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REPORT OF THE 1979 CANUSA COOPERATIVE Bacillus thuringiensis B.T. SPRAY TRIALS

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canusa

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Spruce Budworms
Program

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ABSTRACT

In 1979, a total of 44,499 hectares (109,912 acres) of balsam fir, *Abies balsamea,* white spruce, *Picea glauca* and red spruce, *Picea rubens* forest stands in Eastern Canada and United States infested with the spruce budworm, *Choristoneura fumiferana,* were treated with several commercial preparations of *Bacillus thuringiensis.* The main aim was to limit defoliation to 50% of the current growth in the operational and experimental trials. The geographical locations included Newfoundland, Nova Scotia, New Brunswick, Ontario, Quebec and Maine.

Fifty-five percent of all treatments in balsam fir and white spruce stands achieved the success criterion. Six of the 7 treatments in red spruce stands were also successful. Dipel 88 (Dipel 4L) showed the highest success rate followed by Thuricide followed by Novabac. Limited environmental impact studies in Nova Scotia supported the generally accepted belief that *B.t.* poses no undue hazard to terrestrial and aquatic non-target organisms.

Support studies by Canadian Forestry Service personnel including compatibility of tracer dyes with *B. thuringiensis* var. *kurstaki,* batch quality control bioassays, budworm population quality, aircraft calibration, droplet characterization and deposit analyses are described.

Cost estimates for materials and applications ranged from $15.97 to $21.94/ha in Quebec, Nova Scotia, Ontario and Newfoundland.

The total cost of the Nova Scotia project including temporary personnel salaries, travel, biocide, equipment purchases and rentals, application, accommodation, target and non-target monitoring and egg mass survey was $48.35/ha.

The cooperative effort brought closer to fruition the general acceptability of *B.t.* as an alternative to chemical pesticides in spruce budworm control.

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1. INTRODUCTION

The bacterium, *Bacillus thuringiensis*, has been isolated from a variety of lepidopterous larvae from British Columbia forest but not from the spruce budworm.

The first experimental aerial applications of *Bacillus thruingiensis (B.t.)* against the eastern spruce budworm were carried out in New Brunswick, Canada, in 1960 using Thuricide S075 suspensions in water and in a water-in-oil emulsion[1]. Field trials continued in Canada and USA using a variety of water-based commercial formulations and spray emission equipment up to 1978. The effectiveness of these treatments has always been unpredictable in terms of the short term tree protection from budworm damage and population density reduction. This is explained partly by the fact that water-based formulations of insecticides tend to be highly subject to the meteorological conditions under which they are applied resulting in unpredictable deposit efficiencies and thus unpredictable effectiveness. Another explanation relates to the behaviour of the budworm itself. It inhabits a shelter during its most susceptible stages of development (L_3-L_4). This is complicated by the fact that full bud flushing and L_3-L_4 are not always synchronous and since the bacteria must be ingested to be effective, an asynchronous development of this type may result in reduced effectiveness. Spruce budworm larvae are comparatively highly susceptible to *B.t.* and it is generally agreed that acceptable protection for coniferous trees from budworm damage can be achieved under conditions of moderate population pressure and high deposit efficiency at the feeding site.

In October 1978, the Canadian Forestry Service called a meeting of *B.t.* researchers and applicators with a view to formulating some guidelines for *B.t.* aerial applications and to document information gaps which when filled would contribute to the general acceptability of the product.

Subsequent to this meeting, O.N. Morris (FPMI) was named the Canadian Coordinator for the 1979 *B.t.* spray trials and Chairman of an International *B.t.* Advisory Committee with D.G. Grimble (USDA Forest Service) as Co-Chairman and U.S. Coordinator. The CFS Technical Recommendations were reviewed by the Advisory Committee and submitted to cooperators in both countries with minor changes as the CANUSA Technical Guidelines (Appendix I). The present report summarizes the results of the laboratory and field activities as a whole. It should show certain trends in the effectiveness of *B.t.* for spruce budworm control based on the existing state of the art and confirm certain deficiencies which have been apparent in the past.

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[1]Morris, O.N., Angus, T.A., and Smirnoff, W.A. (1975). In "Aerial Control of Forest Insects in Canada" (M.L. Prebble, ed.) pp. 129-133.

2. SUPPORT ACTIVITIES

Early in the development of the cooperative spray program it was determined that implementation of certain support activities were necessary to assist some cooperators lacking expertise in some aspects of the field program. The support areas included studies on the compatibility of the recommended tracer dye (Erio Acid Red XB 400) with *B.t.*, bioassays of drum samples of commercial products as batch quality control, calibration of spray aircraft, incidence of microsporidia in different geographical areas, droplet characterization of various *B.t.* formulations, and Kromekote card, Millipore filter and glass plate droplet analyses. These activities were financially supported by the eastern component of the CANUSA program.

The reports on the compatibility of the tracer dye and *B.t.*, toxicity of the dye for spruce budworm, droplet analyses, and a comparison of spore deposits on glass slides and Millipore filters are summarized in Appendix II. Included also is a memo relating to environmental concerns with the safety of certain components of the recommended *B.t.* formulation and an NRC report on *B.t.* droplet characterization. The following conclusions were drawn on the basis of these reports:

1. The dye concentration recommended for field application had no significant effect on spore germination or on the toxicity of *B.t.* to the budworm.

2. The tracer dye in the recommended concentration was not toxic for budworm larvae.

3. The use of petri dishes and Millipore filters to estimate deposits at ground level grossly underestimates the actual deposit of viable spores. A more accurate method of estimating *B.t.* deposit is urgently needed. Assessment of deposits on foliage would be most desirable.

4. The generation of uniform droplet sizes which is necessary for determining spread factors of currently used formulations of *B.t.* is almost impossible to achieve with present NRC technology. Also, the NRC Flying Spot Scanner does not accurately record droplets below 50 μm diameter, especially when dye concentrations are below 2.0%. Until these technological problems are solved, manual analysis of deposits on cards are more desirable.

One hundred and twenty-two (122) drum samples of Thuricide, Novabac and Dipel were bioassayed against L_4 spruce budworm and the potencies compared with a standard in-house Thuricide 16B.

The results (Appendix III) indicated wide variations in potencies between batches. However, the wide confidence limits of the bioassays indicates that the precision of the assays was poor. Precision will be improved for future batch quality control assays.

The procedure for sampling *B.t.* from shipping containers, results of viable spore counts and diagnoses of budworm larvae before and after spray applications are reported in Appendix IV. Unfortunately, the samples for analysis arrived later than requested so that the precision of the assays may have been compromised. Also, some samples appeared to have been diluted before submission. It was recommended that spore viability tests be done prior to rather than after application in the future. The greatest variation between presumed activity and established activity based on spore counts occurred in Dipel 88 oil formulation (ABG 6103). This apparent difference was not reflected in the bioassay data, however, (Appendix III) where the pooled relative potency for this product indicated higher activity than 7 pooled batches of Thuricide and 2 pooled batches of Novabac. This suggests that the use of spore counts as a basis of insecticidal activity of *B.t.* is inadequate.

The incidence of microsporidia in budworm populations from Newfoundland and New Brunswick ranged from 20 to 55%. The extent to which this parasite influences treatment mortality in these populations is unknown.

The collaborative study between the Forest Pest Management Institute and the Newfoundland Department of Forest Resources and Lands produced a new technique for aircraft calibration particularly useful at airstrips where even minimal contamination by spray material is prohibited (Appendix V). The report emphasized the need for calibration of aircraft with the spray material (not water) prior to application. It recommended establishment of a standard set of application and deposit efficiency criteria as a baseline for future research on *B.t.* spray deposit/efficacy. A technique using an inexpensive plastic collector tube in the calibration of aircraft is described. Interested parties may request clearer photographic prints from the author, (Randall et al.).

Mixing of commercial water-based *B.t.* was improved by adding the *B.t.* to water rather than vice versa. Distilled water was more efficient than toluene, benzene or several concentrations of ethanol for removing *B.t.* deposits from glass slides. Centrifugation or filtration of washed deposits prior to spectrophotometric analysis increased net deposit estimates by 78% due to light scatter by the suspended *B.t.* particles. The 0.025% dye concentration recommended was too low for the most precise colorimetric analysis. A fluorescent dye would provide a much more precise measurement.

3. EFFICACY OF FIELD TRIALS

Table 1 lists the main technical recommendations as followed by the cooperators and the formulations used. In general, the recommendations were adhered to, and when they were not, extenuating circumstances frequently encountered in aerial applications prevented adherence. For example, ideal budworm development and droplet density for *B.t.* application are not entirely controllable in semi-operational trials. The criterion of "less than 30 larvae/branch" is probably totally impractical under such circumstances. A statement to the effect that *B.t.* treatment can be expected to be less effective under high population pressure would have been more reasonable. Only Nova Scotia and Quebec provided data on bud density so that a comparison of stand quality between all geographic locations is not possible. The number of current balsam fir buds per 45 cm. branch tip ranged from 175 to 227 in Nova Scotia compared with 30 to 98 in Quebec. This will explain partly the greater overall treatment efficacy in Nova Scotia compared with Quebec. The actual reports of the cooperators were too lengthy a package for inclusion in this report. Instead, data from each report are presented on technical data sheets. Full reports are probably available from the individual cooperators.

Ontario - J.R. Carrow

Ontario treated 3,860 ha with Thuricide 16B alone, Novabac alone and acephate (Orthene) followed by Novabac 8 days later (Table 2). Satisfactory results were obtained on balsam fir treated once or twice with 10 BIU/4.7 ℓ/ha of Thuricide 16B. Foliage protection on white spruce was unsatisfactory. Best results were always achieved with early treatments (peak L_4). Many of the failures were apparently due to poor weather conditions during spray time resulting in very mixed deposit efficiencies at ground level. The technique of applying acephate followed by Novabac 8 days later to very high populations (45/branch) was ineffective both in terms of population density reduction and in foliage protection.

Maine - J.Y. Connor and M. Trial

Maine treated 17,895 ha with Thuricide 16B, Thuricide 32B and Dipel 4L(88)(Table 3). Prespray larval densities/45 cm. branch varied between 5 and 18 on white-red spruce and between 13 and 26 on balsam fir. The Thuricide treatments provided satisfactory protection on white spruce but not on balsam fir which is the reverse of teh results from Ontario. Dipel 88 gave satisfactory protection on both tree species. Ground level deposits in all treatments were well below previously set acceptable standards. The generally good results achieved indicate that ground deposit rates do not always correlate with treatment efficacy.

University of Maine - J.B. Dimond and C.J. Spies

UMO applied by helicopter Thuricide 16B alone, Thuricide 16B + Chitinase and Thuricide 16B plus Acephate each to 5 replicates of 40 ha (Table 4). Prespray population densities at peak L_4 were extremely high ranging from 67 to 72/45 cm branch on balsam fir and 25 to 27 on red spruce. Ground deposit rates were well below the set standard of 25 droplets/cm^2 for all treatments. Heavy rain followed some applications. Under the conditions of these tests the addition of chitinase to *B.t.* tank mixes did not significantly enhance the effectiveness of the *B.t.* The addition of Acephate appeared to be slightly superior to *B.t.* alone in population reduction and foliage protection but further evaluation was considered necessary to confirm this. In spite of the very high budworm numbers, all three treatments saved roughly 50% of foliage on both fir and spruce, possibly due to the efficiency of the helicopter spray equipment in depositing the insecticide on the trees.

New Brunswick - FPL - R.G. Lidstone

New Brunswick applied Thuricide 16B (20 BIU/ha) to 5 replicate 40 ha plots and Dipel 88 (40 BIU/ha) and Dipel 45B (22 BIU/ha) to 80 ha plots at L_4-L_5 larval development. Population densities ranged from 38-44/45 cm branch on white spruce, 20-26 on balsam fir and 6-19 on red spruce (Table 5). Heavy rains followed the Thuricide sprays. With the exception of balsam fir in the Thuricide blocks, all treatments limited defoliation of current growth of the three tree species to less than 50%. Foliage protection due to treatment was zero in Thuricide blocks compared with 20-54% in Dipel blocks. The author speculated that the level of protection in the Dipel blocks may have been enhanced by drift from a nearby aminocarb-treated block.

Newfoundland - N.E. Carter

Newfoundland treated 5,650 ha with Thuricide 16B, 60 ha with Thuricide 24BA, 80 ha with Thuricide 24BC and 80 ha with Novabac 45B (Table 6). Population densities in all the balsam fir treatment blocks were low (5-11 larvae/45 cm. branch). Larval development was late (L_5-L_6) for all treatments. Defoliation was limited to less than 50% in the Thuricide 24BA, BC and Novabac treatment blocks but not in the Thuricide 16B block. However, foliage protection due to treatment was low to non-existent in all treatment areas in spite of the low population densities. The author concluded that a single late (L_5-L_6) application of *B.t.* even against low population densities on balsam fir in Newfoundland was inadequate for acceptable tree protection.

Nova Scotia - C.A. Miller

Nova Scotia treated 2,760 ha of red spruce stands in Cumberland County and 1,800 ha of balsam fir in Victoria County, with single or double applications of Thuricide 16B (Table 7). The CANUSA technical recommendations were followed. Prespray bud-density tallies indicated that the test trees were well foliated in spite of previous heavy defoliation. Larval development at spray time was peak L_4 on balsam fir and L_4 to L_6 on red spruce but bud flush was <50% on red spruce compared with 100% on balsam fir. Spray deposit efficiency was high for all applications. Larval densities ranged from 12 to 22/45 cm branch on red spruce and 24-35 on balsam fir. Defoliation on all spray plots ranged from 9% to 19% which was considerably below the 50% limit aimed for. The following conclusions were made by the author:

1. Single or double applications of Thuricide 16B protected both balsam fir and red spruce from unacceptable defoliation of current growth.

2. Two applications of 10 BIU/4.73 ℓ/ha gave better protection of balsam fir than one application of 20 BIU/9.4 ℓ/ha. If one application is proposed for future treatments, consideration should be given to increasing the emitted rate above 20 BIU/ha.

3. Ten or 20 BIU/ha application rate protected red spruce stands from unacceptable defoliation whether applied early when current buds were protected by scales or late when current needles were flaring.

It is noteworthy that good protection of red spruce was also achieved in Maine (Table 4) and in New Brunswick (Table 5). The explanation for these successes on only partially flared red spruce trees is obscure.

Quebec - M.M. Pelletier

Single applications of Thuricide 32B, Novabac and Dipel 88 were applied to a total of 10,674 ha of balsam fir. Formulations of Thuricide and Novabac contained sorbitol and chitinase and Dipel contained chitinase. Population densities were moderate ranging from 10-15 larvae/45 cm branch. Bud densities on balsam fir ranged from 30 to 87/45 cm branch. Dipel and Novabac limited defoliation to 34% and 48% respectively and were most effective in terms of foliage protection due to treatment. Defoliation on the Thuricide 32B plots ranged from 57-58%. The lower efficacy of Thuricide here compared with the results in Nova Scotia was unexpected in view of the higher population densities and higher bud densities in the Nova Scotia balsam fir stands. Ground level colony counts were high in both sets of trials. In Quebec, Dipel 88 treatment gave the best protection in spite of low colony counts on petri dishes at ground level ($6.6/cm^2$). Colony counts on Millipore filters were considerably higher ($40/cm^2$) suggesting that the filters may be more efficient collectors than petri dishes. The extruding strands of the filters are probably efficient collectors of small droplets. Also, the Millipores were incubated on trypticase soy agar which is known to be a better growth medium for *B.t.* than the nutrient agar used in petri dishes. Even with the filters, the colony counts were lowest in the Dipel block. The droplet spectrum of Dipel (97% less than 75 μm) was, however, also the smallest of all treatments and it is expected that such droplet size would deposit at ground level less efficiently than larger ones.

4. ENVIRONMENTAL IMPACT OF THURICIDE 16B

by

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A.S. Ménon, D. MacKay, P. MacDonald,
P. Germain, D.G. Embree & R.M. Bulmer

Concurrently with their tests of efficacy of *B.t.* for spruce budworm, the Nova Scotia Department of Lands and Forests conducted five non-target studies. These included 1) Virus enhancement tests; 2) Recovery of *B. thuringiensis* var. *kurstaki* in the aquatic environment; 3) Environmental monitoring with particular reference to the xylene component of Thuricide; 4) Impact of *B.t.* sprays on songbirds; 5) Impact of *B.t.* sprays on aquatic fauna. The 266-page document detailing this study is available from the senior author (T.D. Smith). The salient points from the report are as follows:

A. *Human Health (Virus Enhancement)*

Although *B.t.k.* is regarded as safe from a human standpoint, when used correctly, viral enhancement data were not available. Therefore, a virus enhancement study was initiated. The virus used for this study was Vesicular stomatitis virus[1] (Indiana Strain). This virus is reported to be the more sensitive, as a biological indicator of disfunctions of the group of viruses associated with Reye's Syndrome (encephalopathey with fatty change or degeneration of the viscera). The optimum concentration levels of chemicals for virus enhancement are just less than toxic. Initial studies of the *B.t.k.* formulation indicated a virus enhancement index of 2.2 at a concentration level of 2,500 ppm. The *B.t.k.* formulation was applied at a concentration level of 0.094 ppm. This application concentration level was 26,315 times less than that required for the virus enhancement index of 2.2. By way of comparison in a similar study, fenitrothion has a virus enhancement index of 2.49 at a concentration level of 2.5 ppm. Based on these data, the risk for virus enhancement associated with the use of *B.t.k.* is minimal.

B. *Water and Shellfish*

I. Presence of *Bacillus thuringiensis kurstaki* in stream water.

(a) Moose River

Bacillus thuringiensis kurstaki was recovered from both surface and sub-surface water samples from the Moose River sampling sites at a level ranging from 20 to 70 colonies per ml of sample during the period of aerial spray application from June 15 to June 18, 1979. No *B.t.k.* was detected in any water samples after June 18, 1979.

(b) MacRae Brook and Baddeck River

Bacillus thuringiensis kurstaki was collected from June 13 to June 26 at MacRae Brook sampling sites and on June 21 at 200 feet above Red Bridge over the Baddeck River. No *B.t.k.* was found at other sampling sites. No *B.t.k.* was found in the McPhee

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[1]Vesicular stomatitis is a disease pathogen of cattle, swine and horses and is communicable to man. The disease, caused by this filterable virus, has similar symptoms to foot-and-mouth disease and is characterized by blisters on the nose, lips and especially the tongue and mucus membrane of the mouth. In Nova Scotia, this disease pathogen is found only in controlled university cultures.

cow's milk samples or Baddeck water supply. The recovery of *B.t.k.* eleven days after the last application of the spray indicates that it is persistent in the aquatic environment.

II. Clams and Oysters

The salt water clam and oyster fisheries are important local industries in Cumberland and Victoria Counties, respectively. Fresh water clams are filter feeders and are sensitive to low concentrations of xenobiotic chemicals and biocides. Fresh water clams were absent in the streams of the experimental areas and estuarine clams and oysters were used to monitor the qualitative and quantitative concentrations of the biocide. No *B.t.k.* was recovered from estuarine clams at Moose River and from estuarine clams and oysters at Brooks Pond.

C. Xylene

Xylene, a hydrocarbon solvent, is included in the *B.t.k.* formulation as a stabilizer to prevent the formulation from separating into different layers and comprises 1.5 percent of the spray formulation. Concern was expressed about the health hazard of this chemical. The xylene component of 1.5 percent represents a volume of 1.71 ml/ha. In a forest canopy of 10 m, this is equal to 1.7 ppb of xylene. The occupational health hazard level is 100 ppm of xylene. The application rate of xylene in the spray formulation in a 10 m deep forest canopy is 59,000 times below the occupational health hazard level.

Analytical techniques used to monitor stream water for the presence of xylene enabled the detection of xylene at a concentration level at or above 10 ppb. No xylene was detected from stream water samples at this level.

D. Aquatic Insects

Aquatic insects are important components of the aquatic food chain and food webs. Chemical insecticides can kill aquatic insects or predispose them to lethal situations. In aquatic systems chemical insecticides can be passed along food chains or become immobilized in the soils of stream beds. Concern was expressed on the effects of *Bacillus thuringiensis kurstaki* on aquatic insect fauna. Observed fluctuations in population densities of insect families could not be associated with that of *B.t.k.* spray formulation.

E. Songbird Behaviour

Many forest songbird species are insectivorous and may be affected by insecticide through direct exposure or by eating contaminated food. One of the better methods of assessing the direct impact of xenobiotic chemicals and biocides on birds is to monitor their singing behaviour before and after spraying. Observed fluctuations in the singing behaviour of songbirds could not be associated with application of *Bacillus thuringiensis kurstaki* spray formulation.

5. COST ESTIMATES

The average cost of materials and application as reported by Quebec, Nova Scotia, Ontario and Newfoundland ranged from $15.97 to $27.98/ha (Table 9). The total operational cost of the Nova Scotia project including temporary personnel salaries, travel, biocide, equipment purchases and rentals, application, accommodation, target and non-target monitoring and egg mass survey was $48.35/ha.

6. DISCUSSION

Comparative Efficacy of B.t.

Slightly more than half (55%) of the 1979 *B.t.* treatments met the previously agreed minimum acceptable standard of 50% or less defoliation in white spruce and balsam fir stands (Table 10). Seven of the 22 white spruce and balsam fir treatments resulted in 50-60% defoliation. Whether these were considered acceptable protection by cooperators was not made clear in their reports. Cooperators might want to compare their success rate with success rates of presently used conventional chemical pesticides as a basis for the acceptability of *B.t.* Six of the 7 (86%) treatments in red spruce stands met the criterion of success. This presents an enigma since bud flaring was generally delayed and larval development was generally advanced in red spruce stands at spray time. It is obvious that research is needed to explain the high levels of protection on this tree species.

Factors which apparently influenced the successful treatments included:

1. Prespray density (with one exception) all less than 28 larvae/45 cm. branch.

2. Larval development at spray time (with one exception), peak L_4.

3. Percent bud flaring (with exception of red spruce), 80-100%.

4. BIU/ha applied ranging from 20-40 in single or double applications.

5. Ground level droplet density (with 3 exceptions) greater than 25/cm^2, based on Millipore filter data.

6. Spray time relative humidity mostly above 65%.

7. Weather conditions following spray application (with one exception), good.

Factors which apparently influenced poor results are:

1. High budworm prespray density - mostly in the 29-72/branch range.

2. Advanced larval development at spray time - mostly L_4 to L_6.

3. BIU/ha applied 20/ha nearly all in single application.

4. Heavy rains following application.

5. Low ground deposit (60% of treatments had 10 or less colonies/cm^2)

Comparative Effectiveness of Commercial Products

Thuricide, Novabac and Dipel were compared as to their overall effectiveness on white spruce, balsam fir and red spruce (Table 11). Thuricide performed well on white spruce and red spruce but not on balsam fir. Novabac appeared to have performed slightly better on balsam fir than on white spruce. Dipel 88 performed well on all three species.

Droplet counts on nutrient agar and on cards were apparently low for all Dipel 88 sprays but the Quebec trials supplied the explanation. For them, Dipel 88 gave good tree protection in spite of apparently low ground deposits on both Kromekote cards and nutrient agar plates. This is explained by the fact that the oil-water droplets released from the aircraft would be expected to lose most or all of the water phase by evaporation by the time they reach the target site leaving essentially a *B.t.*-in-oil droplet. *B.t.* is an obligate aerobe, i.e., it will not grow without air. Thus, the germination and replication of the oil coated bacteria collected directly on nutrient agar would be restricted. The fact that 8 times as many colonies grew on Millipore filters placed next to the agar plates support this view. In the latter case, the oil in the impinging droplet would readily absorb into the filter paper leaving the bacterial spores exposed to the air resulting in good spore germination and replication when placed on nutrient agar. It is evident that while the agar plate collector may be used for water-based formulation (e.g,, Thuricide and Novabac) it should not be used for Dipel 88 oil. When the *B.t.* oil droplet is ingested, the oil would be broken down in the insect gut as readily as are needle waxes, terpenes and other oils of the foliage.

Quebec recovered very low deposits on Kromekote cards in their Dipel 88 plot. Ninety-eight percent of these droplets were less than 75 μm diameter (mean droplet size was not reported) and unless highly stained, which they were not, would be difficult or impossible to observe on cards. Furthermore, such fine droplets do not quickly reach ground surface especially with some turbulence within the canopy after spray. It seems very likely that the droplets which Quebec could not find on the ground were deposited in the tree which would explain the high level of foliage protection with Dipel in that and other jurisdictions.

7. RECOMMENDATIONS FOR 1980 TRIALS

1. Coordination of *B.t.* spray trials should be continued for another year. After that, a meeting of all CANUSA cooperators and scientists should be called to formalize *B.t.* spray recommendations.

2. Support activities carried out in 1979 should continue in 1980, especially for those cooperators who lack appropriate expertise. Batch quality control by bioassay, deposit analysis and population quality checks especially should be supported financially by CANUSA where needed.

3. Aircraft calibration with spray mixes prior to spray applications is of crucial importance and should not be omitted in any spray trial.

4. Rhodamine B tracer dye has recently been confirmed as being mutagenic in *Salmonella* bacteria but Erio Acid Red 400 was not mutagenic (Dr. Ennis, FPMI). EAR-400 does not present any known environmental hazard and can be used as a tracer dye in experimental applications for deposit assessment. The use of any tracer dye with *B.t.*, however, should be avoided in cases where precise ground deposit measurements are not needed.

5. While the Nova Scotia experience indicated no non-target hazard from *B.t.* sprays in 1979, further environmental impact studies are desirable.

6. Research on an accurate method of estimating deposits on coniferous foliage is urgently needed and should be supported on a high priority basis.

7. The mode of effectiveness of *B.t.* in red spruce stands should be explored.

8. Since *B.t.* is an obligate aerobic bacterium, *B.t.*-oil formulations should not be collected directly on agar surface. Millipore filters are superior collecting surfaces since they will absorb the oil leaving spores exposed to air thus facilitating germination and replication of the bacteria.

TABLE I

KEY TECHNICAL RECOMMENDATIONS FOLLOWED BY COOPERATORS

Item	Ontario	Maine	NB-FPL	NF	NS	Quebec	UMO
Formulations							
Thuricide 16B	+	+	+	+	+		+
Thuricide 24BA				+			
Thuricide 24LC				+			
Thuricide 32B		+				+	
Novabac-3	+					+	
Novabac-45B				+			
Dipel ABG 6103		+	+			+	
Dipel 45 B			+				
<30 larvae/br.	±	+	+	+	+	+	-
Bud density	-	-	-	-	+	+	-
L_3-L_4 Timing	-	+	±	-	±	±	+
2 Applications for Mixed stands	+	-	-	NA	+	NA	NA
Min. 25 droplets/cm^2	±	-	-	±	+	+	-
Batch quality check	+	+	+	+	+	+	+
Tracer dye	+	±	±	+	-	+	-
Fettes method-def.	+	+	+	+	+	+	+
Cost estimate	+	+	-	+	+	+	-
Weather data	-	+	+	+	+	+	+

UMO - University of Maine
±, partly achieved
NA = not applicable

TABLE 2

EFFICACY OF 1979 B.t. TRIALS - ONTARIO

FORMULATION	LARVAL DENSITY, Per Br	APPL. RATE, BIU/HA TOTAL (NO. APPLS.)	PERCENTAGE		
			Pop. Red.	Defol.	Protection
NOVABAC-32B (5 plots)	wS 34-55	39.6 (2)	8 - 81	47 - 92	0 - 44
THURICIDE 16B	wS 9; bF 13	10.9 (1)	64; 94	12; 7	84; 91
THURICIDE 16B	wS 29; bF 16	18.8 (2)	19; 64	63; 55	17; 23
ACEPHATE- NOVABAC 32B	wS 47; bF 25	9.9 oz, 20 BIU	65; 86	69; 80	19; 0
THURICIDE 16B	wS 27	18.6 (2)	43 - 63	12 - 38	49 - 84
THURICIDE 16B	wS 42	19.8 (2)	48	91	0

DATA FOR EACH SPRAY BLOCK REQUESTED FOR CANUSA REPORT

1.	Province or State	Ontario
2.	Area - acres (ha)	2,000 (810 ha)
3.	Status - operational or experimental	operational
4.	Pre-spray larval density/18" branch	47 (wS), 25 (bF)
5.	Pre-spray bud density (per m^2)	N/A
6.	Spray time larval development	L_5-L_6 (*B.t.*)
7.	Percent bud flush at spray time (by tree species)	100%
8.	*B.t.* formulation and trade name	Orthene 85 SP + Novobac 32B
9.	BIU applied/acre (ha)	Orthene: 4 oz. a.i./ac (9.9 oz. a.i./ha) Novobac: 8 BIU/ac (19.8 BIU/ha)
10.	Tracer dye used	E.A.R.
11.	Applied volume rate/acre (ha)	Orthene: 20 fl. oz. (0.59 ℓ/ha) Novobac: 0.5 gal. U.S. (4.7 ℓ/ha)
12.	Number of applications	2
13.	Time between applications (days)	8 days
14.	Aircraft type used	Ag Cat
15.	Nozzle system used (boom & nozzle, micronair, etc.)	Micronair
16.	Predominant tree species	white spruce
17.	Date spray started	June 16
18.	Date spray finished	June 24
19.	Met conditions at spray time	acceptable
20.	Met conditions following spray (rain?)	acceptable
21.	Deposit rate	(Novobac), $29/cm^2$
22.	Cost/acre (ha) - optional[(a)]	$6.53 ($16.13/ha) both materials
23.	Percentage control [(b)]	wS 65; bF 86
24.	Percent defoliation (treated/check)	wS 69/85; bF 80/76
25.	Percentage foliage protection[(c)]	wS 19; bF 0

[a] Include costs of materials and application

[b] Abbott's formula: $\frac{\text{\% living untreated} - \text{\% living treated}}{\text{\% living untreated}} \times 100$

[c] $\frac{\text{Expected \% defoliation} - \text{observed \% defoliation} \times 100}{\text{Expected \% defoliation}}$

This list is essentially the same as that requested by the Forest Pest Control Forum.

DATA FOR EACH SPRAY BLOCK REQUESTED FOR CANUSA REPORT

1.	Province or State	Ontario
2.	Area - acres (ha)	490 (198 ha)
3.	Status - operational or experimental	operational
4.	Pre-spray larval density/18" branch	27 (wS)
5.	Pre-spray bud density (per m^2)	N/A
6.	Spray time larval development	L_4-L_5
7.	Percent bud flush at spray time (by tree species)	100%
8.	*B.t.* formulation and trade name	Thuricide 16B
9.	BIU applied/acre (ha)	4 BIU (9.9 BIU/ha) + 3.5 BIU (8.7 BIU/ha)
10.	Tracer dye used	
11.	Applied volume rate/acre (ha)	0.5 gal. U.S. + 0.4 gal. U.S. (4.7 ℓ + 4.1 ℓ/ha)
12.	Number of applications	2
13.	Time between applications (days)	12
14.	Aircraft type used	Ag Cat
15.	Nozzle system used (boom & nozzle, micronair, etc.)	Micronair
16.	Predominant tree species	white spruce
17.	Date spray started	June 12
18.	Date spray finished	June 24
19.	Met conditions at spray time	acceptable
20.	Met conditions following spray (rain?)	acceptable
21.	Deposit rate	N/A
22.	Cost/acre (ha) - optional(a)	$6.30 ($15.56/ha)
23.	Percentage control(b)	58-75%
24.	Percent defoliation (treated/check)	12-38/75
25.	Percentage foliage protection(c)	49-84

[a]Include costs of materials and application

[b]Abbott's formula: $\frac{\text{\% living untreated} - \text{\% living treated}}{\text{\% living untreated}} \times 100$

[c]$\frac{\text{Expected \% defoliation} - \text{observed \% defoliation}}{\text{Expected \% defoliation}} \times 100$

This list is essentially the same as that requested by the Forest Pest Control Forum.

DATA FOR EACH SPRAY BLOCK REQUESTED FOR CANUSA REPORT

1.	Province or State	Ontario
2.	Area - acres (ha)	34 (14 ha)
3.	Status - operational or experimental	operational
4.	Pre-spray larval density/18" branch	42 (wS)
5.	Pre-spray bud density (per m^2)	N/A
6.	Spray time larval development	L_4-L_5
7.	Percent bud flush at spray time (by tree species)	100%
8.	*B.t.* formulation and trade name	Thuricide 16B
9.	BIU applied/acre (ha)	4 BIU (9.9 BIU/ha)
10.	Tracer dye used	-
11.	Applied volume rate/acre (ha)	0.5 gal. U.S. (4.7 ℓ/ha)
12.	Number of applications	2
13.	Time between applications (days)	6
14.	Aircraft type used	Pawnee
15.	Nozzle system used (boom & nozzle, micronair, etc.)	Micronair
16.	Predominant tree species	white spruce
17.	Date spray started	June 13
18.	Date spray finished	June 19
19.	Met conditions at spray time	acceptable
20.	Met conditions following spray (rain?)	acceptable
21.	Deposit rate	N/A
22.	Cost/acre (ha) - optional[(a)]	$13.78 ($34.04/ha)
23.	Percentage control[(b)]	48%
24.	Percent defoliation (treated/check)	91/73
25.	Percentage foliage protection[(c)]	0%

[a] Include costs of materials and application

[b] Abbott's formula: $\frac{\text{\% living untreated} - \text{\% living treated}}{\text{\% living untreated}} \times 100$

[c] $\frac{\text{Expected \% defoliation} - \text{observed \% defoliation}}{\text{Expected \% defoliation}} \times 100$

This list is essentially the same as that requested by the Forest Pest Control Forum.

DATA FOR EACH SPRAY BLOCK REQUESTED FOR CANUSA REPORT

1.	Province or State	Ontario
2.	Area - acres (ha)	550 (223 ha)
3.	Status - operational or experimental	operational
4.	Pre-spray larval density/18" branch	N/A
5.	Pre-spray bud density (per m^2)	N/A
6.	Spray time larval development	L_5-L_6
7.	Percent bud flush at spray time (by tree species)	100%
8.	B.t. formulation and trade name	Novobac 32B
9.	BIU applied/acre (ha)	8 BIU (19.8 BIU/ha)
10.	Tracer dye used	-
11.	Applied volume rate/acre (ha)	0.5 gal. U.S. (4.7 ℓ/ha)
12.	Number of applications	1
13.	Time between applications (days)	-
14.	Aircraft type used	Ag Cat
15.	Nozzle system used (boom & nozzle, micronair, etc.)	Micronair
16.	Predominant tree species	White spruce
17.	Date spray started	June 25
18.	Date spray finished	June 25
19.	Met conditions at spray time	acceptable
20.	Met conditions following spray (rain?)	acceptable
21.	Deposit rate	N/A
22.	Cost/acre (ha) - optional[(a)]	$4.22 ($10.42/ha)
23.	Percentage control[(b)]	N/A
24.	Percent defoliation (treated/check)	
25.	Percentage foliage protection[(c)]	N/A

[a]Include costs of materials and application

[b]Abbott's formula: $\frac{\text{\% living untreated} - \text{\% living treated}}{\text{\% living untreated}} \times 100$

[c]$\frac{\text{Expected \% defoliation} - \text{observed \% defoliation} \times 100}{\text{Expected \% defoliation}}$

This list is essentially the same as that requested by the Forest Pest Control Forum.

TABLE 3

EFFICACY OF 1979 B.t. TRIALS - MAINE

FORMULATION	LARVAL DENSITY, Per Br	APPL. RATE, BIU/HA TOTAL (NO. APPLS.)	PERCENTAGE		
			Pop. Red.	Defol.	Protection
THURICIDE 16B	wS, rS 5-12; bF 13-26	20 (1)	16; 17	29; 66	37; 3
THURICIDE 32B	wS, rS 10; bF 21	20 (1)	0; 56	9; 79	80; 0
DIPEL 88	wS, rS 18; bF 24	20 (1)	0; 25	31; 44	33; 35

DATA FOR EACH SPRAY BLOCK REQUESTED FOR CANUSA REPORT

1.	Province or State	Maine
2.	Area - acres (ha)	16B-29,200 acres, 32B Thuricide - 3,000 32C Dipel - 12,000
3.	Status - operational or experimental	16B - operational, 32B - experimental
4.	Pre-spray larval density/18" branch	(See enclosed report for prespray counts for each treatment variation)
5.	Pre-spray bud density (per m^2)	
6.	Spray time larval development	Peak 4th (Index 3.8-4.0)
7.	Percent bud flush at spray time (by tree species)	Index - 4.0 Fir, 4.4 White spruce, 2.8 Red spruce
8.	*B.t.* formulation and trade name	Thuricide - 16B, Thuricide - 32B, Dipel - 32B
9.	BIU applied/acre (ha)	8 BIU
10.	Tracer dye used	Erio Acid Red in Dipel 32B
11.	Applied volume rate/acre (ha)	16B - 80 fl. oz. 32B - 64 fl. oz.
12.	Number of applications	1
13.	Time between applications ·	
14.	Aircraft type used	Thrush
15.	Nozzle system used (boom & nozzle, micronair, etc.)	Boom & Nozzle - 80-06
16.	Predominant tree species	Fir - White spruce - Red spruce, except Thuricide 32B treatment variation, - Fir - Red-Black - White spruce
17.	Date spray started	*B.t.* spray operation began 5/21/79
18.	Date spray finished.	*B.t.* spray operation completed 5/27/79 16B - 5/23, 32B Dipel - 5/22, 32B Thuricide - 5/27
19.	Met conditions at spray time	1-17 mph wind, 10-22°C; 38-91 RH
20.	Met conditions following spray (rain?)	All Blocks - rain within 36 hours
21.	Deposit rate	*B.t.* 16B Block *Bt*S - 14.13 droplets cm^2, *Bt*L - 17.30 droplets cm^2, *Bt*-U, *Bt*-T, *Bt*-D - Deposit not Adequate
22.	Cost/acre (ha) - optional -	cost figure/acre are not available under current contract arrangement with applicators

Cost figures available:

Cost of formulation as delivered	Acres per unit of formulation	AI/Acre	Cost
16B 9.30/gal.	2	8 BIU	4.65
32B 9.30/gal.	4	8 BIU	2.33

23. Percentage Control

	Fir	Spruce	
Bt-S	13.1	0	
Bt-L	39.0	0	Abbott's Other Questionable Dep.
Bt-D	24.7	0	

24. Percentage foliage protection - *Bt*-S - 34% Saved
Bt-L - 0% Saved
Bt-D - 40% Saved

TABLE 4

EFFICACY OF 1979 B.t. TRIALS - UNIV. OF MAINE

FORMULATION	LARVAL DENSITY, Per Br	APPL. RATE BIU/HA TOTAL (NO. APPLS.)	PERCENTAGE Pop. Red.	Defol.	Protection
THURICIDE 16B	bF 72		bF 71	65	33
	rS 26	20 (1)	rS 19	50	46
THURICIDE 16B + Chitinase	bF 71	20 + 81.5 mg	bF 64	68	31
	rS 25	(1)	rS 13	53	43
THURICIDE 16B + ACEPHATE	bF 67	20 + 56 gm	bF 69	54	47
	rS 27	(1)	rS 41	41	55

Population densitites extreme

DATA FOR EACH SPRAY BLOCK REQUESTED FOR CANUSA REPORT

1.	Province or State	John Dimond - Maine
2.	Area - acres (ha)	40 ha P 1-5
3.	Status - operational or experimental	
4.	Pre-spray larval density/18" branch	bF 72; rS 26
5.	Pre-spray bud density (per m^2)	ND
6.	Spray time larval development	L_3-L_4
7.	Percent bud flush at spray time (by tree species)	100%
8.	*B.t.* formulation and trade name	Thuricide 16B
9.	BIU applied/acre (ha)	20/ha
10.	Tracer dye used	Nil
11.	Applied volume rate/acre (ha)	4.7 ℓ/ha
12.	Number of applications	1
13.	Time between applications (days)	0
14.	Aircraft type used	Bell G5 helicopter
15.	Nozzle system used (boom & nozzle, micronair, etc.)	B&N, Tee Jet 8003
16.	Predominant tree species	Balsam fir - red spruce
17.	Date spray started	June 2
18.	Date spray finished	June 2
19.	Met conditions at spray time	0 mph
20.	Met conditions following spray (rain?)	heavy rain
21.	Deposit rate	7.6 droplets/cm^2
22.	Cost/acre (ha) - optional[(a)]	ND
23.	Percentage control[(b)]	bF 71; rS 18.6
24.	Percent defoliation (treated/check)	bF 65/100; rS 50/96
25.	Percentage foliage protection[(c)]	bF 33; rS 46

[a]Include costs of materials and application

[b]Abbott's formula: $\frac{\text{\% living untreated} - \text{\% living treated}}{\text{\% living untreated}} \times 100$

[c]$\frac{\text{Expected \% defoliation} - \text{observed \% defoliation} \times 100}{\text{Expected \% defoliation}}$

This list is essentially the same as that requested by the Forest Pest Control Forum.

DATA FOR EACH SPRAY BLOCK REQUESTED FOR CANUSA REPORT

1.	Province or State	John Dimond - Maine
2.	Area - acres (ha)	40 ha - P 6-10
3.	Status - operational or experimental	
4.	Pre-spray larval density/18" branch	bF 71; rS 25
5.	Pre-spray bud density (per m^2)	ND
6.	Spray time larval development	L_3-L_4
7.	Percent bud flush at spray time (by tree species)	100
8.	*B.t.* formulation and trade name	Thuricide 16B - chitinase
9.	BIU applied/acre (ha)	20 BIU + 81.5 mg chitinase/ha
10.	Tracer dye used	Nil
11.	Applied volume rate/acre (ha)	4.7 ℓ/ha
12.	Number of applicatons	1
13.	Time between applications (days)	1
14.	Aircraft type used	Bell G5 helicopter
15.	Nozzle system used (boom & nozzle, micronair, etc.)	B&N, Tee Jet 8003
16.	Predominant tree species	Balsam fir and red spruce
17.	Date spray started	June 2
18.	Date spray finished	June 3
19.	Met conditions at spray time	wind 1-2 mph
20.	Met conditions following spray (rain?)	heavy showers
21.	Deposit rate	9.7 droplets/cm^2
22.	Cost/acre (ha) - optional[(a)]	ND
23.	Percentage control[(b)]	bF 64; rS 13.1
24.	Percent defoliation (treated/check)	bF 68/100; rS 53/96
25.	Percentage foliage protection[(c)]	bF 31;rS 43

[a]Include costs of materials and application

[b]Abbott's formula: $\frac{\text{\% living untreated} - \text{\% living treated}}{\text{\% living untreated}} \times 100$

[c]$\frac{\text{Expected \% defoliation} - \text{observed \% defoliation}}{\text{Expected \% defoliation}} \times 100$

This list is essentially the same as that requested by the Forest Pest Control Forum.

DATA FOR EACH SPRAY BLOCK REQUESTED FOR CANUSA REPORT

1.	Province or State	John Dimond - Maine
2.	Area - acres (ha)	40 ha - P 11-15
3.	Status - operational or experimental	
4.	Pre-spray larval density/18" branch	bF 67; rS 27
5.	Pre-spray bud density (per m^2)	ND
6.	Spray time larval development	L_3-L_4
7.	Percent bud flush at spray time (by tree species)	100
8.	*B.t.* formulation and trade name	Thuricide 16B + acephate
9.	BIU applied/acre (ha)	20 BIU + 56 gm acephate/ha
10.	Tracer dye used	Nil
11.	Applied volume rate/acre (ha)	4.7 ℓ/ha
12.	Number of applications	1
13.	Time between applications (days)	0
14.	Aircraft type used	Bell G5 helicopter
15.	Nozzle system used (boom & nozzle, micronair, etc.)	B&N, Tee Jet 8003
16.	Predominant tree species	Balsam fir and red spruce
17.	Date spray started	June 3
18.	Date spray finished	June 4
19.	Met conditions at spray time	wind 1-2 mph
20.	Met conditions following spray (rain?0	no rain
21.	Deposit rate	5.9 droplets/cm^2
22.	Cost/acre (ha) - optional[(a)]	ND
23.	Percentage control[(b)]	bF 69; rS 41.2
24.	Percent defoliation (treated/check)	bF 54/100; 41/96
25.	Percentage foliage protection[(c)]	bF 47; rS 55

[a]Include costs of materials and application

[b]Abbott's formula: $\frac{\%\text{ living untreated} - \%\text{ living treated}}{\%\text{ living untreated}} \times 100$

[c]$\frac{\text{Expected \% defoliation} - \text{observed \% defoliation} \times 100}{\text{Expected \% defoliation}}$

This list is essentially the same as that requested by the Forest Pest Control Forum.

TABLE 5

EFFICACY OF 1979 B.t. TRIALS - NEW BRUNSWICK, FPL

FORMULATION	LARVAL DENSITY, Per Br	APPL. RATE, BIU/HA TOTAL (NO. APPLS.)		PERCENTAGE Pop. Red.	Defol.	Prot.
THURICIDE 16B** (5 plots)	wS 44		wS	86	49	0
	bF 20	20 (1)	bF	67	58	0
	rS 19		rS	75	28	0
DIPEL 88	wS 38		wS	95	33	38
	bF 21		bF	97	22	54
	rS 7	40 (1)	rS	80	23	20
DIPEL 45B + SORBITOL	bF 26		bF	99	22	54
	bS 6	22.2 (1)	bS	58	28	ND

** Heavy rains after spray