of Viable Spores at Three Levels of Balsam Fir Tree Canopy Following Applications of Commercial *Bacillus thuringiensis*

Treatments	No. of Viable Spores Per Acre at		
	10'	15'	25'
Thuricide alone	17×10^8	17×10^8	16×10^8
Thuricide + chitinase	33×10^8	34×10^8	30×10^8
Dipel alone[1]	7.5×10^8	9.6×10^8	-
Dipel + chitinase[1]	3.3×10^8	3.6×10^8	-

1 Data from 25' level accidentally destroyed.

TABLE NO. E-IX

Effect of Weathering on Residual Activity

of Thuricide 16B - Algonquin Park, 1973

No. Days of Weathering	Cumulative[1] Rain (Inches)	Cumulative[1] Radiation Cal/cm^2	No. Larvae Tested[2]	Percent Mortality Larval[3]	Pupal	Total	Percent of dead Larvae Infected by B.t.	Percent Moth Emergence[4]
5	0.45	3042	400 (400)	51.5	18.2	69.7	40.6	26
15	3.0	8420	400 (400)	19.7	25.8	45.5	9.6	33
42	4.79	40709	240 (100)	2¢.5	4.6	32.1	4.2	60

1 Temperature Range: mean min./mean max. temp. (^{O}C) = 44.8/71.5
Humidity Range: mean min./mean max. R.H. (%) = 31.4/97.8

2 Two to four replicates of 100 larvae each. Number of untreated checks in brackets.

3 Corrected for natural mortality (Abbott's formula, 1925).

4 Percentage of live moths from surviving pupae.

TABLE NO. E-X

Corrected[1] Percentage Population Reductions for Spruce Budworm On Balsam Fir Due to Treatment by Commercial *Bacillus thuringiensis* (and/or Chitinase) Or Fenitrothion

Treatments	Pre-Spray Density[2]	5 Days Post-Spray Density[2]	15 Days Post-Spray Density[2]	26 Days Post-Spray Density[2]
Thuricide alone	25.7	7.2	35.3	29.4
Thuricide + chitinase	25.6	40.8	33.5	23.5
Dipel alone	51.7	47.8	75.0	75.5
Dipel + chitinase	69.3	71.2	90.7	93.6
Chitinase alone	18.9	0.0	10.9	0.0
Fenitrothion[3]	12.9	0.0	59.3	54.6
Untreated check A[1]	16.2	46.3	54.3	79.6
Untreated check B[1]	33.3	33.3	47.1	69.4
Untreated check C[4]	8.3	7.2	46.9	79.5

1 Corrected by Abbott's formula. Untreated check A used for chitinase alone. Untreated check B for all other plots except fenitrothion.

2 Number of larvae per 18" branch tip.

3 Operational dosage of 4 oz. AI per acre.

4 For operational fenitrothion spray only.

TABLE NO. E-XI

Larval Mortality on Balsam Fir Sprayed With Commercial *Bacillus thuringiensis* and/or Chitinase

Treatments	Cumulative Total Numbers Collected (dead & alive)				Percent Mortality (Uncorrected)			
	Pre-Spray	5 Days Post-Spray	15 Days Post-Spray	26 Days Post-Spray	Pre-Spray	5 Days Post-Spray	15 Days Post-Spray	26 Days Post-Spray
Thuricide alone	2393	1570	2384	2733	3.3	6.6	5.2	5.3
Thuricide + chitinase	1747	801	1366	1629	9.1	17.0	11.1	9.9
Dipel alone	4748	1886	2517	2752	6.4	20.6	17.2	16.2
Dipel + chitinase	2974	1347	1679	1765	6.8	10.2	9.8	9.3
Chitinase alone	1795	1396	2139	2581	9.6	15.4	13.3	11.7
Untreated check A	362	240	419	497	1.7	20.0	15.5	14.0
Untreated check B	1562	1806	2694	3249	2.0	11.6	9.4	9.8

TABLE NO. E-XII

Incidence of Pathogens Among Larvae From Balsam Fir Trees

Sprayed With Commercial *Bacillus thuringiensis* and/or Chitinase

Treatments	Total No. Larvae Collected[2]	Total Number Dead[2]	Percent of Population Infected By					
			B.t.		NPV*		Microsporidia	
			Totals	Cadavers	Totals	Cadavers	Totals	Cadavers
Pre-spray[1]	15581	955	0.0	0.0	0.0	0.0	0.0	0.0
Thuricide alone	2733	144	1.8	35.2	0.0	0.0	0.3	2.6
Thuricide + chitinase	1629	162	3.2	32.5	0.0	0.0	0.0	0.6
Dipel alone	2752	446	7.2	44.2	0.0	0.0	0.0	0.2
Dipel + chitinase	1765	165	2.0	21.3	0.0	0.0	0.1	1.6
Chitinase alone	2581	303	0.0	0.0	0.0	0.0	0.0	0.6
Untreated check	1924	389	0.0	0.0	0.0	0.0	0.4	1.8

1 Collected from all plots combined.

2 Post-spray collection only.

* Nuclear polyhedrosis virus.

TABLE NO. E-XIII

Relationship Between Vertical Spray Deposits and Population Reduction on Balsam Fir Treated With Thuricide + Chitinase

Tree Height (Ft.)	Spray Deposit (Fl. Oz./Ac	Population Reduction (%) 5 Days Post-Spray
10-15	44.6	69.8
25-30	49.5	61.0

TABLE NO. E-XIV

Mortality of Non-Target Arthropods Collected
Under Balsam Fir Trees in
Treated and Untreated Plots[1]

Treatments	Number Non-Target Arthropods per Acre
Thuricide alone	12197
Thuricide + chitinase	14375
Dipel alone	27443
Dipel + chitinase	4791
Chitinase alone	12632
Fenitrothion	14375
Untreated checks A & B	10890

1 Based on arthropod fall on mats placed under selected sprayed and unsprayed trees.

TABLE NO. E-XV

Percent Defoliation on Balsam Fir Trees Sprayed With Bacillus thuringiensis (and/or Chitinase) or Fenitrothion

Treatment Comparisons Set #1	vs. Set #2	Set #1 No/Set	Set #1 Variance	Set #1 Mean (%)	Set #2 No/Set	Set #2 Variance	Set #2 Mean (%)	T Values
Untreated check B	Thuricide alone	25	386.36	89.51	43	138.43	89.53	0.5001
Untreated check B	Dipel + chitinase	25	386.36	89.51	47	307.8	86.8	.59
Untreated check B	Dipel alone	25	386.36	89.51	44	215.73	84.64	1.1514
Untreated check B	Thuricide + chitinase	25	386.36	89.51	30	921.64	40.57	6.8116*
Thuricide alone	Thuricide + chitinase	43	138.46	89.53	30	921.64	40.57	9.4607*
Dipel + chitinase	Dipel alone	47	307.8	86.8	44	215.73	84.63	0.62
Untreated check A	Chitinase alone	25	580.15	61.72	45	1086.16	62.12	.0520
Untreated check C	Fenitrothion	25	-	42.8	25	-	25.41	-

* Highly significant differences at 99% level of confidence. Confidence interval of mean were 6.99 for Untreated check B vs. Thuricide + chitinase comparison and ±6.05 for Thuricide alone vs. Thuricide + chitinase comparison.

TABLE NO. E-XVI

Effect of Treatments on Feeding Activity of Budworm on Balsam Fir Trees - *Bacillus thuringiensis* and Fenitrothion Aerial Application

Treatments	Population[1] Density	Wt. (mg.) Frass[2] per Sq. Ft.	Ratio Wt./Density
Thuricide alone	13.55	10065	743
Thuricide + chitinase	12.03	1922	159
Dipel alone	19.79	5931	299
Dipel + chitinase	12.39	2425	195
Chitinase alone	11.17	7858	703
Fenitrothion	6.35	813	127
Untreated check	18.86	15682	831

1 Average number of larvae per 18" branch tip for all samples.

2 Total of 36 sq. ft. canvas matting were placed under 4 sample trees per plot. Mats were collected at the end of the pupation period.

TABLE NO. E-XVII

Analysis of Defoliation Estimates Taking Pre-Spray Population Levels and Deposit Differences Into Account

Treatments	Pre-Spray Mean Population Density[1]	Percent Defoliation 26 Days Post-Spray	Ratio of Percent Defoliation to Density (Logs)	Deposit Rate (Fl. oz/ac)	Ratio Adjusted for Deposit Differences
Thuricide alone	25.7	89.5	0.53	21.8	3.0
Thuricide + chitinase	25.6	40.6	0.18	52.1	0.43
Dipel alone	51.7	84.6	0.20	21.4	1.17
Dipel + chitinase	69.3	86.8	0.08	17.1	0.58
Chitinase alone	18.9	62.1	0.51	11.5	5.48
Fenitrothion (Oper. Dosage)	12.9	25.4	0.28	0.54	-
Untreated check A	16.2	61.7	0.58	-	-
Untreated check B	33.3	89.5	0.41	-	-
Untreated check C	8.3	42.5	0.71	-	-

[1] Average number of larvae/18" branch tip.

TABLE NO. E-XVIII

Effect of Treatments on Moth Emergence and Oviposition in Plots Sprayed With *Bacillus thuringiensis* (and/or Chitinase) or Fenitrothion

Laboratory Reared Pupae and Egg Mass Survey

Treatments	Number Pupae (Caged)		Average Pupal Wt (mg.)		% Total Emergence			Av. No. Egg Masses/female (Emerged)		Av. No. Egg Masses/ 100 sq.ft. Foliage[1]
	Males	Females	Males	Females	Males	Females	Total	Total	Viable	
Thuricide alone	240	220	70.2	93.0	97	83	90	3.1	2.8	134
Thuricide + chitinase	207	207	70.7	86.2	52	45	49	3.8	3.3	155
Dipel alone	216	218	50.0	64.2	80	56	68	1.6	1.2	63
Dipel + chitinase*	226	224	64.7	83.9	-	-	-	-	-	132
Chitinase alone	200	200	76.1	110.3	96	85	90	2.5	2.2	93
Fenitrothion	254	189	69.0	90.0	89	57	77	2.5	2.4	195
Untreated check	235	185	73.4	119.9	83	56	71	3.4	3.0	84

* Insect material destroyed by error before some data was recorded.

1 Egg mass survey of 32 to 47 balsam fir trees per plot.

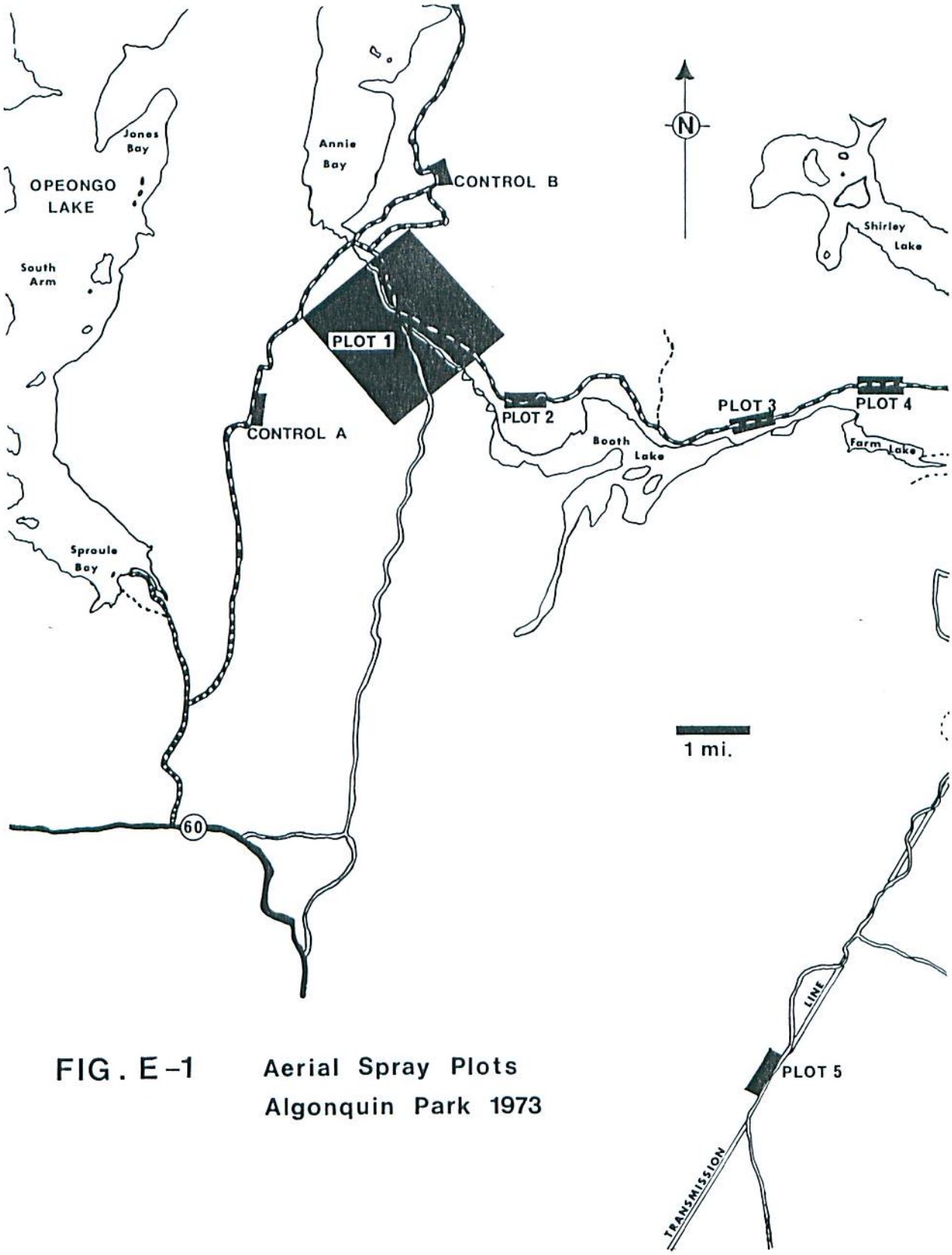


FIG. E-1 Aerial Spray Plots
Algonquin Park 1973

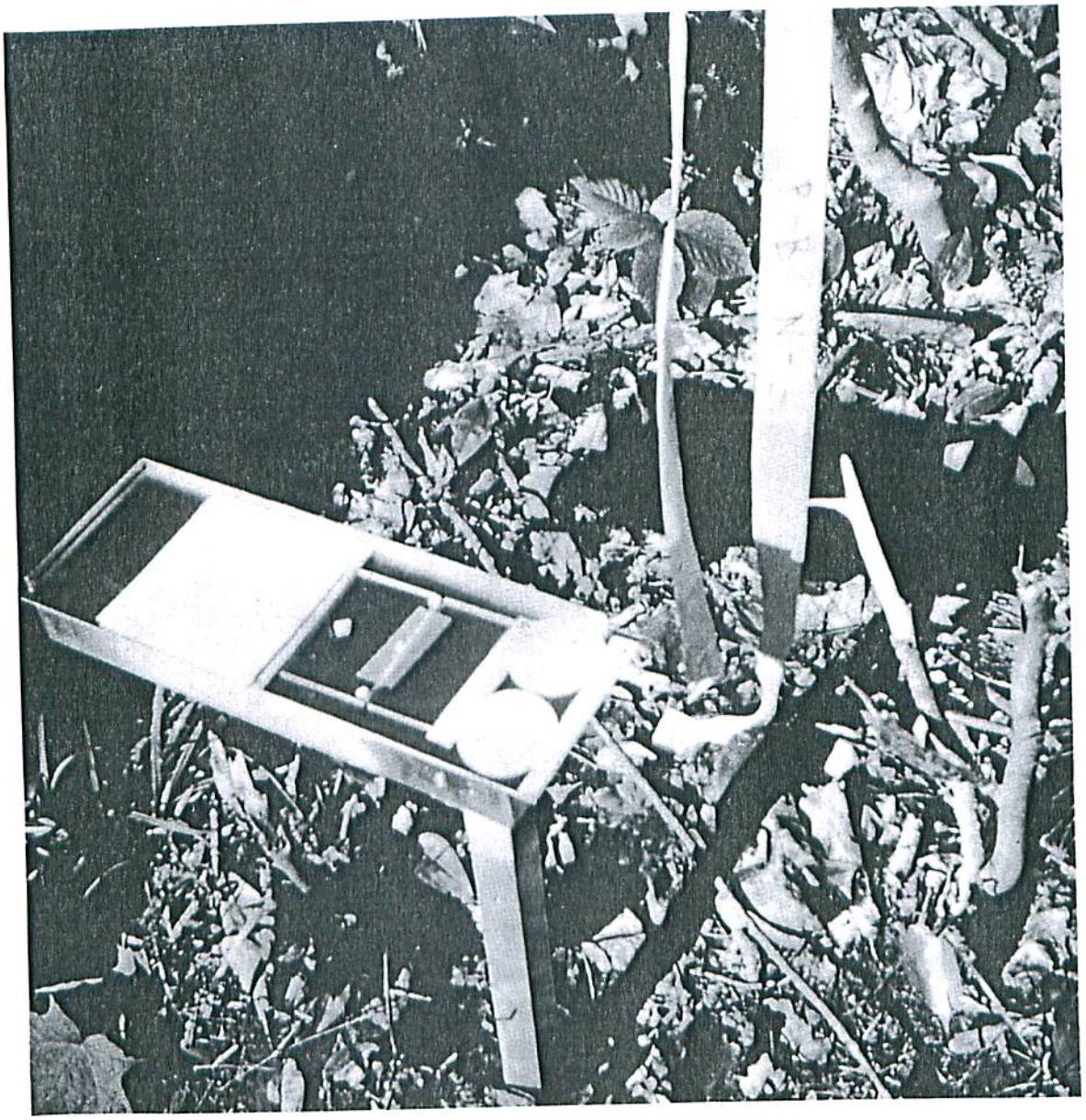


Fig. E-2 Spray deposit sample unit showing (L - R) a Kromekote card, 2 glass plates and 2 Millipore filter membranes.

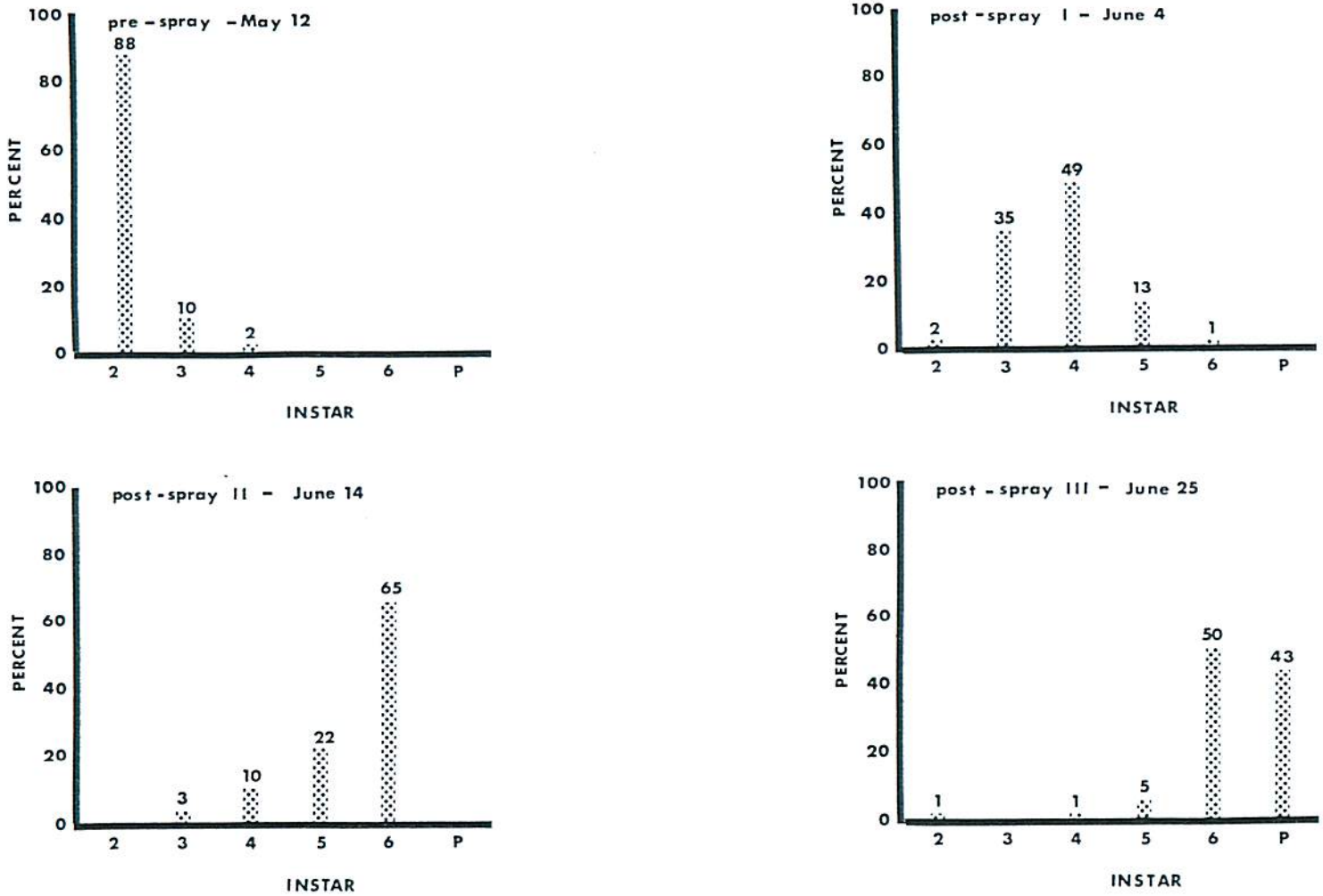


FIG. E - 3 Spruce Budworm development on key dates on Balsam Fir - Thuricide 16B alone treatment

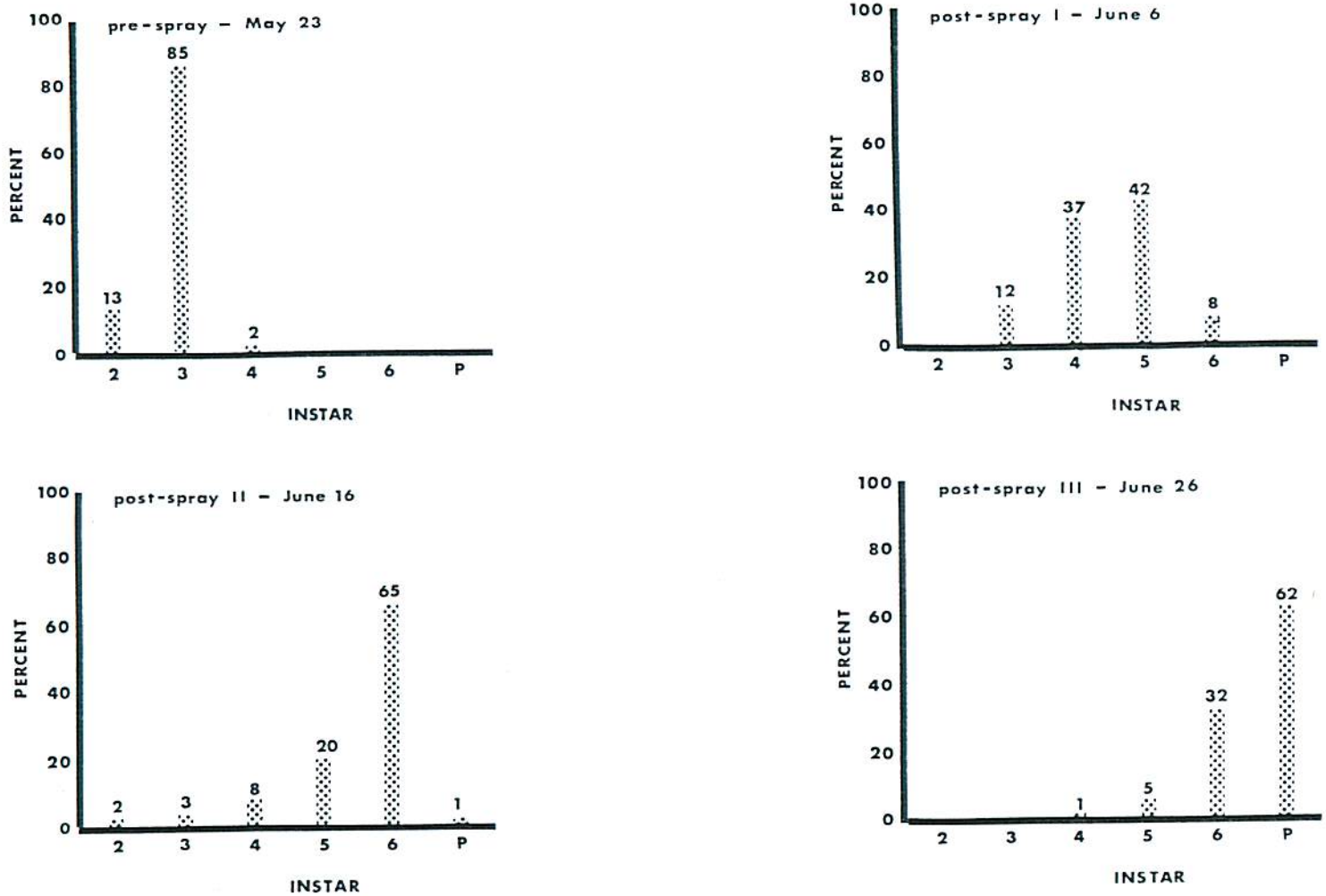


FIG. E-4 Spruce Budworm development on key dates on Balsam Fir – Thuricide 16 B plus Chitinase

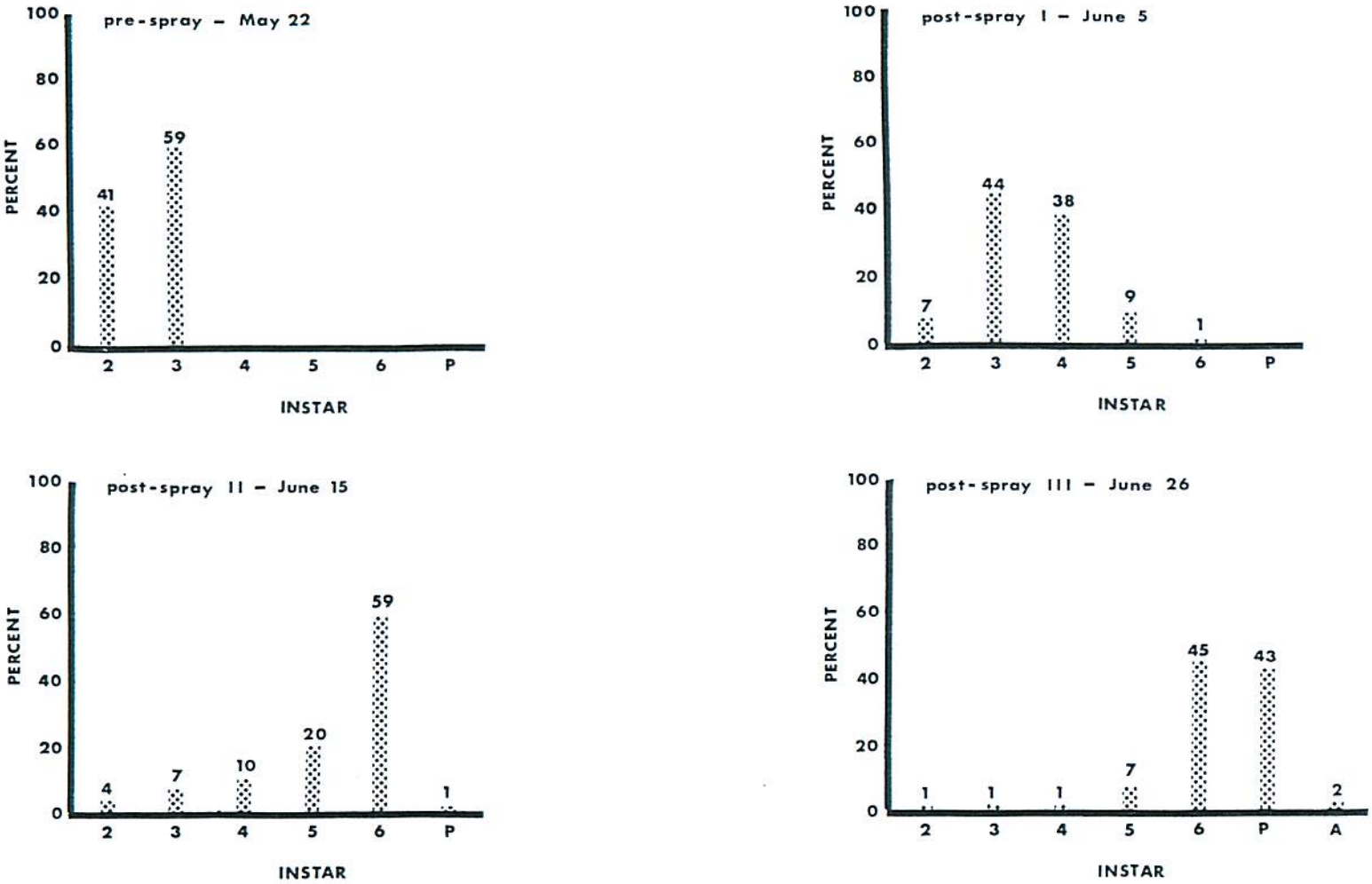
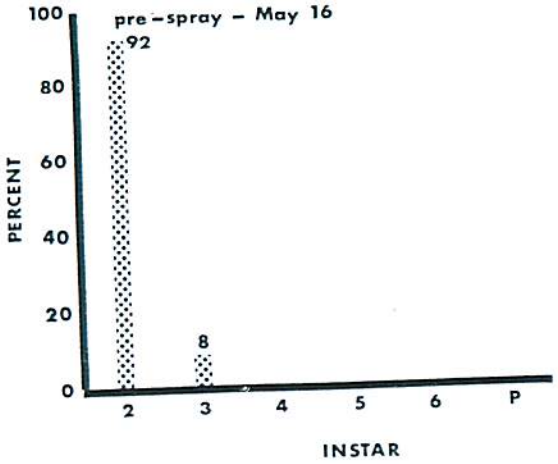
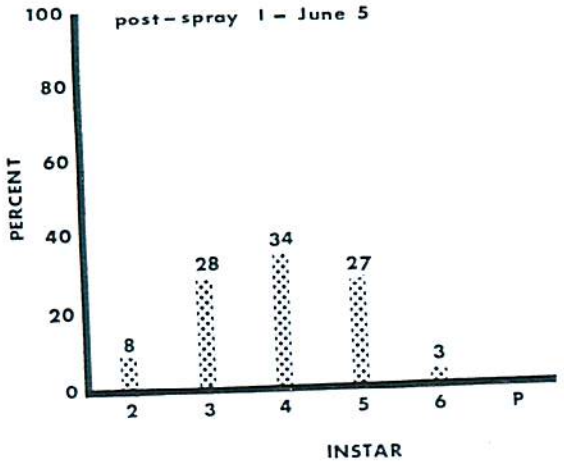
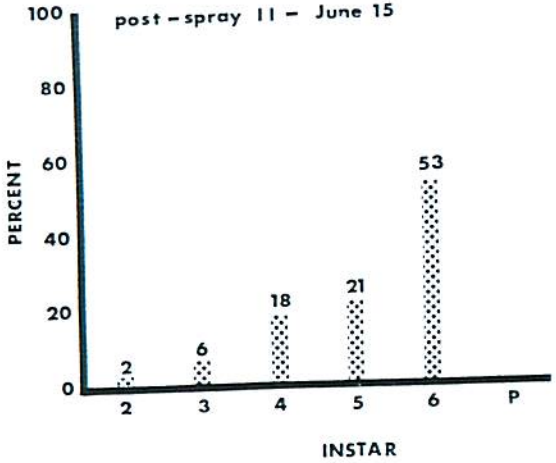


FIG. E-5 Spruce Budworm development on key dates on Balsam Fir – Dipel alone







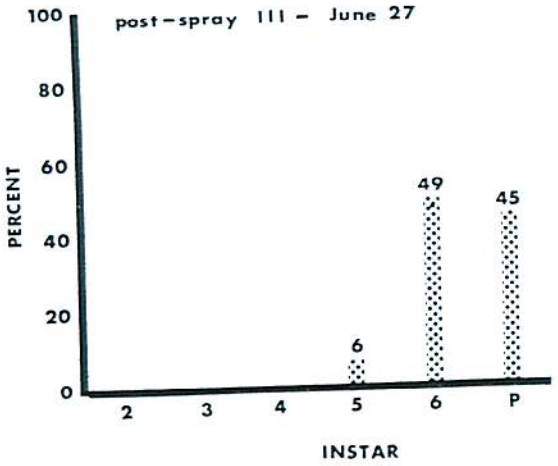


FIG. E-6 Spruce Budworm development on key dates on Balsam Fir - Dipel plus Chitinase

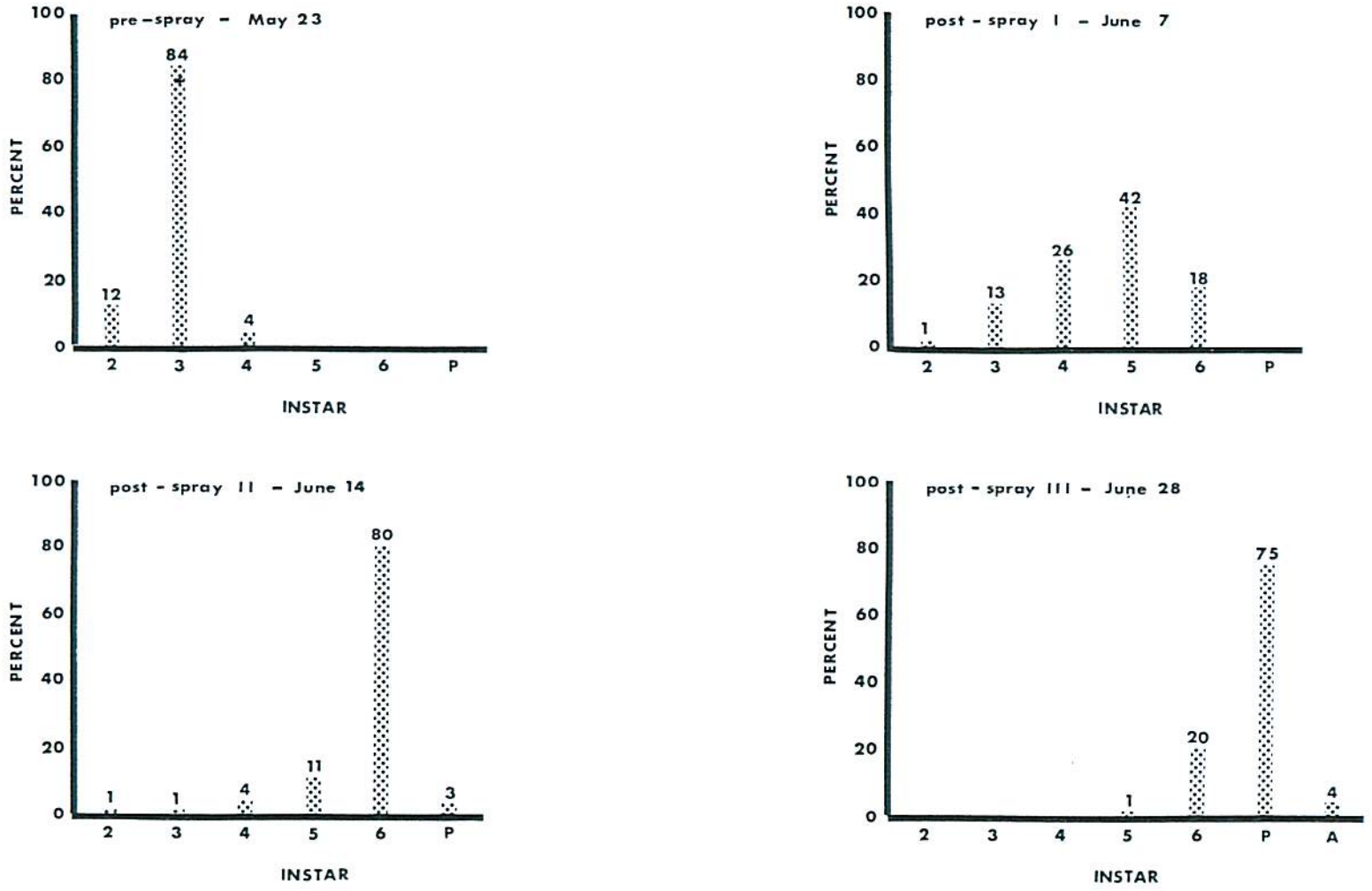


FIG. E-7 Spruce Budworm development on key dates on Balsam Fir - Chitinase alone

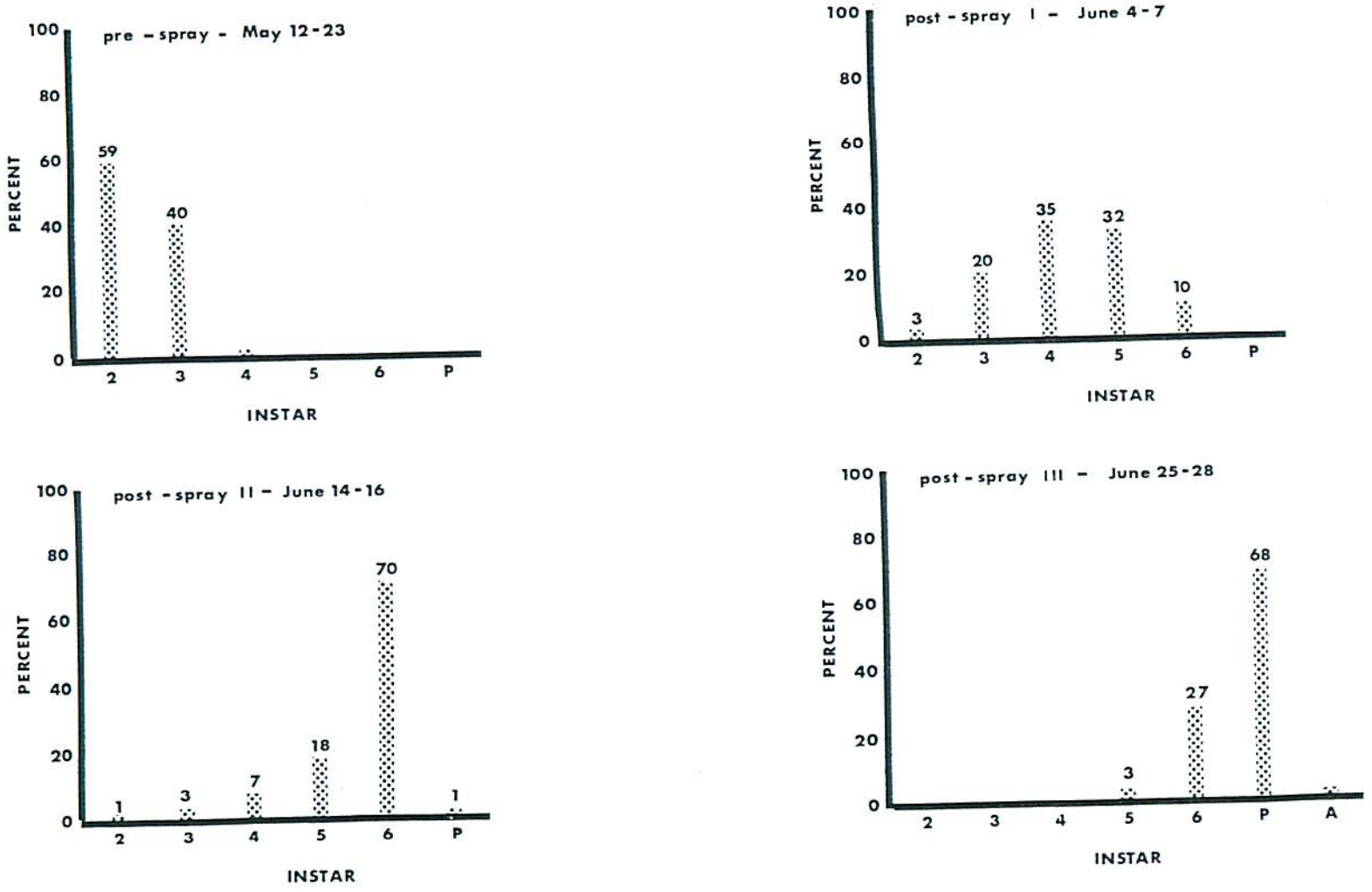


FIG. E-8 Spruce Budworm development on key dates on Balsam Fir - all plots combined

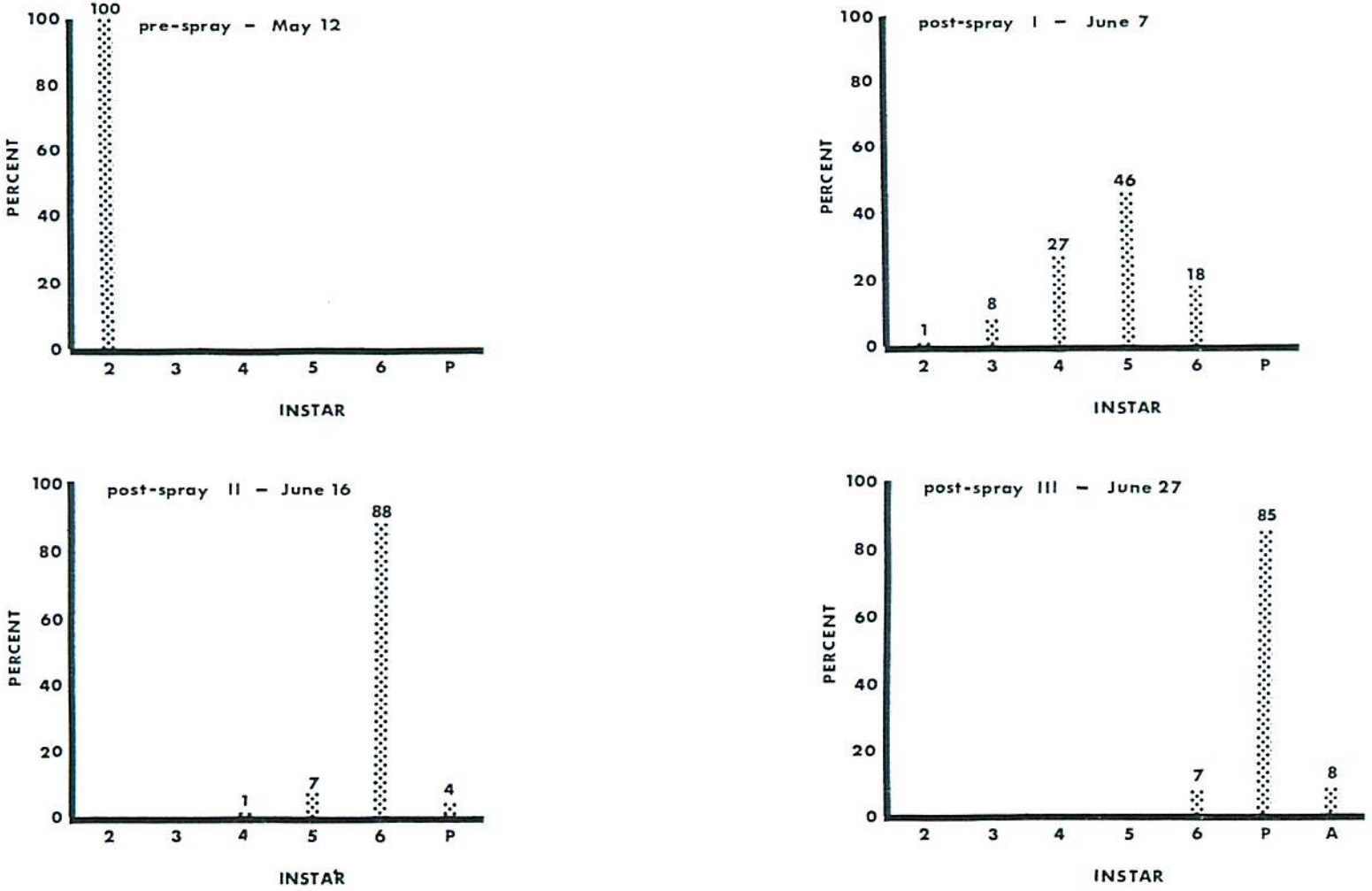
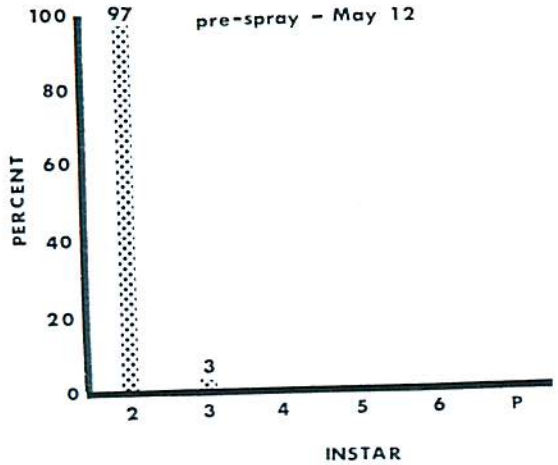
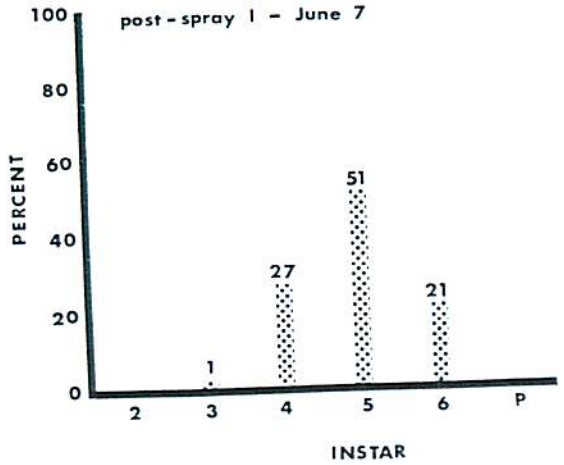
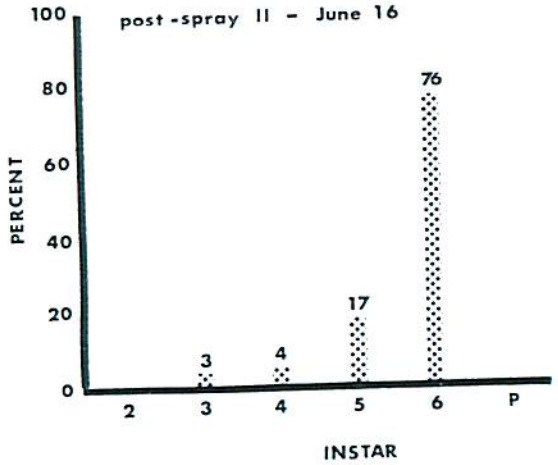


FIG. E-9 Spruce Budworm development on key dates on Balsam Fir - Untreated check A







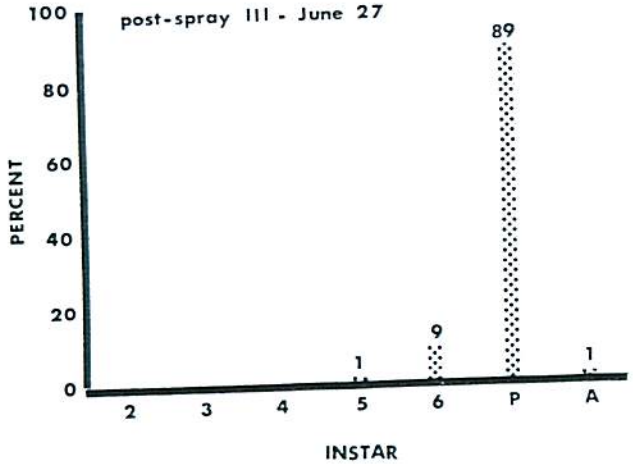


FIG. E-10 Spruce Budworm development on key dates on Balsam Fir - Untreated check B

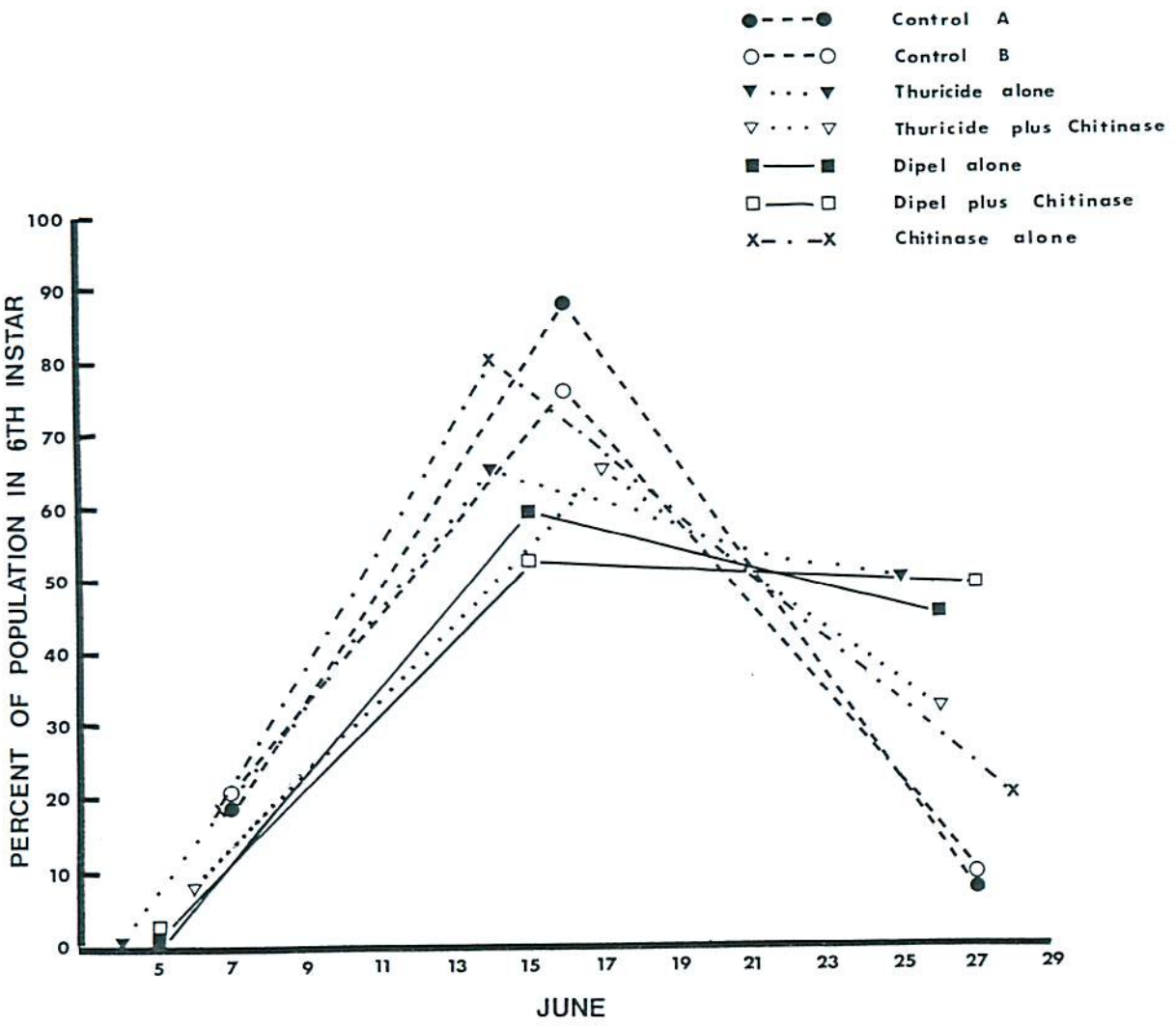


FIG. E-11 Effects of treatments on larval development

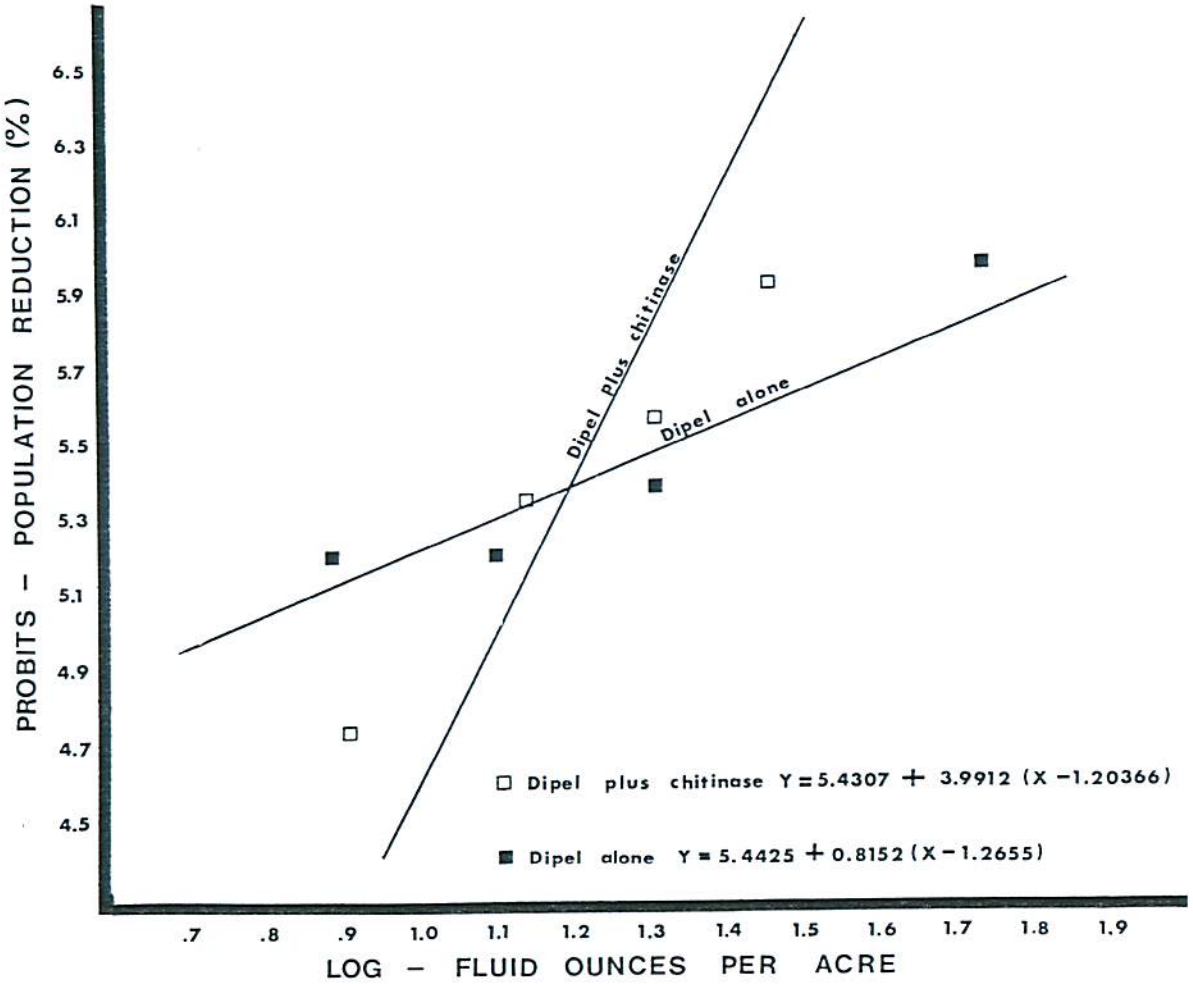


FIG. E-12 Relation of deposits of Bacillus thuringiensis (Dipel) to population reduction - 5 days post spray

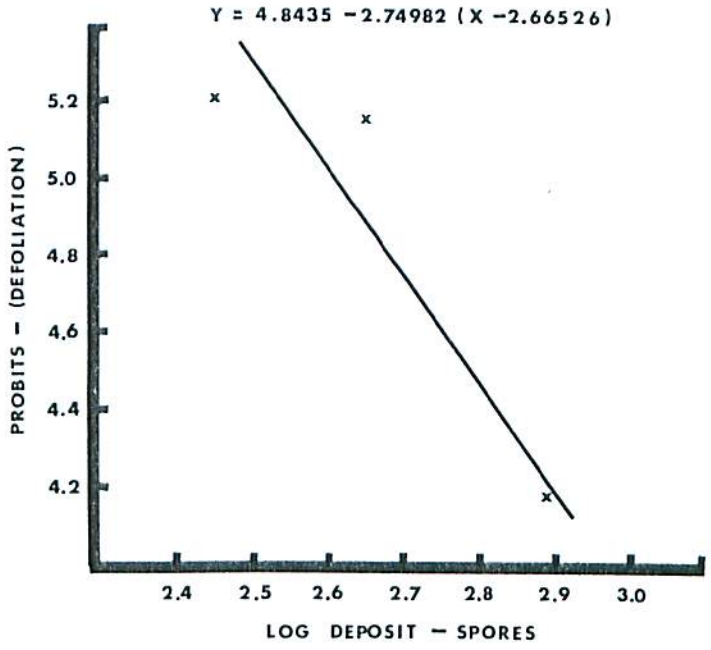


FIG. E-13 Regression analysis of spore deposit of Bacillus Thuringiensis plus Chitinase in relation to defoliation of balsam fir trees

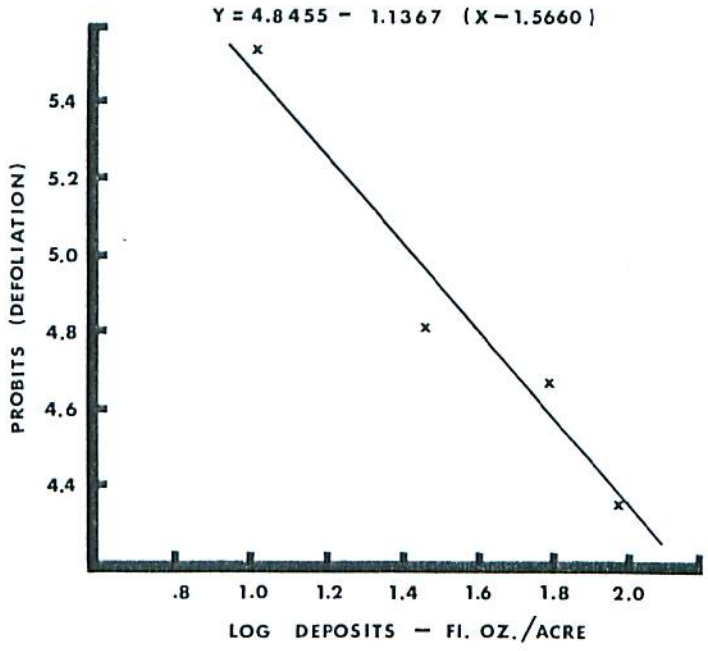


FIG. E-14 Regression analysis of fluid deposit of Bacillus Thuringiensis plus Chitinase in relation to defoliation of balsam fir trees

Evaluation of Commercial Preparations of *Bacillus thuringiensis* with and without Chitinase Against Spruce Budworm

F. Impact of Aerial Treatment on Non-Target Organisms, Algonquin Park, Ontario and Spruce Woods, Manitoba

by C.H. Buckner, P.D. Kingsbury, B.B. Mcleod, K.L. Mortensen and D.G.H. Ray

Chemical Control Research Institute
Canadian Forestry Service
Ottawa, Ontario

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F. Impact of Aerial Treatment on Non-Target Organisms, Algonquin Park, Ontario and Spruce Woods, Manitoba

by

C.H. Buckner, P.D. Kingsbury, B.B. McLeod,
K.L. Mortensen and D.G.H. Ray

INTRODUCTION

Impact studies upon selected components of the environment were carried out on all treatment plots and suitable untreated check plots in Algonquin Park, Ontario and Spruce Woods, Manitoba treated with various formulations containing the bacterial insecticide Bacillus thuringiensis Berliner (B.t.). These experimental treatments were conducted to determine the suitability of B.t. as a biological control agent of spruce budworm, Choristoneura fumiferana (Clem.), the most destructive defoliator of Canadian forests. The commercial preparations of B.t. applied were Thuricide 16B® (International Minerals and Chemicals Corp., Libertyville, Illinois) and Dipel WP® (Abbott Laboratories, North Chicago, Illinois). Both of these products were applied with and without the chitin hydrolysing enzyme chitinase. Formulations applied and methods of treatment are described in Sections C (Spruce Woods, Manitoba) and E (Algonquin Park, Ontario) of this report.

Studies of environmental effects of B.t. on non-target insects have consisted primarily of toxicological studies in laboratories. These

studies have established the non-pathogenicity of B.t. for vertebrates which ingest, inhale or come in contact with the bacteria (Laird, 1973). The thermostable exotoxin produced by some strains of B.t. has been shown to exhibit oral toxicity at high doses in mice, and toxicity to a variety of non-lepidopterous insects including honeybees, however, this exotoxin is not present in the commercial preparations, Thuricide and Dipel. B.t. infects non-target Lepidoptera and may reduce their populations. Very little is known about the fate of B.t. formulations in aquatic ecosystems but monitoring on Moresby Island, British Columbia, in 1960 revealed no adverse effects on coho salmon fry or aquatic insects in streams within experimental B.t. treatment areas (Todd and Jackson, 1961).

The studies described within this report were conducted to determine if any undesirable side-effects on non-target organisms occur when forests are treated with B.t. to control spruce budworm. Studies such as these are essential before B.t. can be registered for operational use against spruce budworm in Canada. The number of components of the forest ecosystem monitored for side-effects was limited by the availability of manpower and suitability of the treatment plots chosen by the entomologists for each type of impact study. Bird and small mammal populations were monitored on all treatment plots in both Algonquin Park and Spruce Woods. Impact studies on other environmental components were limited to treatment plots in Algonquin Park. Domestic honeybees, non-target insects and parasites of spruce budworm were monitored on all Algonquin Park treatment plots. Studies of aquatic fauna were limited to the 2,500 acre Thuricide treatment plot in Algonquin Park as it was

the only treatment area containing an aquatic system suitable for a monitoring program.

METHODS AND MATERIALS

Plot Description - Algonquin Park, Ontario

All the Algonquin Park plots were established in the typical fir-spruce (Abies balsamera L., Picea glauca (Moench) Voss) stands common to the area. Scattered hardwoods were present in all plots. The understory was fairly sparse and the ground cover light. The forest floor was covered with a thin duff layer overlying gravel deposits except where precambrian outcrops emerged.

The treated portion of the Opeongo River consisted of lengthy riffle areas one to three feet deep separated by slower flowing stretches up to ten feet in depth. The river bed consisted of boulders and rocks covering gravel and coarse sand. Sandbars which had been deposited on the inside of some curves in the river and in deeper, slower flowing portions the sand bottom were covered with soft organic debris. The river opening varied from about 30 - 60 feet and was rarely wider than the river itself as dense growth of alders, Alnus spp. and willows, Salix spp. crowded right to the river's edge. The control stream, Costello Creek, was shallow and slow flowing with only one riffle area comparable to the riffle areas of the Opeongo River, (Fig. F-1).

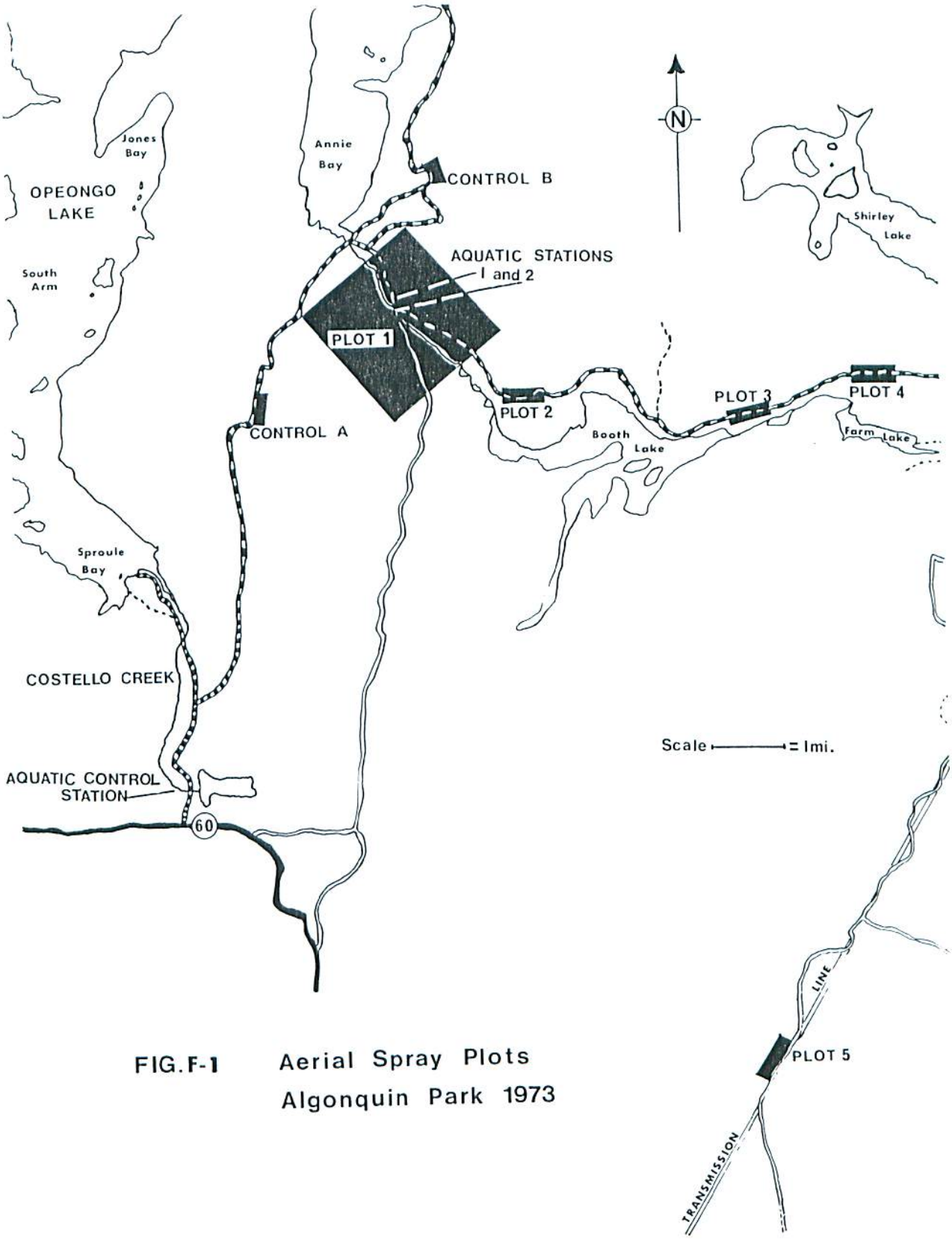

FIG. F-1 Aerial Spray Plots
Algonquin Park 1973

Plot Description - Spruce Woods, Manitoba

Plots in the Spruce Woods area contained mixed habitats including mature spruce stands, poplar (Populus spp.) bluffs, areas of dense brush and open fields. White spruce predominated the treed areas with pockets of poplar, burr oak Quercus macrocarpa Michx. and brush in moist areas. Soils varied from rich leaf and needle litter in heavily forested areas to sandy loam in open areas.

Birds

Breeding bird populations on all Algonquin Park and Spruce Woods treated and untreated check plots were measured using methods and techniques similar to those described by Buckner and Turnock (1965). Twenty acre plots were censused before and after treatment by counting all singing and sighted birds along predetermined parallel lines and recording this information on plot maps. The resulting bird populations were expressed as numbers of birds per 100 acres. Special attention was paid to groups of birds inhabiting certain ecological areas such as upper crown foragers, species inhabiting shrubbery and mid-crown areas of the forest, or others which prefer the forest floor or lower crown habitat.

In Algonquin Park, daily monitoring of populations commenced on the untreated checks, Thuricide alone and Dipel + chitinase plots on May 11-12, on the chitinase-alone plot on May 17 and on the Dipel-alone and Thuricide + chitinase plots on May 20. The pre-spray census terminated approximately one week prior to treatment. The various plots were treated between the evening of May 29 and evening of May 31. The post-spray monitoring commenced immediately after treatment and continued for an additional eight or nine days.

Breeding bird populations on Spruce Woods plots were monitored prior to treatment and at weekly intervals for three weeks after treatment. No census was obtained from the lower dosage treatment plot the week after treatment or from the untreated check plot two weeks after treatment.

Scientific and common names of all birds mentioned in this report are listed in the Appendix.

Small Mammals

Small mammal populations on all Algonquin Park and Spruce Woods treated and untreated check plots were censused approximately 6 weeks after treatment by use of snap-back traps positioned on standard 90 x 4 yard trap lines located in each plot. A standard three consecutive night trap period was used (150 trap nights per line). All small mammal specimens taken were subsequently identified, sexed, aged and dissected to determine breeding condition. The lapse of a six week interval between treatment and trapping ensures that any litter being carried by females at the time of application have time to leave the nest and become available for trapping. The absence of any particular age group from the sample could reflect an impact of the pesticide application upon that portion of the small mammal population.

Domestic Honeybees

Seven colonies of domestic honeybees, Apis mellifera L., were placed on each of the five Algonquin Park treatment plots and on an untreated check plot. The bees were newly purchased packages containing 3 pounds of bees (approximately 12,000 bees) and a mated

queen. All colonies were set up and maintained at the headquarters bee yard near Ottawa until about 4 days prior to treatment. Upon arrival at the treated and untreated check plots, each colony was fitted with a pollen collecting trap attached to the base of the colony. Pollen brought into the colony by foraging bees was collected, bagged, labelled daily and returned to the laboratory for weighing. Daily counts of adult honey bee mortality were recorded by collecting the dead bees which had been removed from the colony and deposited in the dead bee trap at the hive entrance. Relative activity of each hive was measured by an electronic counter placed at the hive entrance to measure the activity of the bees entering and leaving the colony. Hive weights were taken three times during the program period. Each colony was weighed on May 29 (just prior to treatment), on June 7 (just prior to their removal from the treatment area) and again on June 15 (after their return to the headquarters bee yard).

Young brood was observed in each colony throughout the monitoring period to assess the impact of the insecticide upon that portion of the hive.

The bee yard in the Thuricide treatment plot was located in an open area in the centre of a 2 mile square treatment area. This larger plot was employed to assess the impact upon honeybee colonies when the entire normal foraging area is treated with B. thuringiensis.

Non-Target Insects

Non-target insects were sampled from several ecological habitats on all Algonquin Park treatment plots and an untreated check plot. Forest floor fauna was sampled by pit-fall (smooth-walled one

quart oil-can) traps consisting of quart oil cans dug into the forest floor at two chain intervals along a 20 chain line.

Random samples of non-target insects from ground flora were made by use of a sweep net in similar habitat in each of the plots. All specimens collected were preserved immediately and later identified as to Order and Family. Non-target insects were also collected from foliage of three tree species common to all plots. Two 18-inch branches were cut and the insect material was removed carefully, preserved, and later identified to Order.

Collections of this type were made from trembling aspen, Populus tremuloides, alder, Alnus rugosa, and red maple, Acer rubrum.

Pitfall trapping was conducted over six-day periods before treatment, six days after treatment and thirty days after treatment. Foliage samples and sweep net collections were obtained at the same times except that no pre-treatment sweep net collections were made.

Parasites of Spruce Budworm

The incidence of parasitism amongst spruce budworm collected from all treated and an untreated check plots in Algonquin Park was determined from larval collections. Samples of spruce budworm were brought back to the laboratory and were reared to determine the incidence of parasites. Three separate collections were made: pre-treatment (2nd to 3rd-instar larvae), 14 days post treatment (5th to 6th-instar larvae) and pupal collections. All larvae were reared on clean fir or spruce foliage and the numbers of emerged parasites recorded. No 14 day post treatment (5th and 6th instar larvae) collections were obtained from Dipel and Dipel + chitinase plots because of a scarcity of larvae on these plots at this time.

Aquatic Fauna

Bottom fauna populations at two stations in the Opeongo River within the Thuricide-alone treatment plot and at an untreated check station in Costello Creek were monitored from two weeks before to four weeks after treatment by taking periodic groups of foot-square Surber samples (Surber, 1936). Station 1 in the Opeongo River was situated on a shallow sandbar bordering a long riffle area and was several hundred yards upstream from Station 2 which was located in the centre of the treatment plot on a rock-strewn sand and gravel bottom at the foot of a very fast riffle. The untreated check station in Costello Creek was situated on a shallow bottom of coarse gravel and sand at the foot of a small rapids immediately downstream from where the creek emerges from Costello Lake. Organisms were hand picked from samples while live and were preserved and later identified to Order. Bottom fauna populations at Stations 1 and 2 in the Opeongo River were also sampled by collecting rocks from the riverbed and removing all the organisms present on them. A Surber sampler was set in the current at Station 2 to sample drifting organisms before, during and after treatment.

Aquatic insect and fish populations were also monitored by direct observation by divers using scuba equipment. Three days and one month after treatment estimates of fish populations in a half-mile section of river were made by divers floating downstream with the current making visual counts. Divers also collected water, clams and crayfish for examination for the presence of B.t. by microbiological techniques. B.t spores per ml. in water samples were determined by

the plate-dilution frequency technique described by Harris and Sommers (1968). Positive identification of B.t. was made by staining smeared cultures and examining them for the characteristic B.t. crystal. Clams and crayfish collected from an untreated area were set out in buckets of water on the Thuricide + chitinase and chitinase-alone plots to be exposed to the treatments applied there. Mortality in these buckets was compared to mortality amongst untreated check groups and groups collected from the Opeongo River after it was treated.

RESULTS AND DISCUSSION

Birds - Algonquin Park, Ontario

Resident song bird populations were well established in breeding and foraging territories on all Algonquin Park plots by mid-May. Bird populations on all plots throughout the monitoring period are presented in Tables F-I to F-VI.

The small forest song bird complex was fairly evenly distributed over the project area except that fewer numbers of species were recorded on the Dipel-alone plot and larger numbers of species were recorded on the Thuricide-alone plot (Table F-VII).

Population fluctuations on the treatment plots paralleled those recorded on the control plot with few exceptions.

Several species of warblers and sparrows were observed to vary somewhat from the norm in several of the treatment plots. Black and white warbler populations varied in the chitinase-only plot; the chestnut-sided warbler in the Dipel-alone plot; the Magnolia, Nashville and Mourning Warblers in the Thuricide + chitinase plot, the Ovenbird

in the Thuricide + chitinase and Dipel-alone plots; and the white-throated sparrow in the Thuricide-alone, Thuricide + chitinase, Dipel + chitinase and chitinase-alone plots. An analysis of variance for these species (Table F-VIII) indicated that the fluctuations were not significant relative to the treatments.

TABLE F-I

Small Songbird Populations, Untreated Check Plot

Expressed As Birds Per 100 Acres
Algonquin Provincial Park, Ontario

1973

Family	Species	PRE SPRAY DAY												Ave. No. of birds per day	POST SPRAY DAY									Ave. No. of birds per day
		May 11	May 12	May 13	May 14	May 15	May 16	May 17	May 18	May 19	May 20	May 21	May 22		May 30	May 31	Jun 1	Jun 2	Jun 3	Jun 4	Jun 5	Jun 7	Jun 8	
Tetraonidae	Ruffed Grouse	7	9	6	15	9	24	6	6	6	12	0	6	9	6	0	6	6	0	0	0	6	0	3
Scolopacidae	American Woodcock	4	1	0	0	0	0	0	0	6	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Picidae	Hairy Woodpecker	0	1	0	0	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Yellow-bellied Sapsucker	0	1	0	0	0	6	0	3	0	0	0	0	1	0	6	6	6	0	0	0	0	0	2
	Yellow-shafted Flicker	2	7	10	3	12	24	3	2	6	6	0	0	6	0	0	6	0	0	6	0	0	0	1
Tyrannidae	Eastern Phoebe	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Corvidae	Blue Jay	2	1	2	0	3	3	0	6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Common Raven	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Paridae	Black-capped Chickadee	0	4	3	3	0	0	3	0	0	0	0	0	1	0	0	0	0	0	6	0	0	0	1
Sittidae	White-breasted Nuthatch	0	3	4	6	3	18	6	2	6	6	6	18	7	0	0	6	0	0	0	0	0	0	1
Troglodytidae	Winter Wren	0	0	4	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Turdidae	American Robin	11	10	4	4	9	12	9	6	0	0	3	0	6	12	0	3	3	0	6	6	6	0	4
	Wood Thrush	2	1	16	9	0	0	0	2	0	0	0	0	3	0	0	6	12	6	12	0	6	6	5
	Veery	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6	0	6	18	18	5
Sylviidae	Ruby-crowned Kinglet	9	3	0	0	9	0	6	3	6	6	6	0	4	0	0	0	0	0	0	0	0	0	0
Parulidae	Blackburnian Warbler	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	1
	Black and White Warbler	3	1	0	0	12	12	9	18	0	12	12	6	7	30	24	24	18	24	18	18	30	24	21
	Black-throated Green Warbler	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	6	6	12	6	0	6	0	4
	Chestnut-sided Warbler	9	6	0	15	15	24	21	23	24	72	48	54	26	66	30	30	30	36	63	12	12	6	29

TABLE F-1 (Cont'd)

Small Songbird Populations, Untreated Check Plot
Expressed as Birds Per 100 Acres
Algonquin Provincial Park, Ontario
1973

Family	Species	PRE SPRAY DAY May 11	May 12	May 13	May 14	May 15	May 16	May 17	May 18	May 19	May 20	May 21	May 22	Ave. No. of birds per day	POST SPRAY DAY May 30	May 31	Jun 1	Jun 2	Jun 3	Jun 4	Jun 5	Jun 7	Jun 8	Ave. No. of birds per day
Parulidae (Contd)	Magnolia Warbler	0	0	0	0	0	0	0	4	12	0	6	0	2	12	6	24	18	18	6	18	18	24	14
	Nashville Warbler	0	46	14	51	36	78	39	63	36	54	24	30	39	18	18	24	18	42	42	42	36	24	26
	Ovenbird	6	30	2	12	21	36	21	14	18	72	48	54	28	78	66	66	60	42	96	54	48	48	56
	Yellow Warbler	3	0	0	0	0	0	0	0	0	0	0	0	1	6	0	6	0	0	0	0	0	0	1
	Mourning Warbler	0	0	0	0	0	0	0	0	0	0	0	0	0	24	18	6	0	6	0	0	0	0	6
	Bay-breasted Warbler	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Icteridae	Common Grackle	0	0	0	3	4	0	0	0	0	0	0	0	1	6	0	0	0	0	0	0	0	0	1
	Red-winged Blackbird	2	10	1	3	10	0	0	0	0	0	0	0	2	6	0	0	0	0	0	0	0	0	1
Fringillidae	Chipping Sparrow	24	28	15	27	33	18	12	19	90	24	18	12	27	12	6	6	6	0	0	6	12	12	6
	White-throated Sparrow	22	13	14	15	27	33	21	12	6	30	24	21	20	36	30	36	60	24	36	48	30	36	37
	Evening Grosbeak	0	0	0	0	0	0	6	0	0	0	0	0	1	0	0	0	0	30	0	30	12	12	8
	Rose-breasted Grosbeak	0	0	0	0	0	0	0	0	6	0	0	0	1	0	12	6	42	6	18	0	6	0	9
	Purple Finch	0	4	0	6	9	0	0	0	0	0	0	0	2	0	0	0	3	0	0	0	0	0	1
	American Goldfinch	0	3	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Unidentified Species		6	6	0	3	9	9	6	0	3	0	0	0	4	0	0	0	0	0	0	0	0	0	0
	Totals	117	188	96	178	224	297	168	183	225	294	195	201	197	318	216	267	294	252	321	240	246	210	263

TABLE F-II

Small Songbird Population Census, Thuricide Treatment Plot

Expressed As Birds Per 100 Acres

Algonquin Park, Ontario

1973

Family	Species	PRE-SPRAY DAY May 11 -18	May 12 -17	May 13 -16	May 14 -15	May 15 -14	May 16 -13	May 17 -12	May 18 -11	May 19 -10	May 22 -7	Ave. No. of birds per day	POST-SPRAY DAY May 30 +0	May 31 +1	Jun 1 +2	Jun 2 +3	Jun 3 +4	Jun 4 +5	Jun 5 +6	Jun 7 +8	Jun 8 +9	Ave. No. of birds per day
Tetraonidae	Ruffed Grouse	4	0	3	0	2	0	3	6	0	6	2	0	0	0	0	0	0	0	0	0	0
Scolopacidae	American Woodcock	0	0	0	3	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Picidae	Hairy Woodpecker	4	0	0	1	0	6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Black-blacked Woodpecker	6	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Pileated Woodpecker	0	0	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Yellow-bellied Sapsucker	0	3	0	3	3	0	3	6	9	0	3	0	0	6	6	6	0	6	0	0	3
	Yellow-shafted Flicker	0	0	0	3	2	0	0	12	3	0	2	0	0	0	0	0	0	0	6	0	1
Tyrannidae	Eastern Phoebe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	1
	Olive-sided Flycatcher	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	12	0	0	2
Corvidae	Blue Jay	0	3	0	6	4	0	3	3	0	0	2	0	0	0	0	0	0	0	0	0	0
	Gray Jay	0	0	0	3	1	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Common Crow	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Common Raven	0	0	3	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Paridae	Black-capped chickadee	0	0	0	0	0	6	0	12	0	0	2	6	0	0	0	0	0	0	0	6	1
Sittidae	White-breasted Nuthatch	0	0	0	6	2	0	3	9	9	0	3	9	0	0	0	0	0	0	0	0	1
	Red-breasted Nuthatch	2	0	3	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Certhiidae	Brown Creeper	0	0	0	3	0	6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Troglodytidae	Winter Wren	14	6	9	15	2	12	0	12	3	6	8	6	6	6	6	12	12	12	12	12	9
Turdidae	American Robin	0	0	4	12	4	3	4	12	0	0	4	0	0	0	12	9	0	0	0	6	3
	Wood Thrush	0	0	0	6	0	12	3	0	3	0	2	18	18	0	0	12	24	18	12	6	12
	Veery	0	0	0	0	2	0	0	0	0	0	1	6	6	6	0	0	0	12	6	12	5
Sylviidae	Ruby-crowned Kinglet	7	7	3	9	10	12	6	6	0	0	6	6	0	6	6	6	12	12	6	6	7
	Golden-crowned Kinglet	0	0	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Vireonidae	Red-eyed vireo	0	0	0	6	2	12	0	0	3	0	2	0	0	0	0	0	0	0	0	0	0

TABLE F-II - (Cont'd)

Small Songbird Population Census, Thuricide Treatment Plot
Expressed As Birds Per 100 Acres

Algonquin Park, Ontario

1973

Family	Species	PRE-SPRAY DAY May 11 -18	May 12 -17	May 13 -16	May 14 -15	May 15 -14	May 16 -13	May 17 -12	May 18 -11	May 19 -10	May 22 -7	Ave. No. of birds per day	POST-SPRAY DAY May 30 +0	May 31 +1	Jun 1 +2	Jun 2 +3	Jun 3 +4	Jun 4 +5	Jun 5 +6	Jun 7 +8	Jun 8 +9	Ave. No. of birds per day
Parulidae	Blackburnian Warbler	12	3	3	6	1	18	0	6	3	0	5	0	0	0	0	6	6	12	6	12	5
	Black-throated Green Warbler	0	2	0	6	0	0	0	0	6	0	1	24	6	6	6	24	18	24	18	12	15
	Black-throated Blue Warbler	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	2
	Black & White Warbler	12	14	6	15	10	12	18	6	24	72	19	54	42	18	42	18	30	18	18	18	29
	Chestnut-sided Warbler	2	0	3	0	4	0	3	0	0	0	0	0	24	6	6	12	6	0	0	0	6
	Magnolia Warbler	0	0	0	0	0	0	0	0	3	0	1	9	18	18	24	12	48	24	12	12	20
	Mourning Warbler	0	0	0	0	0	0	9	0	0	6	2	0	0	0	0	0	0	0	0	0	0
	Myrtle Warbler	0	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0
	Nashville Warbler	12	14	21	24	10	48	15	24	36	18	22	24	6	6	24	30	30	36	18	18	21
	Ovenbird	10	2	6	18	16	36	21	30	36	51	23	45	60	12	30	60	60	54	66	36	47
	Yellow Warbler	0	0	3	0	0	12	0	0	0	0	2	18	0	6	0	0	0	0	0	0	2
Icteridae	Brown-headed Cowbird	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Common Grackle	0	3	3	0	0	6	0	0	3	0	2	0	0	6	0	0	0	0	0	0	1
	Red-winged Blackbird	0	0	3	0	2	0	0	0	0	0	1	0	0	6	0	0	0	0	0	0	1
	Rusty Blackbird	0	0	0	3	0	0	6	6	9	3	3	0	6	0	0	0	0	0	0	0	1
Fringillidae	Chipping Sparrow	4	13	12	0	2	12	0	0	0	0	4	0	0	0	0	0	0	0	0	6	1
	White-throated Sparrow	8	3	15	6	20	6	21	21	12	24	14	12	6	6	6	18	12	0	0	6	7
	Evening Grosbeak	0	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	6	24	0	0	3
	Rose-breasted Grosbeak	0	0	0	3	12	24	6	18	12	12	9	12	6	6	0	12	6	12	0	0	6
	American Goldfinch	7	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Purple Finch	0	0	0	3	0	0	0	0	12	0	2	0	0	0	0	0	0	6	0	0	1
Unidentified Species		16	6	9	18	9	0	9	12	7	0	9	65	0	9	6	12	6	0	0	0	11
	Total	120	81	112	186	122	246	133	201	199	198	160	314	204	123	174	255	276	282	186	186	222

TABLE F-III

Small Songbird Population Census, Dipel Treatment Plot

Expressed as Birds Per 100 Acres

Algonquin Park, Ontario

1973

Family	Species	PRE-SPRAY DAY May 20 -11	May 21 -10	May 22 -9	May 23 -8	Ave. No. of Birds per day	POST-SPRAY DAY Jun 1 +1	Jun 2 +2	Jun 3 +3	Jun 4 +4	Jun 5 +5	Jun 7 +7	Jun 8 +8	Ave. No. of Birds per day
Anatidae	Black Duck	6	0	0	0	1	0	0	0	0	0	0	0	0
Tetraonidae	Ruffed Grouse	0	0	0	0	0	0	0	6	0	0	0	6	2
Picidae	Yellow-shafted flicker	6	6	0	0	3	0	0	0	6	0	0	0	1
Paridae	Black-capped Chickadee	0	0	0	0	0	6	0	0	0	0	0	0	1
Sittidae	White-breasted Nuthatch	6	0	0	9	4	0	6	6	0	6	6	6	4
Troglodytidae	Winter Wren	18	6	12	12	12	12	6	6	12	18	6	12	10
Mimidae	Brown Thrasher	0	0	0	3	1	0	0	0	0	0	0	0	0
Turdidae	American Robin	15	0	0	1	4	0	0	0	0	0	0	0	0
	Wood Thrush	12	0	6	0	4	12	12	0	0	6	0	0	4
	Swainson's Thrush	0	0	0	0	0	6	0	0	6	0	18	24	8
Sylviidae	Ruby-crowned Kinglet	6	0	6	0	3	12	0	0	0	0	12	0	3
Vireonidae	Red-eyed Vireo	0	0	0	0	0	12	0	6	18	18	0	0	8
Parulidae	Black-throated Green Warbler	24	12	0	3	10	6	12	6	12	6	0	6	7
	Nashville Warbler	54	0	18	9	20	42	0	42	30	12	24	6	22
	Chestnut-sided Warbler	6	12	18	21	14	24	0	0	6	6	12	0	6
	Black and white Warbler	12	36	36	36	30	48	24	36	24	48	36	18	33
	Blackburnian Warbler	6	0	0	0	1	6	0	0	6	6	6	12	5
	Magnolia Warbler	18	0	0	9	7	6	0	0	12	6	12	12	7
	Mourning Warbler	0	12	6	0	4	0	0	0	0	0	0	0	0
	Ovenbird	78	48	42	54	55	30	30	30	18	12	30	18	24

TABLE F-III (Cont'd)

Small Songbird Population Census, Dipel Treatment Plot

Expressed As Birds Per 100 Acres

Algonquin Park, Ontario

1973

Family	Species	PRE-SPRAY DAY May 20 -11	May 21 -10	May 22 -9	May 23 -8	Ave. No. of Birds per day	POST-SPRAY DAY Jun 1 +1	Jun 2 +2	Jun 3 +3	Jun 4 +4	Jun 5 +5	Jun 7 +7	Jun 8 +8	Ave. No. of Birds per day
Fringillidae	Chipping Sparrow	18	0	0	3	5	6	6	0	6	0	6	0	4
	White-throated Sparrow	15	12	12	15	13	18	12	24	0	12	18	24	15
	Rose-breasted Grosbeak	6	0	0	3	2	0	0	6	0	6	6	0	2
	Evening Grosbeak	0	0	0	0	0	6	0	0	0	6	0	0	2
Unidentified Species		12	12	0	3	7	0	0	0	0	0	0	0	0
	Totals	318	156	156	181	202	252	108	168	156	168	192	144	170

TABLE F-IV

Small Songbird Population Census, Dipel Plus Chitinase Treatment Plot

Expressed As Birds Per 100 Acres
Algonquin Park, Ontario

1973

Family	Species	PRE-SPRAY DAY									Ave. No. of birds per day	POST SPRAY DAY							Ave. No. of birds per day
		May 12 -19	May 13 -18	May 14 -17	May 15 -16	May 16 -15	May 17 -14	May 18 -13	May 19 -12	May 20 -11		Jun 1 +1	Jun 2 +2	Jun 3 +3	Jun 4 +4	Jun 5 +5	Jun 7 +7	Jun 8 +8	
Ardeidae	American Bittern	0	0	0	6	6	0	0	0	0	1	0	0	0	6	0	0	0	1
Tetraonidae	Ruffed Grouse	3	0	0	0	0	0	0	0	0	1	0	0	0	0	6	0	0	1
Picidae	Yellow-shafted Flicker	0	0	12	0	0	3	6	3	0	3	0	6	6	0	0	0	0	2
Corvidae	Gray Jay	0	0	0	0	0	0	6	0	0	1	0	0	0	0	0	0	0	0
	Blue Jay	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0
	Common Crow	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0
Paridae	Black-capped Chickadee	0	0	6	0	0	0	3	0	0	1	0	0	0	0	0	3	0	1
Sittidae	Red-breasted Nuthatch	0	6	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	White-breasted Nuthatch	0	0	0	0	0	0	0	3	0	1	0	6	6	6	0	12	0	4
Certhiidae	Brown Creeper	0	0	0	0	6	0	0	0	0	1	0	6	6	6	0	12	0	4
Troglodytidae	Winter Wren	3	24	6	6	18	3	6	12	12	10	0	0	18	6	0	12	0	5
Turdidae	American Robin	10	12	18	6	0	0	21	3	0	8	0	0	0	0	0	0	0	0
	Wood Thrush	0	0	0	6	6	0	0	3	6	2	0	0	0	0	0	0	0	0
	Veery	0	0	0	0	0	0	0	0	0	0	18	6	18	12	0	6	0	9
Sylviidae	Ruby-crowned Kinglet	3	18	6	24	24	9	0	9	24	13	0	0	6	6	0	0	0	2
Bombycillidae	Cedar Waxwing	0	12	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Vireonidae	Red-eyed Vireo	0	0	0	0	0	0	6	0	0	1	0	6	6	6	12	6	0	5
Parulidae	Black and White Warbler	3	6	0	0	6	12	0	9	24	7	18	12	18	36	24	36	18	23
	Tennessee Warbler	0	6	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	Nashville Warbler	3	48	48	108	54	45	42	50	42	49	12	24	42	18	54	54	30	33

TABLE F-IV (Cont'd)

Small Songbird Population Census, Dipel Plus Chitinase Treatment Plot

Expressed As Birds Per 100 Acres

Algonquin Park, Ontario

1973

Family	Species	PRE-SPRAY DAY May 12 -19	May 13 -18	May 14 -17	May 15 -16	May 16 -15	May 17 -14	May 18 -13	May 19 -12	May 20 -11	Ave. No. of birds per day	POST SPRAY DAY Jun 1 +1	Jun 2 +2	Jun 3 +3	Jun 4 +4	Jun 5 +5	Jun 7 +7	Jun 8 +8	Ave.No. of birds per day
Parulidae (Cont'd)	Yellow Warbler	0	0	0	0	0	0	0	0	6	1	6	6	6	0	6	0	0	3
	Magnolia Warbler	0	0	0	0	0	0	0	0	18	2	0	0	0	0	6	6	6	3
	Blackburnian Warbler	0	0	0	0	0	0	0	0	0	0	0	0	12	6	6	18	0	6
	Chestnut-sided Warbler	0	0	0	0	0	0	0	0	0	0	6	12	0	6	0	6	0	4
	Ovenbird	3	0	6	12	12	3	6	27	30	11	12	0	24	18	12	18	24	15
	Yellowthroat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mourning Warbler	0	0	0	0	0	0	0	0	6	1	0	0	0	0	0	0	0	0
	Canada Warbler	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fringillidae	Rose-breasted Grosbeak	0	0	0	6	0	0	0	0	0	1	0	0	0	0	6	0	12	3
	Evening Grosbeak	0	0	0	0	0	0	0	5	0	1	0	0	6	12	0	0	6	3
	Purple Finch	0	12	0	0	24	0	12	3	0	6	0	0	0	0	0	0	0	0
	White-throated Sparrow	21	42	48	42	39	21	30	20	66	38	0	0	30	24	18	24	15	16
	Chipping Sparrow	3	9	12	0	6	12	0	18	12	8	6	12	6	12	6	0	0	6
	Totals	52	195	162	216	195	108	135	168	246	164	72	84	204	174	156	201	111	143

TABLE F-V

Small Songbird Population Census, Thuricide Plus Chitinase Treatment Plot
Expressed As Birds Per 100 Acres
Algonquin Park, Ontario

Family	Species	PRE-SPRAY DAY May 20 -10	May 21 -9	May 22 -8	May 23 -7	Ave. No. of Birds per day	POST-SPRAY DAY May 31 + 1	Jun 1 + 2	Jun 2 + 3	Jun 3 + 4	Jun 4 + 5	Jun 5 + 6	Jun 7 + 8	Ave. No. of birds per day
Tetraonidae	Ruffed Grouse	6	12	9	6	8	0	18	18	6	0	3	0	6
Scolopacidae	American Woodcock	12	0	5	18	9	6	12	15	6	6	18	9	10
Picidae	Yellow-shafted Flicker	0	6	6	0	3	0	0	0	0	0	0	0	0
	Yellow-bellied Sapsucker	0	0	6	0	1	6	0	9	0	0	0	0	2
Paridae	Black-capped Chickadee	0	0	6	0	1	0	0	0	0	0	0	0	0
Sittidae	White-breasted Nuthatch	0	12	0	18	7	6	0	0	0	6	0	0	2
Troglodytidae	Winter Wren	6	6	0	0	3	6	0	0	6	6	6	6	4
Turdidae	American Robin	15	12	0	24	12	3	6	12	9	30	9	0	10
	Wood Thrush	6	0	0	6	3	6	12	18	0	12	6	0	8
	Veery	0	0	0	0	0	6	6	6	6	6	18	6	8
Sylviidae	Ruby-crowned Kinglet	12	0	12	6	7	0	0	6	0	12	0	0	2
Vireonidae	Red-eyed Vireo	6	0	6	0	3	12	6	6	18	24	18	12	14
Parulidae	Black and White Warbler	24	42	24	66	39	54	60	30	12	30	30	30	35
	Nashville Warbler	30	30	18	6	21	36	48	30	60	48	36	21	39
	Yellow Warbler	0	12	0	0	3	6	6	0	0	0	0	6	2
	Magnolia Warbler	12	0	9	42	16	6	6	3	0	0	6	6	4

TABLE F-V (Cont'd)

Small Songbird Population Census, Thuricide Plus Chitinase Treatment Plot

Expressed As Birds Per 100 Acres

Algonquin Park, Ontario

Family	Species	PRE-SPRAY DAY May 20 -10	May 21 -9	May 22 -8	May 23 -7	Ave. No. of Birds per day	POST-SPRAY DAY May 31 + 1	Jun 1 + 2	Jun 2 + 3	Jun 3 + 4	Jun 4 + 5	Jun 5 + 6	Jun 7 + 8	Ave. No. of birds per day
Parulidae (Contd)	Myrtle Warbler	0	3	0	0	1	0	0	0	0	0	0	0	0
	Black-throated Green Warbler	6	0	6	0	3	0	0	0	0	0	0	0	0
	Blackburnian Warbler	0	0	0	0	0	0	0	6	3	24	24	18	10
	Chestnut-sided Warbler	18	24	48	6	24	72	66	60	42	24	54	36	36
	Ovenbird	42	36	45	24	37	24	24	18	12	6	12	12	15
	Mourning Warbler	0	24	18	36	19	12	0	0	0	0	0	0	2
	Yellowthroat	0	0	0	0	0	0	6	6	0	0	0	6	2
Icteridae	Common Grackle	0	0	0	0	0	0	0	9	15	0	6	0	4
	Rusty Blackbird	0	6	6	0	3	0	0	0	0	0	0	0	0
	Red-Winged Blackbird	0	0	3	0	1	0	0	0	0	0	0	0	0
Fringillidae	Rose-breasted Grosbeak	6	0	3	0	2	6	12	0	0	0	6	0	3
	Purple Finch	6	3	0	0	2	0	0	18	6	0	0	0	3
	Evening Grosbeak	0	0	3	21	6	0	6	6	6	9	12	18	8
	White-throated Sparrow	36	36	39	36	37	18	42	18	24	18	34	24	27
	Chipping Sparrow	0	6	24	12	10	0	21	15	6	18	0	6	9
	Song Sparrow	0	0	0	0	0	0	6	0	12	0	6	0	3
Unidentified Species		0	15	6	6	7	6	0	0	0	6	18	12	6
	Totals	243	285	296	333	289	285	363	309	249	285	322	222	290

TABLE F-VI

Small Songbird Population Census, Chitinase Only Treatment Plot

Expressed As Birds Per 100 Acres

Algonquin Park, Ontario

1973

Family	Species	PRE SPRAY DAY May 17 -14	May 18 -13	May 19 -12	May 20 -11	Ave. No. of birds per day	POST SPRAY DAY Jun 1 +1	Jun 2 +2	Jun 3 +3	Jun 4 +4	Jun 5 +5	Jun 7 +7	Jun 8 +8	Ave. No. of birds per day
Picidae	Hairy Woodpecker	0	6	0	0	2	0	0	0	0	0	0	0	0
	Yellow-bellied Sapsucker	0	0	0	0	0	0	0	6	0	12	6	0	3
	Yellow-shafted Flicker	12	6	0	0	4	0	0	6	0	0	0	0	1
Tyrannidae	Crested Flycatcher	0	0	0	0	0	6	0	0	0	0	0	0	1
Corvidae	Blue Jay	9	0	0	0	2	0	0	0	0	0	0	0	0
Paridae	Black-capped Chickadee	0	18	0	0	4	0	0	0	0	0	6	0	1
Sittidae	White-breasted Nuthatch	6	0	0	0	1	0	0	0	0	0	0	0	0
Troglodytidae	Winter Wren	24	6	30	18	19	24	6	0	6	6	6	12	9
Mimidae	Brown Thrasher	0	0	0	0	0	6	0	0	0	0	0	0	1
Turdidae	American Robin	6	0	0	0	2	6	0	6	6	6	0	6	4
	Wood Thrush	6	3	6	12	7	0	0	12	6	12	0	0	4
	Veery	0	0	0	0	0	6	6	6	6	6	18	32	11
Sylviidae	Ruby-crowned Kinglet	0	12	0	0	3	0	6	6	6	6	0	0	3
Vireonidae	Red-eyed Vireo	6	0	0	0	2	0	0	0	0	0	0	0	0
Parulidae	Blackburnian Warbler	0	0	0	0	0	0	0	0	0	6	18	6	4
	Black and White Warbler	36	66	6	66	43	12	18	18	12	6	12	6	12
	Black-throated Green Warbler	0	0	24	0	6	12	30	30	12	18	0	24	18
	Chestnut sided Warbler	0	0	0	0	0	36	24	24	12	18	12	6	19

TABLE F-VI (Cont'd)

Small Songbird Population Census, Chitinase Only Treatment Plot

Expressed as Birds Per 100 Acres

Algonquin Park, Ontario

1973

Family	Species	PRE SPRAY DAY				Ave. No. of birds per day	POST SPRAY DAY							Ave. No. of birds per day
		May 17 -14	May 18 -13	May 19 -12	May 20 -11		Jun 1 +1	Jun 2 +2	Jun 3 +3	Jun 4 +4	Jun 5 +5	Jun 7 +7	Jun 8 +8	
Parulidae (Contd)	Magnolia Warbler	0	0	0	6	1	18	8	6	12	18	30	12	15
	Nashville Warbler	48	66	30	42	46	12	24	24	30	18	12	24	21
	Yellow Warbler	0	6	6	0	3	0	6	6	0	0	0	12	3
	Yellowthroat	12	0	0	12	6	6	12	12	18	6	0	6	9
	Ovenbird	30	42	36	60	42	84	48	66	48	42	48	64	57
Icteridae	Common Grackle	3	0	0	0	1	0	0	0	0	0	0	0	0
Fringillidae	Chipping Sparrow	18	0	6	6	7	6	6	6	0	0	12	0	4
	White-throated Sparrow	30	12	12	27	20	6	12	12	18	6	6	0	9
	Song Sparrow	12	0	12	0	6	0	0	6	0	0	0	0	1
	Evening Grosbeak	0	0	0	0	0	0	12	0	18	18	48	18	16
	Rose-breasted Grosbeak	12	0	0	6	4	18	12	0	0	6	0	6	6
	Purple Finch	24	0	0	0	6	0	0	0	0	0	0	0	0
	American Goldfinch	3	0	0	0	1	0	0	0	0	0	0	0	0
Unidentified Species		3	0	0	0	1	0	0	6	0	0	0	0	1
	Totals	300	243	168	255	241	258	230	258	210	210	234	234	233

TABLE NO. F-VII

Number of Families and Species of Birds Recorded on Plots

Algonquin Park, Ontario, 1973

	No of Families	No. of Species
Untreated Check	13	33
Thuricide-alone	15	44
Dipel + chitinase	15	31
Dipel-alone	12	24
Thuricide + chitinase	13	32
Chitinase-alone	14	31

TABLE NO. F-VIII

Analysis of Variance of Seven Species of Birds that Apparently Diverged From the Norm in *Bacillus thuringiensis* Treated Areas, Algonquin Park, Ontario, 1973

Source of Variation	Sums of Squares	Degrees Freedom	Mean Squares	F Ratio
Total about mean	8778.00000	42.000		
Mean	3.42857	1.000		
Total about origin	8774.57031	41.000		
Due to treatments	1396.85693	5.000	279.37134	1.3632
Analytical error	7377.71094	36.000	204.93640	

Birds - Spruce Woods, Manitoba

Bird populations on the Spruce Woods plots throughout the monitoring period are presented in Tables F-IX and F-X.

Population fluctuations of breeding birds on the 4 gal/ac treatment plot paralleled those on the untreated check plot among most species over the pre-treatment and post-treatment census period. Populations of chipping sparrows, Vesper sparrows, Cowbirds, Cape May warblers, Orange-crowned warblers, Red-eyed Vireos, the two species of Chickadees, Blue Jay, and Mourning Dove all remained nearly constant. Similar results were obtained from the 2 gal/ac treatment plot except that data for the first week post spray was not collected.

Small Mammals - Algonquin Park, Ontario

A total of 53 specimens representing 6 species were trapped from the 6 plots. This represents a low population level in the park area. Twenty-one specimens were trapped on the untreated check plot, 4 on the Thuricide-alone plot, 14 from the Dipel-alone plot, 7 from the Thuricide + chitinase plot, and 6 from the chitinase-alone plot. Only one specimen was trapped on the Dipel + chitinase plot.

The woodland jumping mouse, Napaeozapus insignis (Miller) was the most abundant species trapped with a total of 27 specimens taken, followed by deer mouse, Peromyscus maniculatus (Wagner) with 12 specimens. Less abundant species taken were: the short-tailed shrew, Blarina brevicauda (Soy) (7 specimens), the common or masked shrew, Sorex cinereus Kerr (4 specimens), the red-backed vole, Clethrionomys gapperi (Vigors) (2 specimens), and the eastern chipmunk, Tamias striatus (Linnaeus) (1 specimen), see Tables F-XI to F-XV.

TABLE F-IX

Breeding Bird Populations, Dipel 4 gal/acre Plot

(Breeding Birds Per 100 Acres)

Sprucewoods Provincial Forest, Manitoba

1973

FAMILY	SPECIES	CONTROL				TREATMENT PLOT			
		Pre Spray	Week 1	Week 2	Week 3	Pre Spray	Week 1	Week 2	Week 3
Columbidae	Mourning Dove	10	5	-	0	0	5	5	5
Cuculidae	Black-billed Cuckoo	0	0	-	0	0	2	0	0
Picidae	Pileated Woodpecker	0	1	-	0	1	0	0	0
	Yellow-shafted Flicker	0	0	-	0	1	0	0	0
Tyrannidae	Eastern Kingbird	5	0	-	0	0	0	0	0
	Great-crested Flycatcher	0	0	-	0	5	0	0	5
	Least Flycatcher	0	0	-	0	10	0	0	0
Corvidae	Gray Jay	1	0	-	1	0	0	0	0
	Blue Jay	0	2	-	1	1	2	0	1
Paridae	Black-capped Chickadee	5	0	-	5	5	5	5	0
	Boreal Chickadee	5	0	-	5	0	0	0	5
Sittidae	Red-breasted Nuthatch	5	0	-	5	5	0	0	0
Troglodytidae	House Wren	5	0	-	0	0	0	0	0
Turdidae	Eastern Bluebird	0	0	-	0	0	0	5	0
Sylviidae	Golden-crowned Kinglet	0	0	-	0	5	0	5	0
	Ruby-crowned Kinglet	0	0	-	0	5	0	0	0
Bombycillidae	Cedar Waxwing	1	46	-	17	0	0	0	0
Vireonidae	Red-eyed Vireo	0	10	-	0	10	0	5	10

TABLE F-IY (Contd)

Breeding Bird Populations, Dipel 4 gal/acre Plot

(Breeding Birds Per 100 Acres)

Sprucewoods Provincial Forest, Manitoba

1973

FAMILY	SPECIES	CONTROL				TREATMENT PLOT			
		Pre Spray	Week 1	Week 2	Week 3	Pre Spray	Week 1	Week 2	Week 3
Parulidae	Orange-crowned Warbler	15	10	-	10	35	10	5	10
	Nashville Warbler	0	0	-	0	5	0	0	0
	Cape May Warbler	0	0	-	10	25	15	5	10
	Myrtle Warbler	0	5	-	0	0	0	5	15
	Ovenbird	5	5	-	0	0	0	0	0
Icteridae	Brewer's Blackbird	0	0	-	0	4	0	0	0
	Brown-headed Cowbird	4	10	-	4	5	0	0	5
Fringillidae	Purple Finch	0	0	-	0	5	0	0	0
	Pine Siskin	14	0	-	8	60	0	0	4
	American Goldfinch	0	0	-	2	7	0	0	0
	Red Crossbill	0	0	-	8	0	125	85	0
	Rufous-sided Towhee	5	0	-	0	0	0	0	0
	Vesper Sparrow	10	0	-	10	5	5	5	0
	Slate-coloured Junco	0	5	-	15	0	0	5	5
	Chipping Sparrow	60	45	-	35	60	55	45	90
	Clay-coloured Sparrow	5	0	-	5	0	0	0	5

TABLE F-X

Breeding Bird Population Census, Dipel 2 gal/acre Plot

(Breeding Pairs Per 100 Acres)

Sprucewood Provincial Forest, Manitoba
1973

FAMILY	SPECIES	CONTROL				TREATMENT PLOT			
		Pre Spray	Week 1	Week 2	Week 3	Pre Spray	Week 1	Week 2	Week 3
Columbidae	Mourning Dove	10	5	-	0	0	-	0	5
Picidae	Pileated Woodpecker	0	1	-	0	0	-	0	0
	Yellow-bellied Sapsucker	0	0	-	0	0	-	0	2
Tyrannidae	Eastern Kingbird	5	0	-	0	0	-	0	0
	Great-creasted Flycatcher	0	0	-	0	0	-	0	5
Corvidae	Gray Jay	1	0	-	1	2	-	10	5
	Blue Jay	0	2	-	1	4	-	0	2
Paridae	Black-capped Chickadee	5	0	-	5	0	-	0	5
	Boreal Chickadee	5	0	-	5	5	-	5	15
Sittidae	Red-breasted Nuthatch	5	0	-	5	10	-	5	15
Troglodytidae	House Wren	5	0	-	0	0	-	0	0
Sylviidae	Golden-crowned Kinglet	0	0	-	0	5	-	5	0
	Ruby-crowned Kinglet	0	0	-	0	10	-	5	10
Bombycillidae	Cedar Waxwing	1	46	-	17	0	-	5	0
Vireonidae	Red-eyed Vireo	0	10	-	0	10	-	0	0
Parulidae	Tennessee Warbler	0	0	-	0	0	-	0	5
	Orange crowned Warbler	15	10	-	10	10	-	0	0
	Cape-May Warbler	0	0	-	10	40	-	15	20
	Myrtle Warbler	0	5	-	0	25	-	5	20
	Ovenbird	5	5	-	0	5	-	0	0

TABLE F-X (CONT'D)

Breeding Bird Population Census, Dipel 2 gal/acre Plot

(Breeding Pairs Per 100 Acres)
Sprucewood Provincial Forest, Manitoba

1973

FAMILY	SPECIES	CONTROL Untreated Check Plot				TREATMENT PLOT			
		Pre Spray	Week 1	Week 2	Week 3	Pre Spray	Week 1	Week 2	Week 3
Icteridae	Brown-headed Cowbird	4	10	4		4	-	2	14
Fringillidae	Purple Finch	0	0	0		5	-	5	5
	Pine Siskin	14	0	8		11	-	30	8
	American Goldfinch	0	0	2		0	-	0	2
	Red Crossbill	0	0	8		75	-	0	42
	White-winged Crossbill	0	0	0		0	-	0	5
	Rufous-sided Towhee	5	0	0		0	-	0	0
	Vesper Sparrow	10	0	10		5	-	0	10
	Slate-colored Junco	0	5	15		0	-	10	20
	Chipping Sparrow	60	45	35		90	-	60	65
	Clay-colored Sparrow	5	0	5		0	-	0	5
	White-throated Sparrow	0	0	0		0	-	5	0

TABLE NO. F-XI

Small Mammal Population Census, Untreated Check Plot

Algonquin Park, Ontario, 1973

	MALES				FEMALES						
							Adults				
SPECIES	Juv.	Sub. A	Adult	Total	Juv.	Sub. A	Preg.	N. Preg.	With P. Scars	Total	Totals
Sorex cinereus	0	0	0	0	0	0	0	1	0	1	1
Blarina brevicauda	0	0	0	0	0	1	0	0	0	1	1
Peromyscus maniculatus	1	0	2	3	0	0	0	0	0	0	3
Napaeozapus insignis*	1	4	3	8	2	1	0	0	4	7	15
Totals	2	5	5	12	2	2	0	1	4	9	21

* One specimen of N. insignis trapped but was so badly mutilated by predators that sex and age could not be determined

Preg. - Pregnant

N. Preg. - Not Pregnant

With P. Scars - With Placental Scars

TABLE NO. F-XII

Small Mammal Populations Census, Thuricide Treatment Plot

Algonquin Park, Ontario, 1973

SPECIES	MALES				FEMALES						Totals
							Adults				
	Juv.	Sub. A	Adult	Total	Juv.	Sub. A	Preg.	N. Preg.	With P. Scars	Total	
Sorex cinereus	0	0	1	1	0	0	0	0	0	0	1
Peromyscus maniculatus	0	0	0	0	0	0	0	0	1	1	1
Napaeozapus insignis	0	0	0	0	1	0	0	0	1	2	2
Totals	0	0	1	1	1	0	0	0	2	3	4

TABLE NO. F-XIII

Small Mammal Population Census, Dipel Treatment Plot

Algonquin Park, Ontario, 1973

SPECIES	MALES				FEMALES						
							Adults				
	Juv.	Sub. A	Adult	Total	Juv.	Sub. A	Preg.	N. Preg.	With P. Scars	Total	Totals
Sorex cinereus	0	0	1	1	0	0	0	1	0	1	2
Peromyscus maniculatus	0	2	2	4	0	0	0	0	2	2	6
Napaeozapus insignis	0	0	2	2	0	0	0	0	3	3	5
Tamias striatus	0	0	0	0	0	1	0	0	0	1	1
Totals	0	2	5	7	0	1	0	1	5	7	14

TABLE NO. F-XIV

Small Mammal Population Census, Thuricide Plus Chitinase Treatment Plot

Algonquin Park, Ontario, 1973

SPECIES	MALES				FEMALES						Totals
							Adults				
	Juv.	Sub. A	Adult	Total	Juv.	Sub. A	Preg.	N. Preg.	With P. Scars	Total	
Peromyscus maniculatus	0	0	2	2	0	0	0	0	0	0	2
Blarina brevicauda	0	0	0	0	0	0	0	0	2	2	2
Napaeozapus insignis	0	1	0	1	1	0	0	0	1	2	3
Totals	0	1	2	3	1	0	0	0	3	4	7

TABLE NO. F-XV

Small Mammal Population Census, Chitinase Only Treatment Plot

Algonquin Park, Ontario, 1973

SPECIES	MALES				FEMALES						
							Adults				
	Juv.	Sub. A	Adult	Total	Juv.	Sub. A	Preg.	N. Preg.	With P. Scars	Total	Totals
Blarina brevicauda	0	0	0	0	0	3	0	0	0	3	3
Clethrionomys gapperi	0	0	1	1	0	0	0	0	1	1	2
Napaeozapus insignis	0	1	0	1	0	0	0	0	0	0	1
Totals	0	1	1	2	0	3	0	0	1	4	6

Individual animals representing the three main age groups (juvenile, sub-adult and adult) were trapped and approximately 80% of all adult female specimens contained placental scars indicating the recent birth of a litter.

This indicates that small mammal populations continued breeding throughout the treatment period.

An analysis of variance of small mammal populations on the treated and untreated check plots indicated no significant differences relative to treatment (Table F-XVI).

TABLE NO. F-XVI

Analysis of Variance of Small Mammal Populations

on Treated and Untreated Check Plots

in Algonquin Park

Source of Variance	Sums of Squares	Degrees Freedom	Mean Squares	R Ratio
Total about mean	338.00000	30.000		
Mean	83.33333	1.000		
Total about origin	254.66667	29.000		
Due to treatments	31.66667	4.000	7.91667	.8875
Analytical error	223.00000	25.000	8.92000	

Small Mammals - Spruce Woods, Manitoba

Small mammal populations were very low throughout the Spruce Woods area with only two specimens trapped from the 2 gal/ac treatment plot and 3 specimens from the 4 gal/ac treatment plot. No specimens were taken on the untreated check plot. In all, 4 specimens of P. maniculatus and a single specimen of the western chipmunk, Eutamias minimus (Backman) were trapped (Tables F-XVII and F-XVIII).

TABLE NO. F-XVII

Small Mammal Population Census, Dipel 4 gal/ac

Spruce Woods Provincial Forest, Manitoba, 1973

SPECIES	MALES				FEMALES						Totals
							Adults				
	Juv.	Sub. A	Adult	Total	Juv.	Sub. A	Preg.	N. Preg.	With P. Scars	Total	
Peromyscus maniculatus	0	0	2	2	0	0	0	0	1	1	3
Totals	0	0	2	2	0	0	0	0	1	1	3

TABLE NO. F-XVIII

Small Mammal Population Census, Dipel 2 gal/ac

Spruce Woods Provincial Forest, Manitoba, 1973

SPECIES	MALES				FEMALES						Totals
							Adults				
	Juv.	Sub. A	Adult	Total	Juv.	Sub. A	Preg.	N. Preg.	With P. Scars	Total	
Peromyscus maniculatus	0	0	1	1	0	0	0	0	0	0	1
Eutamias minimus	0	0	1	1	0	0	0	0	0	0	1
Totals	0	0	2	2	0	0	0	0	0	0	2

As this portion of the Spruce Woods Provincial Forest is poor habitat for many species of small mammals and since populations were generally depressed throughout the area, the data collected probably reflects the normal population fluctuation rather than the result of the treatment of the area with B.t. This conclusion is supported by a chi-square analysis of the data (Table F-XIX).

TABLE NO. F-XIX

Chi-Square Analysis of Spruce Woods Provincial Forest

Small Mammal Data

Species	Contribution to Chi-Square: Untreated Check Plot	Dipel at 2 gal/ac	Dipel at 4 gal/ac	X^2
P. maniculatus	.000	.150	.225	.375
E. minimus	.000	.600	.900	1.500
Total	.000	.750	1.125	1.875

Chi-Square = 1.875 with 2 degrees of Freedom

Domestic Honeybees

Adult honeybee flight activity recorded from colonies located on the treatment plots paralleled those taken from the untreated check plot. Flight activity did not decrease as a result of the treatments (Table F-XX) and increased considerably when removed to a more favourable habitat upon being returned to the headquarters bee yard.

TABLE NO. F-XX

Adult Flight Activity Counts (Average of Seven Colonies)

Algonquin Park, Ontario, 1973

Date	Untreated Check	Thuricide-Alone (Plot 1)	Dipel + Chitinase (Plot 2)	Dipel-Alone (Plot 3)	Thuricide + Chitinase (Plot 4)	Chitinase-Alone (Plot 5)
May 29	4200	5222	3584	6874	20288	3266
30	48333	*43430	45682	29862	*16294	50522
31	33856	14752	*40346	*23718	44378	*21594
June 1	40154	47618	36966	28198	38694	29709
2	22490	26445	14630	9088	13286	20685
3	30146	37210	38630	26112	39603	48987
4	21466	37850	47616	29312	49139	25600
5	33766	38989	60006	27520	89600	68096
6	29914	49114	53722	35942	63232	24192
		Colonies Transferred to Headquarters Bee Yard				
12	41344	24102	42458	83891	65920	81203
13	68608	85402	127488	72883	121946	123648
14	67686	90918	127168	73150	82150	79411
15	18112	19056	22362	16192	19878	20352
16			Rain			
17**	22528	14840	12800	8448	13568	17400
18	90982	68326	73536	56486	84634	95514
19	158822	46118	67904	56742	116506	73574
20	85094	156890	135920	94950	120806	102758

* Indicates treatment date

** Indicates two day cumulative total

Mortality of adult bees caused by their transfer from the headquarters bee yard to the treament area declined to a normal level by the time the first treatment was applied. There was no significant increase in mortality among foraging bees as a result of the application of the insecticides (Table F-XXI). Observations of young brood within the colonies revealed that it also was unaffected. Again some bees were destroyed as a result of the move (Junt 12) but mortality rate quickly returned to a normal low and no further significant mortality occurred.

The weight of pollen collected daily by foraging bees and gathered in pollen traps within each colony is presented in Table F-XXII.

Pollen collections from the treatment plots paralleled those from the control, but some variation was recorded for collections taken from the Thuricide treatment plot. An analysis of variance was applied to the data which indicated that the variations were insignificant (Table F-XXIII).

The colonies from each of the treatment plots gained weight comparable to the untreated check plot through the post spray period in Algonquin Park. This same situation continued when the colonies were relocated and the more rapid increase in weight reflects the more suitable foraging habitat offered by the headquarters bee yard as compared to a forest habitat (Table F-XXIV).

TABLE NO. F-XXI

Adult Honeybee Mortality (Average of Seven Colonies)

Algonquin Park, Ontario, 1973

Date	Untreated Check	Thuricide-Alone (Plot 1)	Dipel + Chitinase (Plot 2)	Dipel-Alone (Plot 3)	Thuricide + Chitinase (Plot 4)	Chitinase-Alone (Plot 5)
May 29	20.5	30.0	19.0	15.8	8.9	29.6
30	2.6	* 4.0	11.2	2.2	* 9.9	8.6
31	0.3	11.8	* 4.6	* 3.3	5.0	* 3.8
June 1	0.8	6.5	4.3	1.7	2.7	6.9
2	0.5	3.8	3.8	3.7	3.7	2.7
3	1.2	4.7	2.2	3.0	1.6	6.6
4	4.5	7.5	2.0	2.0	3.3	2.3
5	1.3	3.7	0.0	1.0	0.4	1.4
6	1.5	2.3	1.7	0.2	1.7	1.8
Total	33.2	74.3	48.8	32.9	37.2	63.7
		Transferred to Headquarters Bee Yard				
12	12.0	4.0	9.7	0.9	2.6	4.3
13	0.8	1.0	0.5	2.3	0.7	0.6
14	9.3	1.7	0.5	2.2	2.7	0.4
15	5.3	5.8	0.7	1.7	5.6	2.1
16			Rain			
(1) 18	5.8	11.2	5.8	3.3	7.7	6.3
19	7.2	7.3	3.8	3.7	4.1	4.4
20	1.8	3.0	1.8	1.5	2.0	6.6
Sub-Total	42.2	34.0	22.8	15.6	25.4	24.7

* Indicates treatment date

(1) Cumulative total (three day)

TABLE NO. F-XXII

Weight of Pollen Collected (grams) (Average of Seven Colonies)

Algonquin Park, Ontario, 1973

Date	Untreated Check	Thuricide-Alone (Plot 1)	Dipel + Chitinase (Plot 2)	Dipel-Alone (Plot 3)	Thuricide + Chitinase (Plot 4)	Chitinase-Alone (Plot 5)
May 30	52.9	* 60.6	52.6	59.6	* 17.2	30.7
31	80.9	7.3	* 34.8	* 39.1	54.6	* 33.0
June 1	70.1	26.7	29.8	24.4	51.7	33.6
2	12.0	16.3	15.5	6.7	9.4	22.2
3	46.2	9.1	18.6	11.3	32.2	38.3
4	26.5	26.3	42.6	10.9	20.5	11.0
5	63.3	18.1	30.8	19.8	57.6	72.5
6	36.2	37.3	38.1	17.7	30.8	19.2
Total	388.1	201.7	262.8	189.5	274.0	260.5
Transferred to Headquarters Bee Yard						
12	69.6	30.7	42.5	44.1	61.5	52.8
13	72.6	61.5	75.6	59.9	79.3	49.9
14		No Pollen Collected				
(1) 15	41.5	36.9	45.7	33.6	28.2	18.9
16			Rain			
(2) 18	81.8	109.0	98.5	82.5	94.3	65.0
19	62.3	69.1	70.7	56.9	64.4	46.5
20	100.0	96.6	103.1	84.6	98.5	70.1
Total	427.8	403.8	436.1	361.6	426.2	303.2

* Indicates treatment date

(1) Cumulative total

(2) Cumulative total

TABLE F-XXIII

T-Test for Significant Differences in Pollen Collection

Between Hives on Control and Treatment Plots

Plot	Mean weight	Variance	Degrees of freedom	T-Value
Untreated Check	48.5125	465.944		
Thuricide Alone	25.1375	258.765	14	-2.29731
Dipel + Chitinase	32.8500	129.260	14	-1.69855
Dipel Alone	23.6875	273.034	14	-2.41614
Thuricide + Chitinase	34.2500	297.155	14	-1.36601
Chitinase Alone	32.5625	297.942	14	-1.52685

TABLE NO. F-XXIV

Bee Hive Weights (pounds) (Average of Seven Colonies)

Algonquin Park, Ontario, 1973

Date	Untreated Check	Thuricide-Alone (Plot 1)	Dipel + Chitinase (Plot 2)	Dipel-Alone (Plot 3)	Thuricide + Chitinase (Plot 4)	Chitinase-Alone (Plot 5)
May 29 Pre-spray weight	46.8	44.7	48.2	47.0	46.8	45.4
June 7 1st. Post-spray weight (Algonquin Park)	49.2	48.2	52.0	49.4	49.2	47.8
Percent weight gain on treatment plot	4.9	7.8	7.4	5.1	5.1	5.3
June 15 2nd. Post-spray weight (H.Q. Bee Yard)	55.2	55.2	56.8	57.6	57.8	55.1
Percent weight gain on H.Q. Bee Yard	12.2	14.5	9.2	16.7	17.5	15.3
Overall post-treatment percent weight gain	17.9	23.4	17.4	22.6	23.5	21.4

Non-Target Insects

Results from the three series of pitfall collections made on each plot are presented in Table F-XXV. The great majority of the organisms collected were spiders (Araneida), ground beetles (Coleoptera: Carabidae) and ants (Hymenoptera: Formicidae). All of these groups declined in numbers to much the same extent on the untreated check plot as on the treated plots indicating that they were relatively unaffected by the treatments applied. Ground beetles on plots treated with Dipel only, and with B.t. and chitinase mixtures did decline to a greater extent than on the untreated check plot. Millipeds (Diplopoda) on all B.t. treated plots declined after treatment but none were found on the untreated check plot to compare these changes with.

Insects hand picked from foliage samples of trembling aspen, alder, and red maple were primarily lepidopterous larvae except for large numbers of ants and bugs (Hemiptera) found in aggregations on individual branches. Almost all insect groups were far more abundant on trembling aspen than on alder or red maple (Table F-XXVI) but by the last sampling period insects were scarce on all types of foliage on both untreated check and treated plots. Lepidoptera larvae on treatment plots showed little decline in the first week after treatment but dropped considerably by a month after treatment. A similar drop was seen in Lepidoptera on the untreated check plot so there is no evidence that B.t. affected non-target Lepidoptera to a significant extent.

Sweep net collections taken on all plots after treatment (Table F-XXVII) contained a wide range of insect groups but revealed no significant differences between populations on the untreated check and treated plots.

TABLE NO. F-XXV

Number of Forest Floor Organisms Collected in Pitfall Traps on Untreated Check and Treatment Plots, Algonquin Park, Ontario, 1973

Sampling Period	Untreated Check			Thuricide			Dipel + Chitinase			Dipel			Thuricide + Chitinase			Chitinase		
	Spray Day			Spray Day			Spray Day			Spray Day			Spray Day			Spray Day		
	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30
Diplopoda	0	0	0	5	2	0	6	5	0	12	3	0	5	3	0	1	0	2
Chilopoda	0	0	0	0	1	0	1	1	0	0	0	0	0	2	0	0	0	0
Arachnida	33	34	13	10	30	2	13	41	14	22	19	3	29	34	8	11	37	9
Gastropoda	0	10	3	0	0	0	0	1	0	0	1	1	0	3	1	0	0	0
Orthoptera	2	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2
Hemiptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Homoptera	0	0	0	0	0	5	0	0	0	0	0	0	0	0	2	0	0	0
Coleoptera	8	10	5	10	5	5	24	7	1	25	18	1	24	20	7	6	14	4
Lepidoptera	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Diptera	0	0	0	0	1	2	0	0	0	0	1	0	1	3	4	0	1	3
Hymenoptera	241	26	66	51	20	32	26	11	18	6	17	5	4	10	6	50	4	3
Total	284	81	92	76	59	46	70	66	33	66	59	10	63	75	28	70	56	24

TABLE NO. F-XXVI

Number of Insects Collected From Foliage of Three Tree Species on Untreated Check and Treatment Plots, Algonquin Park, Ontario, 1973

Sampling Period	Untreated Check Spray Day			Thuricide Spray Day			Dipel + Chitinase Spray Day			Dipel Spray Day			Thuricide + Chitinase Spray Day			Chitinase Spray Day		
	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30
Populus tremuloides																		
Lepidoptera	15	29	11	11	7	1	3	3	2	9	11	2	8	4	2	35	39	5
Hymenoptera	229	139	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0
Coleoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
Homoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemiptera	0	0	0	0	1	0	0	43	0	0	0	0	0	0	0	0	0	0
Alnus rugosa																		
Lepidoptera	1	0	0	0	5	3	10	5	1	3	0	1	2	4	1	2	1	0
Hymenoptera	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0
Coleoptera	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0
Homoptera	0	0	0	0	0	0	4	0	0	1	0	0	0	0	0	1	0	0
Hemiptera	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	5	0	0
Acer rubrum																		
Lepidoptera	0	2	0	4	1	1	4	3	0	2	0	0	2	1	0	4	2	0
Hymenoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Coleoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Homoptera	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Hemiptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE NO. F-XXVI (Cont'd)

Number of Insects Collected From Foliage of Three Tree Species on Untreated Check and Treatment Plots, Algonquin Park, Ontario, 1973

Sampling Period	Untreated Check			Thuricide			Dipel + Chitinase			Dipel			Thuricide + Chitinase			Chitinase		
	Spray		Day	Spray		Day	Spray		Day	Spray		Day	Spray		Day	Spray		Day
	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30	Pre	+6	+30
Total																		
Lepidoptera	16	31	11	15	13	5	17	11	3	14	11	3	12	9	3	41	42	5
Hymenoptera	229	139	0	0	0	0	0	0	0	0	0	13	0	0	0	0	37	0
Coleoptera	0	0	0	0	0	0	0	1	0	1	0	0	0	3	1	0	0	0
Homoptera	0	0	0	3	0	0	5	0	0	1	0	0	0	0	0	1	0	0
Hemiptera	0	0	0	1	1	1	1	43	1	0	0	0	0	0	0	5	0	0

TABLE NO. F-XXVII

Number of Insects Collected After Treatment by Sweep Net on Untreated Check and Treatment Plots, Algonquin Park, Ontario, 1973

Sampling Period	Untreated Check		Thuricide		Dipel + Chitinase		Dipel		Thuricide + Chitinase		Chitinase	
	Spray +6	Day +30	Spray +6	Day +30	Spray +6	Day +30	Spray +6	Day +30	Spray +6	Day +30	Spray +6	Day +30
Odonata	0	1	0	0	0	1	0	1	0	2	0	1
Orthoptera	1	0	1	0	1	0	1	0	1	0	0	1
Hemiptera	0	1	1	7	1	4	3	1	2	3	3	5
Homoptera	1	0	1	2	1	0	2	0	1	1	3	1
Coleoptera	2	1	2	8	0	1	2	0	11	3	17	6
Trichoptera	0	0	1	1	0	0	0	0	0	0	1	0
Lepidoptera	1	3	1	2	3	4	0	0	1	1	3	2
Diptera	5	0	7	8	6	2	6	0	3	2	9	11
Hymenoptera	3	1	0	3	4	0	0	3	0	1	7	4
Total	13	7	14	31	16	12	14	5	19	13	43	31

Parasites of Spruce Budworm

The incidence of parasites in spruce budworm larvae and pupae collected from the untreated check and treatment plots in Algonquin Park is presented in Table F-XXVIII. All the parasites which emerged from the budworm belonged to the Order Hymenoptera.

The incidence of parasites decreased steadily after treatment on the Thuricide plot but to a lesser extent than on the untreated check plot. The incidence of parasites on the Thuricide + chitinase plot showed an opposite trend with an increase from no parasitism amongst the sample of 2nd- and 3rd-instar larvae to a high incidence of parasites among pupae collected from this plot. Results from the Dipel and Dipel + chitinase plots are too incomplete to show a definite trend. The incidence of parasites among budworm on the chitinase-alone plot first increased and then decreased. Some of these changes may be due to the variation in sample size used or may result from changes in the budworm populations themselves. There is, however, no evidence that the treatments had significant effects upon this group of spruce budworm parasites.

TABLE NO. F-XXVIII

Incidence of Hymenopterous Parasites in Spruce Budworm Larvae and Pupae From Untreated Check and Treatment Plots, Algonquin Park, Ontario, 1973

Plot	Pre-Treatment (larvae)		14 Day Post-Treatment (larvae)		Post-Treatment Pupal Collection	
	No. Budworm	% Parasitized	No. Budworm	% Parasitized	No. Budworm	% Parasitized
Untreated Check	91	12.1	320	2.8	276	1.8
Thuricide	51	9.8	240	5.0	324	3.1
Dipel + Chitinase	88	8.0	No Samples		300	5.0
Dipel	174	2.3	No Samples		293	6.1
Thuricide + Chitinase	31	0.0	400	0.3	147	11.6
Chitinase	39	7.7	372	12.9	215	6.0

Aquatic Fauna

Pre-spray Surber samples showed significant differences between the benthic invertebrate populations at Stations 1 and 2 in the Opeongo River. The bottom fauna at Station 1 was twice as abundant in terms of the total number of organisms per square foot than at Station 2. Midge larvae (Diptera: Chironomidae) were the most abundant organisms, but there were also very dense populations of caddisfly larvae (Trichoptera) and mayfly nymphs (Ephemeroptera). Midge larvae were also the most abundant organisms at Station 2, but populations of other aquatic insects at this station were relatively low. The bottom fauna of the untreated check station and Station 2 were very similar when sampled two weeks before treatment.

Significant reductions in the bottom fauna populations of Station 2 and the untreated check station occurred before treatment began. Station 2 was located on a flat bottom and during the two-weeks period between the first pre-treatment series of bottom samples and the samples taken the day before treatment depth of water dropped from 18 in. to 6 in. and the direction and velocity of the current over the bottom was radically altered. These changes resulted in reduction of the midge larvae and mayfly nymph populations at this station to very low levels. During the same period before treatment began a freshet at the untreated check station caused by heavy rains caused a similar severe reduction in bottom fauna when a log lying across the creek bottom was undermined and much of the substrate held back by this log was swept downstream. Populations of aquatic insects at the untreated check station were greatly reduced when this occurred. As a result of these fluctuations in water

level bottom fauna populations at Station 2 and the untreated check station were at very low levels when treatment occurred. Station 1 was relatively unaffected by changes in water level as it was located on an elongated sandbar sloping gently from the bank of the river down to about four feet of water. Bottom organisms could escape the effects of decreasing water levels by moving along the sandbar to deeper water as the level of the river dropped.

There were no immediate effects of the bacterial spray on the bottom fauna populations at Station 1 or 2 (Tables F-XXX and F-XXXI). Over the next four weeks the total number of benthic organisms per square foot at Station 1 declined steadily but this was directly attributable to the emergence of adult caddisflies, mayflies and stoneflies. The mass emergence of mayflies and caddisflies during this period was evident from the swarms of adults seen over the Opeongo River. Swarms of mayflies were seen from the day before treatment to two weeks after treatment. Adult caddisflies appeared in large numbers shortly after treatment and were still present four weeks later. The large number of caddisfly larvae and pupae and mayfly nymphs found on rocks during this period (Table F-XXXII) also show that large numbers of these organisms were emerging at this time. Caddisfly larvae at Station 1 could be seen to have been moving from the sandbar onto rocks where they pupated before emerging as adults. Mayfly nymphs also moved onto rocks in large numbers subsequent to swimming from them to the surface and emerging as subimagos. Stonefly nymphs were not found in large numbers on rocks during their period of emergence as they crawled out at the river's edge and emerged as adults from dry land.

Bottom fauna populations at Station 2 slowly increased from their low pretreatment levels in the four weeks after treatment, but not to the same extent as at the untreated check station (Table F-XXIX). At both stations increases in the number of benthic organisms per square foot were due primarly to increases in midge larvae populations. Mayfly nymph populations also increased at the untreated check station, but the emergence of surviving mayflies, stoneflies and caddisflies at Station 2 kept the populations of these groups at levels close to or below pre-treatment levels.

Drift net samples taken at Station 2 before, during and after treatment showed that the number of larval aquatic insects caught per hour during treatment was from four to five times greater than before or after treatment (Table F-XXXIII). This appears to have been due to the short (half-hour) sampling periods using during treatment and the much longer (nine to thirty-eight hours) sampling periods used at other times. With the longer sampling periods, the net's efficiency was reduced as it became clogged with algae and debris. Also, when the net was left in place for long periods of time many organisms escaped from it as was seen by the large numbers of live mayflies and stoneflies found clinging to the outside of the net when it was recovered after long sampling periods. The number of adult mayflies and midges captured per hour in the drift net during treatment increased by from 70 to 110 times and from 20 to 360 times, respectively, over the pre-treatment averages. This is strong evidence that the spray products affected adult aquatic insects swarming over the river, but this knockdown was caused by physical interaction between the spray formulation and the swarming insects and wasn't the result of a toxic

TABLE NO. F-XXIX

Bottom Fauna Populations at the Untreated Check Station, Costello Creek, as Mean Numbers and Standard Deviations of Organisms/Sq. Ft.

Algonquin Park, Ontario, May 14 to June 28, 1973

Number of Days Before or After Treatment	-14	+2	+14	+28
Number of Samples	3	3	2	2
Trichoptera	39.7 ± 9.1	7.3 ± 5.3	7.0 ± 2.0	9.0 ± 4.0
Ephemeroptera	29.7 ± 16.1	3.0 ± 2.9	10.0 ± 5.0	45.0 ± 9.0
Plecoptera	31.0 ± 12.3	7.0 ± 7.9	3.0 ± 1.0	13.0 ± 6.0
Coleoptera	0.3 ± 0.5	0.7 ± 0.5	-	2.0 ± 1.0
Diptera	140.0 ± 52.3	26.7 ± 24.6	165.0 ± 22.0	233.5 ± 3.5
Turbellaria	0.3 ± 0.5	-	-	4.5 ± 2.5
Nematoda	11.0 ± 3.6	4.0 ± 0.8	5.0 ± 5.0	2.5 ± 2.5
Oligochaeta	4.0 ± 2.4	7.3 ± 4.0	5.0 ± 4.0	0.5 ± 0.5
Hirudinea	2.0 ± 1.4	-	19.5 ± 11.5	7.5 ± 3.5
Amphipoda	5.0 ± 6.4	0.3 ± 0.5	-	-
Hydracarina	1.3 ± 0.5	1.3 ± 1.2	-	-
Gastropoda	1.3 ± 0.9	0.3 ± 0.5	0.5 ± 0.5	-
Pelecypoda	3.3 ± 1.7	2.0 ± 2.2	2.5 ± 1.5	8.0 ± 5.0
Total	269.0 ± 58.2	60.0 ± 43.7	217.5 ± 24.5	325.5 ± 10.5

TABLE NO. F-XXX

Bottom Fauna Populations at Station 1, Opeongo River, Thuricide Treatment Plot

as Mean Numbers and Standard Deviations of Organisms/Sq. Ft.

Algonquin Park, Ontario, May 14 to June 28, 1973

Number of Days Before or After Treatment	-14	-1	+4	+14	+28
Number of Samples	3	2	2	2	2
Trichoptera	172.3 ± 85.5	153.5 ± 3.5	122.0 ± 100.0	122.0 ± 52.0	25.5 ± 8.5
Ephemeroptera	75.3 ± 47.9	61.0 ± 28.0	72.0 ± 53.0	28.5 ± 7.5	15.5 ± 1.5
Plecoptera	20.3 ± 3.8	21.5 ± 0.5	25.5 ± 12.5	12.0 ± 7.0	-
Odonata	6.3 ± 1.7	6.0 ± 1.0	2.0 ± 1.0	1.0 ± 0.0	2.5 ± 1.5
Coleoptera	5.7 ± 5.2	29.0 ± 1.0	15.0 ± 9.0	10.0 ± 5.0	5.5 ± 4.5
Diptera	270.3 ± 77.0	192.5 ± 65.5	242.0 ± 71.0	266.5 ± 84.5	188.0 ± 19.0
Turbellaria	6.3 ± 1.7	14.0 ± 6.0	4.5 ± 4.5	4.0 ± 4.0	0.5 ± 0.5
Nematoda	-	-	-	0.5 ± 0.5	1.0 ± 1.0
Oligochaeta	2.3 ± 0.9	1.5 ± 0.5	4.5 ± 4.5	2.0 ± 1.0	1.0 ± 1.0
Hirudinea	-	-	7.0 ± 7.0	1.0 ± 0.0	14.5 ± 14.5
Amphipoda	0.7 ± 0.5	-	-	-	-
Hydracarina	0.3 ± 0.5	0.5 ± 0.5	-	-	-
Gastropoda	0.3 ± 0.5	-	-	-	-
Pelecypoda	19.0 ± 7.9	23.5 ± 10.5	19.5 ± 3.5	32.0 ± 14.0	6.5 ± 1.5
Total	579.3 ± 213.4	503.0 ± 105.0	514.0 ± 259.0	432.5 ± 172.5	260.5 ± 40.5

TABLE NO. F-XXXI

Bottom Fauna Populations at Station 2, Opeongo River, Thuricide Treatment Plot

as Mean Numbers and Standard Deviations of Organisms/Sq. Ft.

Algonquin Park, Ontario, May 14 to June 28, 1973

Number of Days Before or After Treatment	-14	-1	+4	+14	+28
Number of Samples	3	2	2	2	2
Trichoptera	14.0 ± 6.6	17.0 ± 3.0	14.5 ± 5.5	20.0 ± 8.0	9.5 ± 5.5
Ephemeroptera	36.0 ± 24.9	6.0 ± 0.0	2.5 ± 2.5	9.0 ± 2.0	6.5 ± 3.5
Plecoptera	17.3 ± 7.5	18.0 ± 10.0	11.0 ± 5.0	16.5 ± 3.5	1.5 ± 1.5
Odonata	2.3 ± 1.2	0.5 ± 0.5	–	–	0.5 ± 0.5
Coleoptera	3.3 ± 4.0	3.0 ± 0.0	3.5 ± 3.5	8.0 ± 3.0	11.5 ± 11.5
Diptera	146.7 ± 119.2	7.5 ± 4.5	8.0 ± 6.0	20.5 ± 5.5	55.0 ± 7.0
Turbellaria	0.3 ± 0.5	–	–	–	–
Nematoda	1.7 ± 2.4	0.5 ± 0.5	0.5 ± 0.5	1.0 ± 1.0	1.0 ± 1.0
Oligochaeta	9.3 ± 11.8	0.5 ± 0.5	3.0 ± 3.0	–	14.5 ± 11.5
Hirudinea	8.3 ± 5.2	9.0 ± 6.0	7.5 ± 0.5	10.5 ± 4.5	9.5 ± 7.5
Amphipoda	0.7 ± 0.9	–	–	0.5 ± 0.5	–
Hydracarina	0.7 ± 0.9	0.5 ± 0.5	–	–	–
Gastropoda	2.3 ± 3.3	–	0.5 ± 0.5	–	1.0 ± 1.0
Pelecypoda	17.0 ± 4.5	25.5 ± 23.5	52.0 ± 19.0	35.0 ± 6.0	15.2 ± 2.5
Total	260.0 ± 147.7	88.0 ± 42.0	103.0 ± 46.0	121.0 ± 4.0	126.0 ± 5.0

TABLE NO. F-XXXII

Number of Aquatic Insects Picked From Rocks* at Two Stations In The Opeogo River, Thuricide Treatment Plot

Algonquin Park, Ontario, May 14 to June 12, 1973

	Station 1			Station 2		
Days Before or After Treatment	-14	-1	+14	-14	-1	+14
Trichoptera - Fam. Hydropsychidae	45	154	120	61	136	102
- % pupae	0.0%	1.3%	13.3%	41.0%	33.1%	19.6%
- Other families	8	5	7	11	28	22
Ephemeroptera	3	45	55	9	27	49
Plecoptera	-	3	1	2	5	1
Odonata	-	-	4	2	-	-
Coleoptera	-	-	-	2	3	-
Diptera	7	25	37	41	43	34
Total	63	232	224	128	242	208

* All organisms picked by hand in the field from two rocks 8" to 10" in diameter taken from one foot of fast flowing water near Surber sampling sites

TABLE NO. F-XXXIII

Number of Organisms/Hour Collected in a Drift Net at Station 2, Opeongo River, Before and After Treatment With Thuricide

Algonquin Park, Ontario, May 28 to June 2, 1973

Hours before (-) or after (+) treatment started sampling period begun*	-26½	-8½	+½	+1	+1½	+2	+40
Duration of sampling period (hrs.)	18	9	½	½	½	38	16
Trichoptera - larvae	0.9	1.8	12	16	16	0.6	1.2
Ephemeroptera - nymphs	3.5	2.7	8	8	12	2.8	3.5
Plecoptera - nymphs	0.8	0.4	-	2	2	0.6	1.3
Diptera - larvae & pupae	0.3	0.6	-	2	-	0.1	-
Total aquatic insect larvae, nymphs & pupae	5.6	5.6	20	28	30	4.0	6.1
Ephemeroptera - adults	0.3	0.1	22	14	16	0.03	0.6
Diptera - adults	0.7	0.4	10	12	200	0.4	0.8
Total - adult aquatic insects	1.1	0.6	32	26	216	0.4	1.5

* Duration of spraying period was about two hours

reaction. The effect of such a knockdown on populations of these insects would be negligible as they emerge over considerable periods in tremendous numbers and are probably more severely affected by heavy rains than by a non-toxic spray of very short duration. In addition, the knockdown was only partial as swarms of midges and mayflies were observed in the spray area immediately after treatment.

Observations made by scuba divers floating along a half mile stretch of the river revealed normal fish and aquatic invertebrate populations three days and one month after treatment. Twenty brook trout, Salvelinus fontinalis (Mitchill) and thirty common white suckers, Catostomus commersoni (Lacépède) were seen three days after treatment and twenty-one brook trout, fifteen common white suckers and twelve smallmouth bass, Micropterus dolomieui (Lacépède), were seen one month after treatment. On both occasions observations of normal abundance and behaviour were made upon sponge, hydra, planarian, crayfish, aquatic insect, clam and fish populations.

Culturing of water, clam and crayfish samples collected by divers revealed the presence of viable B.t. spores in river water and clams for a short period after treatment. A sample of river water from Station 2 taken thirty minutes after treatment began contained 1,730 B.t. spores per ml. but the bacteria had disappeared from the river water a month later. Clams collected from the same location two days after treatment contained viable B.t. spores, but no spores were found in clams collected a month later. A bucket of water exposed to treatment on Plot 4 contained 22,800 B.t. spores per ml. and heavy growth of the bacteria was cultured from clams which lived in this water for two weeks without suffering noticeable pathological effects. After being

refrigerated in darkness for two months 7,800 B.t. spores per ml. were cultured from this same water. No viable B.t. spores were found in crayfish collected from the Opeongo River or exposed to treatment in a bucket of water on Plot 4. There were no significant differences in the survival times of untreated check groups and groups of crayfish exposed in buckets to the treatments on Plots 4 and 5 or collected from the Opeongo River after Plot 1 was treated.

SUMMARY AND CONCLUSIONS

Birds

There were no significant reductions in bird populations apparent in areas treated with B.t. with and without chitinase. Fluctuations in the populations of some species of warblers and sparrows on Algonquin Park treatment plots were found not to be significant when subjected to statistical analysis. No significant consistent declines were found when similar species and families exposed to treatments of Dipel on Algonquin Park and Spruce Woods plots were compared.

Mammals

Small mammal populations were at low levels on both Algonquin Park and Spruce Woods plots. Evidence that small mammals continued breeding through the treatment periods and statistical analysis of trapping data indicate that under the conditions of application B.t. treatments with or without chitinase did not harm the small mammal complex inhabiting treatment areas.

Domestic Honeybees

There was no evidence from the data collected that any of the formulations of B.t. with or without chitinase applied in Algonquin Park affected domestic honeybee colonies, even when the entire foraging area of the colonies was treated.

Non-Target Insects

No dramatic reduction of non-target insects occurred on any of the Algonquin Park treatment areas. There was some suggestion of impact upon ground beetle, milliped and lepidopterous larvae populations on some treatment plots, but these groups also declined on the untreated check plot and in light of this population reductions on the treatment plots were not significant.

Parasites of Spruce Budworm

There was no evidence that B.t. treatments with or without chitinase significantly affected hymenopterous parasites of spruce budworm.

Aquatic Fauna

Fish and bottom fauna populations in a river exposed to aerial treatment with Thuricide suffered no adverse effects up to four weeks after treatment, but an insignificant knockdown of adult midges and to a lesser extent of adult mayflies occurred due to a physical interaction with the spray products. Viable B.t. spores were present in river water and clams immediately after treatment but had disappeared after four weeks.

A third of the B.t. present in a bucket exposed to an aerial application of Thuricide and chitinase remained viable after being kept refrigerated and in darkness for two months.

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APPENDIX

Scientific and Common Names of Small Forest Birds
Monitored in Algonquin Park, Ontario and
Spruce Woods Provincial Forest, Manitoba
1973

Family	Scientific Name	Common Name
Ardeidae	Botaurus lentiginosus (Rackett)	American Bittern
Anatidae	Anas rubripes Brewster	Black Duck
Tetraonidae	Bonasa unbellus (Linnaeus)	Ruffed Grouse
Scolopidae	Philohela minor (Gmelin)	American Woodcock
Columbidae	Zenaidura macroura (Linnaeus)	Mourning Dove
Cuculidae	Coccyzus erythropthalmus (Wilson)	Black-billed Cuckoo
Picidae	Colaptes auratus (Linnaeus)	Yellow-shafted Flicker
	Sphyrapicus varius (Linnaeus)	Yellow-bellied Sapsucker
	Dryocopus pileatus (Linnaeus)	Pileated Woodpecker
	Dendrocopus villosus (Linnaeus)	Hairy Woodpecker
	Picoides arcticus (Swainson)	Black-breasted three-toed Woodpecker
Tyrannidae	Myiarchus crinitus (Linnaeus)	Creasted Flycatcher
	Soyornis phoebe (Latham)	Eastern Phoebe
	Tyrannus tryannus (Linnaeus)	Eastern Kingbird
	Empidonax minimus (Baird & Baird)	Least Flycatcher
Corvidae	Perisoreus canadensis (Linnaeus)	Gray Jay
	Cyanocitta cristata (Linnaeus)	Blue Jay
	Corvus brachyrhynchos Brehm	Common Crow
	Corvus corax Linnaeus	Common Raven
Paridae	Parus atricapillus Linnaeus	Black-capped Chickadee
	Parus hudsonicus Forster	Boreal Chickadee
Sittidae	Sitta carolinensis Latham	White-breasted Nuthatch
	Sitta canadensis Linnaeus	Red-breasted Nuthatch
Certhiidae	Certhia familiaris Linnaeus	Brown Creeper
Troglodytidae	Troglodytes troglodytes (Linnaeus)	Winter Wren
	Troglodytes aedon Vieillot	House Wren

APPENDIX (Cont'd)

Family	Scientific Name	Common Name
Mimidae	Toxostoma rufum (Linnaeus)	Brown Thrasher
Turdidae	Turdus migratorius Linnaeus	American Robin
	Hylocichla mustelina (Gmelin)	Wood Thrush
	Hylocichla fuscescens (Stephens)	Veery
	Hylocichla ustulata (Nuttall)	Swainson's Thrush
	Sialia sialis (Linnaeus)	Eastern Bluebird
Sylviidae	Regulus calendula (Linnaeus)	Ruby-crowned Kinglet
	Regulus satrapa Licktenstein	Golden-crowned Kinglet
Bombycillidae	Bombycilla cedrorum Vieillot	Cedar Waxwing
Vireonidae	Vireo olivaceus (Linnaeus)	Red-eyed Vireo
Parulidae	Dendroica fusca (Muller)	Blackburnian Warbler
	Mniotilta varia (Linnaeus)	Black and White Warbler
	Dendroica virens (Gmelin)	Black-throated Green Warbler
	Dendroica pensylvanica (Linnaeus)	Chestnut-sided Warbler
	Dendroica magnolia (Wilson)	Magnolia Warbler
	Vermivora ruficapilla (Wilson)	Nashville Warbler
	Seiurus aurocapillus (Linnaeus)	Overbird
	Dendroica petechia (Linnaeus)	Yellow Warbler
	Oporornis philadelphia (Wilson)	Mourning Warbler
	Dendroica castanea (Wilson)	Bay-breasted Warbler
	Geothlypis trichas (Linnaeus)	Yellowthroat
	Dendroica caerulescens (Gmelin)	Black-throated Blue Warbler
	Vermivora celata (Say)	Orange-crowned Warbler
	Dendroica tigrina (Gmelin)	Cape May Warbler
	Vermivora peregrina (Wilson)	Tennessee Warbler
	Wilsonia canadensis (Linnaeus)	Canada Warbler
Icteridae	Quiscalus quiscula (Linnaeus)	Common Grackle
	Agelaius phoeniceus (Linnaeus)	Red-winged Blackbird
	Molothrus ater (Boddaert)	Brown-headed Co#bird
	Euphagus carolinus (Muller)	Rusty Blackbird
	Euphagus cyanocephalus (Wagler)	Brewer's Blackbird
Frangillidae	Spizella passerina (Bechstein)	Chipping Sparrow
	Zonotrichia albicollis (Gmelin)	White-throated Sparrow
	Hesperiphona vespertina (Cooper)	Evening Grosbeak
	Pheuctictus ludovicianus (Linnaeus)	Rose-breasted Grosbeak
	Carpodacus purpureus (Gmelin)	Purple Finch

APPENDIX (Cont'd)

Family	Scientific Name	Common Name
	Spinus tristis (Linnaeus)	American Goldfinch
	Spinus pinus (Wilson)	Pine Siskin
	Loxia curvirostra Linnaeus	Red Crossbill
	Pipilo erythrophthalmus (Linnaeus)	Rufous-sided Towhee
	Pooecetes gramineus (Gmelin)	Vesper Sparrow
	Junco hyemalis (Linnaeus)	Slate-coloured Junco
	Spizella pallida (Swainson)	Clay-coloured Sparrow
	Melospiza melodia (Wilson)	Song Sparrow
	Loxia leucoptera Gmelin	White-winged Crossbill

Evaluation of Commercial Preparations of *Bacillus thuringiensis* with and without Chitinase Against Spruce Budworm

G. Summary, Conclusions and Recommendations

by R.F. DeBoo and O.N. Morris

Chemical Control Research Institute
Canadian Forestry Service
Ottawa, Ontario

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G. Summary, Conclusions and Recommendations

by

R.F. DeBoo and O.N. Morris

The spruce budworm situation in Canada and border regions of the United States has proven to be one of the most difficult to manage from the viewpoint of sound silvicultural practice. With the rapid advent of multiple-use concepts in forestry, the budworm has become a major problem in the conservation of forest stands for both economic and aesthetic purposes. The implementation of protection programs, notably the aerial application of synthetic organic insecticides, still remains as the forester's major alternative to leaving important forests to depredation by the insect.

Forest protection policy in Canada varies from Province-to-Province, from year-to-year, and even in certain contiguous forest zones within a province during budworm attack years. Reasons for this variability are many, but decisions to implement spray programs are related basically to economics. Values attached to fibre-producing forests and to aesthetic areas such as parks may be sufficiently high to warrant consideration for spray treatment. In recent years, for example, fibre forests have been successfully treated with chemical sprays at costs of less than $1.00/acre. Practical alternatives to chemical insecticides are few, however, and even though costs of $2.00/acre or more might be economically justified, the use of chemicals for the protection of certain forest stands (e.g. national parks) is not permitted. One

acceptable approach to alleviating the budworm problem under such conditions at present is through the use of highly selective and non-hazardous insecticides. The bacterial insecticide Bacillus thuringiensis (B.t.) has shown the greatest potential to fill this requirement.

Research by staff of the Canadian Forestry Service during the past decade has indicated that B.t., particularly the new and more potent strains currently produced commercially, has good infectivity against spruce budworm larvae. Fieldwork by the Laurentian Forest Research Centre also has indicated that the addition of the enzyme chitinase to B.t. spray preparations results in accelerated septicemia of infected larvae and subsequently may provide a higher degree of foliage protection during the year of application. Also, mistblower and aerial trials by the Chemical Control Research Institute have shown that B.t. plus chemical insecticide combinations are potentially effective in protecting trees from excessive defoliation.

At the request of the Directorate of Program Coordination, Canadian Forestry Service, the Chemical Control Research Institute (C.C.R.I.) initiated an intensive study of B.t. and B.t. + chitinase sprays during 1973. The study was designed specifically to determine the suitability of B.t. for registered use against the spruce budworm in Canada utilizing to the greatest degree possible all expertise and facilities of the Institute. The core study for aerial spray applications was established within the boundaries of Algonquin Park, one of Ontario's largest park areas. Concurrent investigations were undertaken at C.C.R.I. (laboratory toxicological studies using spray towers) in Ottawa, at a registered tree farm plantation near Shawville, Quebec, utilizing a device for applying measured dosages of simulated aerial sprays to individual

trees, and at the Spruce Woods Provincial Park and Forest area of southern Manitoba where both aircraft and mistblower applications were evaluated. In addition, close liaison was maintained with other establishments conducting B.t. experiments during 1973 (Laurentian Forest Research Centre, Insect Pathology Research Institute, University of Maine) to compare results and experiences.

The results of the interdisciplinary studies by C.C.R.I. are summarized as follows:

Laboratory toxicological experiments using fifth-instar larvae and B.t. treated larch foliage indicated that, at those dosages evaluated, the potency of Dipel WP® (Abbott Laboratories) was greater than that of Thuricide 16B® (currently marketed by Sandoz-Wander). The toxicity of both products increased with volume (gal/ac) and deposit (drops/cm^2). The addition of chitinase increased the toxicity of Thuricide approximately 1.5 to 3 times, but did not appreciably change the efficacy of Dipel sprays. In summary, the results of the 1973 laboratory study indicated the following descending order of toxicity:

Dipel = Dipel + chitinase > Thuricide + chitinase > Thuricide

Field experiments utilizing a device for simulating aerial spray applications to young white spruce trees near Shawville, Quebec, suggested that neither commercial preparation induced significant reduction in larval population densities when examinations were made two weeks after treatment. Also, the addition of chitinase to the B.t. sprays did not appear to increase their activity within this same timespan. The B.t. treatments retarded larval development, however, and apparently

also reduced pupation. The addition of a small amount of fenitrothion (0.4%) to the B.t. sprays gave a mean population reduction as great as that obtained using 10 times that amount of fenitrothion applied alone, as well as a significant improvement of budworm control using B.t. alone. The ranges of droplet density (15-40 drops/cm^2) and size (110-150μ) were similar to those encountered with aerial spray deposits, and the device and technique for applying simulated aerial sprays were considered to have provided good application results.

Applications of Thuricide, Dipel and fenitrothion by mistblower to white spruces at the Spruce Woods Provincial Park, Manitoba, did not provide satisfactory protection of foliage due primarily to the very high spruce budworm population levels on the trees. Also, sprays were applied approximately one week late due to inclement weather conditions during the L_3 larval period. Highly significant population reduction (75%) was attributed to the Thuricide treatment, however. The failure of the Dipel treatment was attributed to rainfall (wash-off) which occurred immediately after application.

Aerial applications of Dipel (4 billion IU) at 2 and 4 gal/ac at the Spruce Woods Provincial Forest also did not provide acceptable levels of foliage protection on white spruce trees. Population reductions of 43% and 61% were attained, but these levels were inadequate in terms of the control requirement for the larval density situation during 1973. Many dead and sickly larvae were observed on trees in both treatment plots, and significantly fewer pupae were found than in the companion untreated area. Good protection of foliage (<20% defoliation) was obtained, however, where optimal spray deposit was attained and/or where larval density was low.

Results of the aerial applications at 4 billion IU in 0.5 gal/ac of Dipel and Thuricide with and without chitinase at Algonquin Park indicated that:

1. To ensure a good deposit of a B.t. formulation and attain good results, an emission system that will produce spray droplets of less than 100 microns in diameter should be used and the droplet numbers should be at least $50/cm^2$. The addition of an anti-evaporant (e.g. molasses) to the spray mixture is considered essential to ensure a good deposit under less than ideal conditions. The spray must be applied under stable weather conditions. The physical characteristics of B.t. are such that adequate mixing can only be achieved with a mechanical agitation system and a large capacity (approximately 50 gallons per minute) positive displacement pump is required to pump the material.

2. Using Brilliant Sulfo Flavine FFA (Chemical Developments of Canada Ltd., Toronto) as tracer dye, deposits on target trees varied from 18% (chitinase alone) to 81% (Thuricide + chitinase) of the amount emitted from the aircraft. Drop densities ranged from 16 - $98/cm^2$ and drop diameter sizes from 91 - 111 microns. Residual activity of B.t. was drastically reduced after 5 days of weathering. Dipel and Dipel + chitinase (but not Thuricide or Thuricide + chitinase) were highly effective (75% and 94%, respectively) in reducing very high spruce budworm population densities on balsam fir trees. Thuricide + chitinase treatment resulted in significant foliage protection on balsam fir trees with moderate population densities in the year of application.

B.t. treatments with and without chitinase retarded development of the budworm and reduced their pupal weights, oviposition rates and egg viability.

Populations of small mammals and birds at Algonquin Park and at the Spruce Woods were closely monitored before and after B.t. aerial spray application. At both locations, the sprays were found to have no impact on the densities and activities of the resident animals. Similarly, chitinase when applied with B.t. or alone, had no effect. At Algonquin Park, studies of domestic bees (established artificially in the spray areas), other non-target insects, and aquatic fauna, at Algonquin Park also indicated the absence of detrimental effects caused by B.t. or B.t. + chitinase airsprays.

The results of the 1973 study by C.C.R.I. on B.t. and the spray additive chitinase have indicated that successful treatment of spruce budworm populations is dependent upon four major variables:

1) Availability of chitinase at acceptable cost.
2) Spray concentration and deposit as affected by meteorological conditions.
3) Timing of spray application.
4) Budworm population density.

Since the addition of chitinase increased the potency of B.t. sprays in most experiments, its use should be considered where optimum protection is required. Chitinase, as available at present, however, is extremely expensive, and the cost prohibits its use as a spray adjuvant. Current efforts to produce an inexpensive preparation may soon improve this situation, however.

The C.C.R.I. studies in Quebec, Ontario and Manitoba have also shown that droplet density ca 50/cm^2 (100μ MMD) over budworm feeding sites is required to effect good control of infestations. Conditions of temperature inversion, high relative humidity and cloud cover should be considered for optimum deposition of spray. Alternatively, recent advances in aircraft guidance have shown that night-time spraying may be the most suitable period for insecticide application, and this could be an extremely important step towards ensuring the efficacy of B.t. sprays against the spruce budworm.

Because of the lapse between spray application and disease establishment the target stadium for spruce budworm larvae should be L_3. Later applications against larger larvae may be ineffective in providing the desired levels of foliage protection.

Similarly, when larval population densities exceed 20-30/18 in. branch tip (as in Manitoba and certain areas treated in Algonquin Park) chemical sprays or B.t. plus chemical combinations may provide better results. In each of the experiments where population levels ranged from 50-90/18 in. branch, unacceptable levels of defoliation resulted after the B.t. treatment. Accordingly, population trends should be followed closely so that applications of B.t. can be made at the beginning of a numerical increase rather than at the peak of the infestation.

The mixing of B.t. sprays during May and early June may pose serious problems at the airstrip. When cold, the viscosity of the Thuricide 16B formulation increases; likewise, if the concentration of the molasses anti-drift/anti-evaporant is high in the Dipel WP spray, mixing will be difficult. Paddle-agitators in mixing tanks or power

mixers for 45-gal. drums are required to promote rapid mixing, particularly with powder preparations of B.t.

The nature of the forest stands to be treated also should be considered in planning B.t. sprays by aircraft. As for most chemical insecticides, it was indicated that B.t. is more effective on spruce budworm infesting balsam fir than on spruce. For this reason, and with due consideration for population density, several of the following alternatives should be considered where spruce composition of the forest is high:

1) Double applications - 4 billion IU at 0.5 gal/ac spaced 3-5 days apart.
2) Increased concentration of B.t. - 6 or 8 billion IU at 0.5 gal/ac.
3) Increased volume of spray mixture and/or B.t. concentration - 4, 6, 8 billion IU at 1-4 gal/ac.
4) Ground spray application when area is small and trees accessible - by mistblower, hydraulic sprayer.

The following program[1] for studies of B.t. spray efficacy outlines both the problem areas and planning for additional research by the C.F.S. during the period 1974-1976:

(1) Biochemical studies of toxic derivatives.
(2) Laboratory evaluation of the role of microsporidia.
(3) Toxicological studies (laboratory).
(4) Laboratory evaluation of sublethal effects.

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[1] From minutes of inter-agency discussions held at C.C.R.I., Nov. 15, 1973, on use of Bacillus thuringiensis against spruce budworm (prepared by C.J. Sanders).

(5) Field evaluation of sublethal effects (follow-up).

(6) Search for improved formulations.

(7) Evaluation of interaction with insecticides (stress factor).

(8) Evaluation of interaction with virus.

(9) Further field evaluation of the importance of chitinase as additive.

(10) Further evaluation of environmental impact.

(11) Standardization of field evaluation (methodology).

In view of the toxicological specificity of B.t. as indicated by the intensive studies by C.C.R.I. and other agencies, it is apparent that B.t., as produced commercially by Abbott Laboratories (Dipel WP) and Sandoz-Wander (Thuricide 16B), should be registered for use in Canada against the spruce budworm. It should be expected, at this time at least, that the efficacy of treatment will depend largely on the parameters influencing application as discussed above. Currently, parks (where treatment with chemical insecticides is prohibited) and watershed areas are logical choices for B.t. spray treatment. Until future studies definitively establish the role of B.t. for control of spruce budworm, results of treatments most probably will continue to be highly variable. In view of the urgent need for insecticides of assured environment acceptability, the Canadian Forestry Service and the Chemical Control Research Institute will continue to promote research on Bacillus thuringiensis in the interests of modern forest management.

Evaluation de Préparations Commerciales de *Bacillus thuringiensis* avec et sans Chitinase Employés Contre la Tordeuse des Bourgeons de l'Épinette

H. Sommaire, Conclusions et Recommandations

par R.F. DeBoo et O.N. Morris

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H. Sommaire, Conclusions, et Recommandations

par

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Les invasions de tordeuses de bourgeons de l'épinette au Canada et dans les régions frontières des Etats-Unis sont devenues un des problèmes les plus difficiles à résoudre en sylviculture. Avec l'adoption rapide des notions d'aménagement forestier polyvalent, la tordeuse est devenue un problème majeur dans la conservation des peuplements forestiers à des fins tant économiques qu'esthétiques. L'exécution de programmes de protection, notamment de programmes d'application aérienne d'insecticides synthétiques organiques, demeure encore le meilleur moyen dont dispose le forestier pour protéger les forêts importantes contre les ravages de cet insecte.

Pendant les années d'invasions de tordeuses de bourgeons de l'épinette, les politiques de protection varient au Canada d'une province à l'autre et d'une année à l'autre et peuvent même varier entre deux zones forestières contigues au sein d'une même province. Il y a plusieurs raisons à cela, mais le facteur prédominant lorsque vient le moment de décider d'entreprendre un programme de pulvérisation aérienne est le facteur économique. La valeur qu'on accorde aux forêts productrices de fibres et aux régions d'agrément comme les parcs peut être assez élevée pour qu'on envisage le recours à la pulvérisation aérienne. Au cours des dernières années, par exemple, on a traité avec succès des forêts productrices de fibres au moyen de pulvérisations d'insecticides chimiques pour moins de $1 l'acre. A part les insecticides chimiques cependant, il existe peu de méthodes de traitement vraiment pratiques, et même si des frais de l'ordre $2 l'acre peuvent se justifier du point de vue économique, l'emploi d'insecticides chimiques est interdit pour certains peuplements forestiers (les parcs nationaux, par exemple). Un des moyens présentement acceptables de réduire le problème de la tordeuse est le recours aux insecticides hautement sélectifs et non dangereux. L'insecticide Bacillus thuringiensis (B.t.) est celui qui semble offrir les meilleurs possibilités à cet égard.

Les recherches effectuées par le personnel du Service canadien des forêts au cours de la dernière décennie ont montré que le B.t., en particulier les nouvelles souches plus puissantes que l'industrie produit actuellement, est efficace contre les larves de tordeuses de bourgeons de l'épinette. Des travaux sur le terrain menés au Centre de recherche forestière des Laurentides ont également montré que l'addition d'enzyme chitinase aux solutions de B.t. entraîne une septicémie accélérée chez les larves infectées et peut par la suite assurer au feuillage une meilleure protection pendant l'année d'application. En outre, des essais de pulvérisation par atomiseur et de pulvérisation aérienne effectués par l'Institut de recherche en répression chimique ont révélé que des mélanges de B.t. et d'insecticides chimiques peuvent prévenir la défoliation excessive des arbres.

A la demande de la Direction générale de la coordination des programmes du Service canadien des forêts, l'Institut de recherche en répression chimique (I.R.R.C.) a entrepris en 1973 une étude poussée de pulvérisations de B.t. et de mélanges B.t./chitinase. L'étude avait pour object précis d'analyser, en utilisant au maximum tous les moyens et connaissances techniques de l'Institut, la possibilité d'employer le B.t. comme agent de répression de la tordeuse des bourgeons de l'épinette au Canada. La principale étude portant sur les pulvérisations aériennes a été faite à l'intérieur du parc Algonquin, un des plus grands parcs de l'Ontario. Des études parallèles ont été entreprises à l'I.R.R.C. (études toxicologiques menées en laboratoire au moyen de tours de pulvérisation) à Ottawa, dans une ferme sylvicole située près de Shawville au Québec (où l'on a employé un dispositif permettant d'appliquer à des arbres individuels des doses de B.t. au moyen de pulvérisations aériennes simulées), ainsi qu'au parc provincial et à la forêt de Spruce Woods du sud du Manitoba (où l'on a évalué des applications faites par atomiseur et par aéronef). En plus, on est resté en liaison étroite toute l'année avec d'autres établissements expérimentant le B.t. (Centre de recherche forestières des Laurentides, Institut de recherche en pathologie des insectes, Université du Maine) afin de pouvoir comparer les résultats et les expériences.

Voici un résumé des résultats des études interdisciplinaires menées par l'I.R.R.C.:

Des expériences toxicologiques faites en laboratoire sur des larves au 5^{e} stade de développement et du feuillage de mélèze traité au B.t. ont montré qu'aux doses évaluées, le Dipel WP® (laboratoires Abbott) était plus efficace que le Thuricide 16B® (actuellement vendu par Sandoz-Wander). La toxicité des deux produits augmentait en fonction du volume (gallons/acre) et du dépôt (gouttes/cm^2). L'addition de chitinase a rendu le Thuricide de 1.5 à 3 fois plus toxique, mais n'a pas eu d'effet notable sur l'efficacité des pulvérisations de Dipel. En résumé, les résultats de l'étude en laboratoire de 1973 ont permis d'établir l'ordre de toxicité décroissant suivant:

Dipel = Dipel + chitinase > Thuricide + chitinase > Thuricide

Des expériences sur le terrain ont été effectuées près de Shawville au Québec au moyen d'un dispositif qui simulait la pulvérisation aérienne d'insecticides sur de jeunes épinettes blanches. Des examens faits deux semaines après le traitement ont semblé indiquer qu'aucune des deux préparations commerciales n'avaient entraîné de réduction notable de la densité des populations de larves. On a également constaté que l'addition de chitinase aux pulvérisations de B.t. ne semblait par avoir accru leur efficacité, après le même laps de temps. Par contre, les traitements au B.t. ont retardé le développement des larves et, apparemment, auraient également réduit la pupation. L'addition d'une faible quantité de fénitrothion (0.4%) aux pulvérisations de B.t. a entraîné la même réduction moyenne de population que si l'on avait employé 10 fois la même quantité de fénitrothion sans B.t., et a marqué une nette amélioration sur le traitement contre la tordeuse des bourgeons de l'épinette qui n'utilise que le B.t. Comme les variations de densité (15-40 gouttes /cm^2) et de grosseur (110-150µ) des gouttelettes étaient du même ordre que celles des dépôts de pulvérisation aérienne, on a jugé que le dispositif et la méthode employés

pour simuler la pulvérisation aérienne donnaient de bons résultats d'application.

Les applications de Thuricide, de Dipel et de fénitrothion faites au moyen d'un atomiseur sur les épinettes blanches du parc provincial Spruce Woods n'ont pas réussi à protéger le feuillage de façon satisfaisante. Cet échec s'explique surtout par la très dense population de tordeuses des bourgeons de l'épinette qui ravageait les arbres. En outre, les conditions atmosphériques défavorables qui prévalaient lors de la période larvaire < 3 ont retardé les applications d'une semaine environ. Néanmoins, le traitement au Thuricide aurait entraîné une très forte réduction de la population (75%). L'échec du traitement au Dipel a été attribué à une averse (délavage) qui a immédiatement suivi l'application.

Des pulvérisations aériennes de Dipel (4 milliards U.T.I.) à des doses de 2 et 4 gallons l'acre n'ont pas non plus fourni de protection acceptable au feuillage des épinettes blanches de la forêt provinciale Spruce Woods. On a obtenu des réductions de population de l'ordre de 43% et 61%, mais ces niveaux étaient insuffisants par rapport à ceux qu'exigeait la situation de densité larvaire en 1973. De nombreuses larves mortes ou malades ont été trouvées sur les arbres des deux zones de traitement. En outre, on y a relevé beaucoup moins de pupes que dans la zone voisine qui n'avait pas subi de traitement. On a obtenu une bonne protection du feuillage (défoliation < 20%) dans les endroits où les insecticides s'étaient parfaitement bien déposés ou dans ceux où la densité larvaire était faible.

Les résultats de la pulvérisation aérienne au parc Algonquin de 4 milliards d'U.I. de Dipel et de Thuricide avec ou sans chitinase à des doses de 0.5 gallon l'acre ont montré que:

1. Pour assurer un bon dépôt d'une solution de B.t. et obtenir de bons résultats, il faut employer un système d'émission capable de pulvériser des gouttelettes d'un diamètre inférieur à 100 microns. De plus, il devrait y avoir un moins 50 gouttelettes par cm^2. L'addition au mélange d'un antiévaporant (la mélasse, par exemple) s'impose lorsque les conditions de pulvérisation ne sont pas idéales. L'insecticide doit être appliqué dans des conditions atmosphériques stables. Etant donné les caractéristiques physiques du B.t., le mélange ne peut être fait qu'au moyen d'un système d'agitation mécanique, de plus, il faut se servir d'une pompe à déplacement positif et à grande capacité (environ 50 gallons à la minute) pour pomper le liquide.

2. L'emploi d'un colorant traceur, le Brilliant Sulfo Flavine FFA (Chemical developments of Canada Ltd., Toronto), a révélé que les insecticides pulvérisés ont atteint les arbres cibles dans des proportions allant de 18% (chitinase seule) à 81% (Thuricide + chitinase). La concentration de gouttes variait de 16 à 98/cm^2 alors que le diamètre se situait entre 91 et 111 microns. L'action résiduelle du B.t. se trouvait fortement réduite après 5 jours

d'exposition. Le Dipel ainsi que le mélange Dipel/chitinase (mais non le Thuricide ni le mélange Thuricide/chitinase) ont bien réussi à réduire (réductions de 75% et 94% respectivement) des populations très denses de tordeuses des bourgeons de l'épinette sur les sapins baumiers. Le traitement au mélange Thuricide/chitinase, appliqué une année où les concentrations de population étaient modérées, a assuré une bonne protection du feuillage des sapins baumiers.

Les traitements au B.t., avec et sans chitinase, ont retardé le développement des tordeuses et ont réduit le poids des pupes, les taux de ponte ainsi que la viabilité des oeufs.

Des populations de petits mammifères et d'oiseaux du parc Algonquin et de Spruce Woods ont été étroitement surveillées avant et après l'application aérienne de B.t.

Ont a constaté aux deux endroits que les pulvérisations n'avaient eu aucun effet sur les densités de population ni sur les activités des animaux résidents, De même, la chitinase appliquée seule ou mélangée au B.t. n'a eu aucun effet sur la faune. Au parc Algonquin, des études portant sur des abeilles domestiques (établies artificiellement dans les zones cibles), d'autres insectes non visés et sur la faune aquatique ont également montré que les pulvérisations de B.t. et de B.t. + chitinase n'entraînaient pas d'effets secondaires nuisibles.

Les résultats de l'étude de 1973 de l'I.R.R.C. sur le B.t. et la chitinase ont montré que la réussite d'un traitement contre les populations de tordeuses de bourgeons de l'épinette est reliée à quatre variables importantes:

1) La disponibilité de la chitinase à un coût abordable;
2) L'effet des conditions atmosphériques sur la concentration et le dépôt de l'insecticide;
3) le moment de l'application de l'insecticide;
4) La densité des populations de tordeuses de bourgeons de l'épinette.

Comme l'addition de chitinase a accru l'efficacité des pulvérisations de B.t. dans la plupart des expériences, on devrait en envisager l'emploi dans les situations qui nécessitent un degré de protection maximum. Pour le moment, cependant, le coût de la chitinase est beaucoup trop élevé pour qu'on puisse s'en servir comme adjuvant. Néanmoins, les efforts actuels en vue de produire une préparation peu coûteuse pourraient bien améliorer la situation d'ici peu.

Les études de l'I.R.R.C. au Québec, en Ontario et au Manitoba ont également montré que la densité de gouttelettes requise dans les régions attaquées par les tordeuses pour réprimer efficacement les invasions est d'environ 50/cm^2 (diamètre médian selon la masse de 100µ).

Pour obtenir un dépôt d'insecticide optimal, il faut tenir compte des conditions d'inversion de la température, de l'humidité relative élevée et de la nébulosité. Par ailleurs, de récents perfectionnements dans le domaine de la navigation aérienne ont montré que la nuit est peut-être la période qui convient le mieux à la pulvérisation d'insecticides, et cette découverte pourrait contribuer grandement à l'efficacité des pulvérisations de B.t. contre la tordeuse des bourgeons de l'épinette.

Vue le laps de temps qui sépare l'application du pesticide de l'apparition de la maladie, le traitement devrait être appliqué lorsque les larves atteignent leur troisième stade de développement. Des applications faites après ce stade pourraient ne pas offrir le degré de protection du feuillage voulu.

De même, un pulvérisation d'agents chimiques ou de mélanges B.t./agent chimique devrait donner de meilleurs résultats lorsque les densités de populations larvaires dépassent 20-30/18 po de bout de branche (comme c'était le cas au Manitoba et dans certaines régions traitées du parc Algonquin). Dans chacun des expériences faites avec des niveaux de population variant de 50 à 90/18 po de bout de branche le traitement au B.t. s'est soldé par des niveaux de défoliation inacceptables. Par conséquent, les tendances de population devraient être suivies de près afin que les applications de B.t. puissent être faites dès le début d'un accroissement numérique plutôt qu'au pire de l'invasion.

Le mélange des insecticides au B.t. au terrain d'atterrissage en mai et au début juin peut poser de graves problèmes. Lorsqu'il fait froid, la viscosité du Thuricide 16B augmente; de plus, une forte concentration du Dupel WP en mélasse servant d'anti-dérivant et d'anti-évaporant rendra le mélange difficile. Pour un mélange rapide, surtout lorsqu'il s'agit de préparations de B.t. en poudre, on devra se servir d'agitateurs à palettes pour les réservoirs à mélange ou de malaxeurs mécaniques pour barils de 45 gallons.

Un autre facteur à considérer dans la planification d'un programme de pulvérisation aérienne de B.t. est la nature des peuplements forestiers à traiter. On a constaté en effet que le B.t., comme la plupart des insecticides chimiques, est plus efficace contre les tordeuses qui s'attaquent au sapin baumier que contre celles qui s'attaquent à l'épinette. Aussi, lorsque les forêts à traiter ont une forte proportion d'épinettes, il faudrait envisager plusieurs des possibilités mentionnées ci-dessous tout en tenant compte, par ailleurs, de la densité de la population.

1) Deux applications successives de 4 milliards UI à 0.5 gallon/acre à un intervalle de 3 à 5 jours;

2) Concentration accrue de B.t. - 6 à 8 milliards UI à 0.5 gallon/acre;

3) Volume accru de mélange insecticide ou centration accrue en B.t. - 4,6,8 milliards UI à 1-4 gallons/acre;

4) Application d'insecticide au sol lorsque la zone à traiter est peu étendue et que les arbres sont accessibles-pulvérisation par atomiseur ou par pulvérisateur hydraulique.

Le programme[1] d'étude de l'efficacité du B.t. comme insecticide donné ci-après expose les secteurs-problèmes et les projects de recherche supplémentaire du Service canadien des forêts pour la période 1974-1976:

(1) Etudes biochimiques des dérivés toxiques;

(2) Evaluation en laboratoire du rôle des microsporidies;

(3) Etudes toxicologiques (en laboratoire);

(4) Evaluation en laboratoire des effets sublétaux;

(5) Evaluation sur le terrain des effets sublétaux (suite donnée);

(6) Recherche de mélange améliorés;

(7) Evaluation de l'interaction avec les insecticides (rôle du stress);

(8) Evaluation de l'interaction avec les virus;

(9) Nouvelle évaluation sur le terrain de l'importance de la chitinase comme adjuvant;

(10) Nouvelle évaluation de l'effet environnemental;

(11) Uniformisation des méthodes d'évaluation sur le terrain.

Compte tenu des propriétés toxicologiques du B.t. qu'ont confirmées les études poussées menées par l'I.R.R.C. et d'autres organismes, il ressort que les préparations commerciales du B.t. fabriquées par Abbott Laboratories (Dipel WP) et Sandoz-Wander (Thuricide 16B) devraient être autorisées pour emploi au Canada contre la tordeuse des bourgeons de l'épinette. Il faut s'attendre, pour le moment du moins, à ce que l'efficacité du traitement dépende dans une large mesure des paramètres d'application mentionnés plus haut. A l'heure actuelle, les parcs (où l'emploi d'insecticides chimiques est interdit) de même que les bassins hydrographiques sont des endroits tout désignés pour le traitement au B.t. Tant que d'autres études n'auront pas établi de façon définitive le rôle du B.t. dans la répression de la tordeuse des bourgeons de l'épinette, les résultats des traitements continueront probablement d'être hautement variables. Devant le besoin urgent d'insecticides non nuisibles à l'environnement, le Service canadien des forêts et l'Institut de recherche en répression chimique va continuer à préconiser la recherche sur le Bacillus thuringiensis en vue d'une gestion forestière moderne.

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[1] Tiré du compte rendu de la rencontre inter-organismes sur l'emploi du Bacillus thuringiensis contre la tordeuse des bourgeons de l'épinette, laquelle a eu lieu à l'Institut de recherche en répression chimique le 15 novembre 1973 (préparé par C.J. Sanders).