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CENTRE DE RECHERCHES FORESTIÈRES DES LAURENTIDES
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CONTENTS

ABSTRACT

A 10,000 acre stand of mature balsam fir, in Temiscouata County, Quebec, which had a spruce budworm population of about 21 larvae per 18-inch branch tip was sprayed with a Bacillus thuringiensis + chitinase formulation. Aerial spraying was carried out by 3 TBM aircraft between June 4-7, 1972, when insect development was at the peak of the third instar. Where the quantity of B. thuringiensis colonies per cm^2 was higher than 77 and spray deposit higher than 0.4 gal (US)/acre, larval mortality was between 84% and 93%.

Mortality in the control plot was between 39% and 53%. Foliage protection was 47%. The results indicate the possibilities of using B. thuringiensis + chitinase to control spruce budworm infestations.

RESUMÉ

Dix milles acres d'un peuplement de sapin baumier ayant un rendement de 15 cordes de bois de pulpe à 1'acre ont été aspergés avec une formule à base de Bacillus thuringiensis et de chitinase. L'application aérienne eut lieu entre le 4 et le 7 juin 1972 alors que la majorité des larves avait atteint le 3ième âge larvaire. Trois appareils de type TBM équipés d'un système d'arrosage à gicleur ont été utilisés pour répandre la formule au rythme de 2 gal (US)/acre. Dans les places échantillons où le nombre de colonies de B. thuringiensis par cm^2 était supérieur ou égal à 77, et où le volume déposé excédait 0.4 gal (US)/acre, la mortalité larvaire varia de 84 à 93%. Dans les places témoins la mortalité s'échelonna de 39 à 53%. La protection accordée au feuillage fut de 47%. Les résultats de cette expérience sur 10,000 acres démontrent clairement les possibilités concrètes offertes par la formule B. thuringiensis + chitinase pour le contrôle des épidémies de la tordeuse des bourgeons de l'épinette.

FORWORD

Throughout the world and particularly in the Canadian Forestry Service research projects are being developed to reduce the severe damage caused by insect outbreaks to the forest environment. Along with chemical methods of control, biological methods are being investigated.

We, of the Laurentian Forest Research Centre, are very proud to bring our contribution to such an endeavour. Although our activities within the field of biological control of forest insect constitute only part of our total research effort, imaginative thinking by our scientific staff has lead to investigations on means of controlling insect outbreaks by integrated and biological methods. This present report deals with the results of one such investigation, which consists of an aerial spraying operation with Bacillus thuringiensis + Chitinase for the control of the spruce budworm. We have been very fortunate to obtain the help and expertise of:

a) La Direction de la Conservation du Ministère des Terres et Forêts du Québec,

b) The Chemical Control Research Institute of the Canadian Forestry Service,

c) The Forest Insect and Disease Survey of our own establishment.

At this time, it is obvious that the cost of commercial application of Thuricide^{R}-HPC-Chitinase is still high and this may prevent its use over large areas. However, as this has been the case with all

newly developed methods, refinements in techniques of insecticide production and aerial application will undoubtedly reduce the cost. Hence we feel that the methods developed by Dr. W.A. Smirnoff will be readily accepted in the future.

Marcel Lortie,
Director,
Laurentian Forest Research Centre,
Canadian Forestry Service,
Ste. Foy, Quebec.

During the last two years, in an attempt to find a substitute to chemical insecticides for the control of the spruce budworm outbreaks, the Department of Lands and Forests of Quebec agreed to participate in biological control experiments conducted in the vicinity of Lake Temiscouata, Quebec, by Dr. W.A. Smirnoff, Laurentian Forest Research Centre, Canadian Forestry Service.

The Department of Lands and Forests contribution consisted in providing the services of a technician and seasonal workers, and of supplying aircraft required for the spraying of the bacterial suspension against the spruce budworm.

In 1971, with the use of a Stearman aircraft, the first aerial experimental treatment was made on two 100 acre plots, one was sprayed with Bacillus thuringiensis + the enzyme chitinase, the other was sprayed with B. thuringiensis alone. The addition of the enzyme chitinase was found to increase the B. thuringiensis efficiency against the spruce budworm.

The results of this first treatment were very satisfactory and a second test was performed this year with the same bacillus + enzyme chitinase formulation. This aerial spraying was made with three T.B.M. Avenger aircraft. The results of this second test are reported in this publication.

These two bacteriological control operations mark a real progress in the search of a way to control the spruce budworm. To date, aerial application of chemical insecticides were the only way to protect

our forests from the destructive action of the spruce budworm. If treatment with the bacillus + enzyme demonstrate the possibility of controlling populations of spruce budworm efficiently and economically, the research conducted by Dr. Smirnoff with assistance from the Department of Lands and Forests, will have found a solution which does not present any danger to the environment.

Gérard Paquet,
Director,
Forest Entomology and Pathology Branch,
Department of Lands and Forests,
Quebec.

INTRODUCTION

The action of Bacillus thuringiensis Berliner on spruce budworm (Choristoneura fumiferana (Clem.)) larvae has been under investigation by the insect pathology unit of the Laurentian Forest Research Centre, Department of the Environment, Canada, for several years. Initial field tests against the insect were made in 1959 with the first available commercial preparations of B. thuringiensis (Angus, et al., 1961, Smirnoff, 1963a, b).

Commercial preparations of the organism contain two active agents, spores which initiate septicemia in the host larvae of Lepidoptera, and parasporal crystals which are toxic to certain species of Lepidoptera. Our studies showed the crystals play only a minor role in spruce budworm mortality, and that larval mortality is mostly the result of a typical septicemia enterotoxinosis. The incubation period and the virulence of the disease are affected by environmental conditions, particularly temperature (Smirnoff, 1967). Low temperatures (14 to 18 C), usual during larval development in the field, are unfavourable to the action of B. thuringiensis for spruce budworm control.

Studies on the action of the bacteria in spruce budworm larvae showed the septicemia did not begin until the organism had penetrated the gut wall and entered the hemolymph (Smirnoff, 1971). It was thought that disruption of the chitinous lining of the gut lumen would allow a better contact of ingested spores with cells of the gut wall, and facilitate penetration. Tests made in the winter of 1971, showed that traces

of the enzyme chitinase (which hydrolyzes chitin) in B. thuringiensis preparations administered to spruce budworm larvae could accelerate development of the disease and increase the mortality rate attributable to the bacillus, even at temperatures in the normally critical range of 14 to 18 C (Smirnoff, 1971).

On the basis of these results, an experimental aerial spraying with Thuricide HPC + Chitinase and Thuricide HPC alone was carried out on two 100-acre blocks of a spruce budworm infested balsam fir forest in the Lower St. Lawrence region of Quebec. The experiment carried out with the co-operation of the Department of Lands and Forests, Quebec, and the technical assistance of the Chemical Control Research Institute, Ottawa, was a success. Larval mortality was 93% in the plot treated with Thuricide + Chitinase, 85% in the plot treated with Thuricide alone and 54% in the control plot (Smirnoff et al. 1972, Smirnoff et al. in press). The tested formulation also proved its value as far as foliage protection is concerned since only 24% of the current year shoots were destroyed in the plot treated with Thuricide + Chitinase, compared to 65% in the plots treated with Thuricide alone and 87% in the control plot. In addition to demonstrating the feasibility of the Thuricide - Chitinase formulation as a way of controlling the spruce budworm, the results of this experiment showed that Thuricide can be applied by aircraft, that B.t. can survive for 30 days on foliage, and that no pollution occurred from spraying with the B.t. formulations.

The success of the experiment led to the aerial spraying of the Thuricide - Chitinase formulation over 10,000 acres of infested balsam fir forest in 1972. This operation was realized through the cooperation of different agencies: the insect pathology unit of the Quebec Laboratory prepared the report on laboratory tests and all biological, bacteriological and biochemical data; the Chemical Control Research Institute provided calibration of aircraft and assessment of spray deposit; the Conservation Branch of the Quebec Department of Lands and Forests provided the data on defoliation and foliage protection resulting from treatments with B.t. and chemical insecticides.

METHODS AND MATERIALS

The Territory

A 10,000 acre area (6.3 x 2.5 miles) was selected in Seigneurie de Madawaska, midway between Cabano and St. Michel de Squatec, Témiscouata County, Quebec. The forest, of the Abietum hylocomietosum type, was fully stocked and composed of 80% balsam fir, Abies balsamea, 55 to 65 years of age, the other tree species being eastern white cedar, Thuja occidentalis, black spruce, Picea marinana, and white spruce, Picea glauca. Twenty-five sample plots were selected at random throughout the area. At each plot 5 codominant balsam fir trees were selected at 2-chain intervals, the first sample tree was at least 3 chains from the edge of the forest. Nine sample plots were established in a similarly infected stand, a distance of 5 miles from the sprayed areas to serve as controls. Trees and shrubs

were cleared from around the sample trees to ensure even spray coverage. Numbered floor tiles were placed in the cleared area near each sample tree. Larval-drop tables, measuring 30 sq. ft., were placed under the crown of two trees in each plot to collect falling insects and to provide information on progress of the disease in the budworm population and its possible effect on associated insects.

Larval population sampling

Larvae were collected daily, so as to determine insect development. Population changes were monitored before and at intervals after spraying. Branches from the mid-crown of sample trees were obtained with a pole pruner equipped with a collecting bag. One 18-inch branch tip per tree constituted a sample; each sample was enclosed in a polyethylene bag for transport to the laboratory. Counts of larvae and pupae were made by hand picking or by shaking branches within a horizontal screened metal drum (Martineau and Benoit, 1973). The population was recorded in terms of larvae per 18-inch branch tip and per sq. ft. of foliage.

Dead larvae from sample branches, and from larval drop tables, were examined microscopically for the presence of B. thuringiensis cells. Weight and length of larvae and pupae were compared at various periods during larvae development. Also, biochemical analyses were conducted to determine the effect of the bacterial infection on the physiological condition of larvae and pupae. Frass that fell on the cloth tables was weighed to determine the relative amount of food eaten by infected and

control larvae. An egg count was made during the fall of 1972 to obtain an index of the following year's population.

The estimation of defoliation was usually made by visual observations. This was done by having a few observers estimate the percentage of buds remaining on tree crowns and averaging the figures given by each. Last year an attempt was made to develop a more accurate method of estimating tree defoliation. The 18-inch branch tips obtained for the pupal check were examined and the current year foliage was classified as: completely destroyed, partially destroyed or intact. This year, the entomology division of the Conservation Branch, Quebec Department of Lands and Forests, and the Department of Forest Management and Sylviculture, Faculty of Forestry, Laval University proposed a new method. This consists in cutting a branch from the upper part of the middle third of sample trees in both the test and the control stands. The percentage of needles on nine current year shoots and the proportion of the 27 buds which should produce the 1973 growth are estimated. This method permitted the classification of needle defoliation into 11 categories, from intact needles, number 1, to complete defoliation, number 11.

Spray application

Spraying was done by means of 3 T.B.M. aircraft each of which carried 625 US gallons of formulation. Each aircraft was equipped with boom and nozzles spray delivery system. These aircraft were guided by Cessna pointers as commonly used for the application of chemical insecticides. Spraying took place between June 4 and 7, 1972. The formulation

per acre was:

Thuricide HPC concentrate[1]	0.5 gal (US)
Polyglycol 400[2]	0.5 gal (US)
Chevron spray sticker[3]	0.16 oz (US)
Water	1 gal (US)
Chitinase (activity, 950 nephelemetric units)	10 mg.

With the exception of the Chevron spray sticker the formulation was similar to the one used in 1971. This sticker proved to be more efficient, less expensive and can be used in smaller quantities than the Nu-Film-Bt. This formulation gave a high quality suspension and was easily mixed and pumped with the equipment used in commercial aerial spraying.

Spray deposit sampling

Spray deposit was assayed physically and bacteriologically. Prior to the spray application, a 10 cm petri dish of nutrient agar and two 50 sq. cm Kromekote cards were placed on a numbered floor tile under each sample tree. The cards were recovered immediately after spraying; one set of cards was submitted to the Chemical Control Research Institute, Ottawa, for analysis, the other was retained as a record. Bacillus thuringiensis colonies on the petri dishes were counted after incubation

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[1] International Minerals Corporation, Libertyville, Illinois, U.S.A.

[2] Dow Chemical of Canada, Limited, Sarnia, Ontario.

[3] Chevron Chemical (Canada) Limited, Oakville, Ontario.

by means of a Quebec Bacteria Counter[1].

Distribution and persistence of B. thuringiensis was also monitored at the plots. Several days after treatment, the foliage was tested for viable spores on culture medium. Air in the plots was sampled with a Luckiesh-Holladay-Taylor electrostatic bacterial air sampler[2], and cultured on nutrient agar plates. A small particle detector[2], type CN, was used to sample the concentration of air-borne particles in the plots. After 5 days incubation at 30 C, bacterial colonies were counted and the presence of B. thuringiensis verified by microscopic examination of smears.

Calibration of Grumman Avenger (T.B.M. aircraft)

The Chemical Control Research Institute, represented by Mr. A.P. Randall, accepted the responsibility for the calibration and performance of aircraft assigned to the project. The area treated proved to be too large to permit use of ultra low volume equipment, and the sprays were applied with three T.B.M. aircraft flying in formation.

The aircraft were equipped with boom and nozzles spray delivery systems and were calibrated to deliver the required dose of acceptably fine droplets according to a plan approved by the Quebec Laboratory and CCRI.

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1 Fisher Scientific Co. Montréal, Québec.

2 Gardiner Associates Inc., Schenectady 3, New York, U.S.A.

Estimation of deposit

The system of sampling the deposit by means of special deposit cards was designed and assessed by Mr. W. Haliburton of CCRI. Six sets of sample cards were exposed at pre-determined locations for each of the seven flight periods. The nominal emission rate for each flight was 0.5 gal/acre (U.S.). The entire plot being covered four times for a total emission rate of 2 gals/acre (U.S.).

Assessment of spray deposit on the Kromekote cards was difficult because marker dye could not be used in the spray formula for fear of spore inactivation. However, because of its solid content, dried spray drops on the cards could be seen as dull spots under magnification by reflected specular light. The drop traces were made readily visible by rubbing the cards lightly with dry Rhodamine B dye, which adhered to the solids and gradually stained the residual low volatile liquid component of the drops which had soaked into the cards.

The dyed spots were projected onto a Microcard[1] reader screen, measured with a calibrated transparent wedge-scale, and tallied sequentially in 20 μm diameter classes on successive square centimeter areas on the cards. Class mean numbers per square centimeter were totalled to give drop density values for each card. The factor 0.5 was used to convert spot size to drop size. This factor was obtained by a rough determination of the drop diameter to spot diameter ratio of a narrow range

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[1]Present equivalent: National Cash Register Co., Micro-opaque Card, Reader Model 456-722.

of laboratory produced drops, sorted to size in a wind tunnel. The total formula of size class densities and appropriate volume conversion values yielded deposited volume values for each card in gallons per acre. It was recognized these values were relative, and much below the emitted rate, as there was no way to measure evaporation from the drops between emission and deposition.

RESULTS

Spray coverage

The mean of the summated deposit estimate was 0.37 ± 0.32 gal/acre with a range from 0.005 to 1.33 gal/acre. The coverage was quite variable, indicating varying degrees of sample exposure to the open sky or chance variation in swath spacing and overlapping. The low mean deposit estimates appear low but are probably attributable to evaporation of possibly up to 70% of water from the formulation. Furthermore, an unknown portion of the spray cloud may not have reached the cards because of screening by foliage and perhaps a basic discrepancy in the arbitrary stain-to-drop-size conversion value of 1 to 1.8. The low estimate of deposit represents a normal situation where deposit on the ground can be expected to produce only 25-50% of the nominal emission rate of the aircraft, depending on foliage screening, meteorological conditions and evaporation rates.

Biological observations

Populations in the treated and control areas were about the same prior to treatment; 19.5 larvae per sq. ft of foliage in the treated area and 21.3 larvae per sq. ft of foliage in the control plots.

Spraying was done at the peak of the third instar; when 5.2% of the larvae were at the second, 75.5% at the third, 18.6% at the fourth and 0.7% at the fifth instar.

Larval mortality figures were established for the entire area sprayed and for the control plots. In the total sprayed territory the deposit averaged 0.4066 gal/acre ranging from 0.0045 to 1.3237 gal/acre and mortality was 73.1% with a low of 51% and a high of 93%. In the treatment category where spray deposit was lower than 0.4 gal/acre, it averaged 0.1626 gal/acre ranging from 0.0045 to 0.3477 gal/acre and mortality was 64% with a low of 51% and a high of 76%. In the plots where the deposit was higher than 0.4 gal/acre the deposit averaged 0.6506 gal/acre ranging from 0.4037 to 1.3237 gal/acre and larval mortality was 88.2% with a low of 84% and a high of 93%. No traces of deposit was registered in control plots neither on spray cards nor, on agar plates, and larval mortality averaged 48.5% with a low of 39% and a high of 53% (Table 1). The number of dead larvae recovered in the buds during estimation of the larval population averaged 27.2% (4 to 43%) in the treated area and 8% (3 to 18%) in controls (Table 1).

No dead defoliating insects, other than spruce budworm, were found on the drop tables. Puparia of dipterous parasites of spruce budworm found on the tables were in good health.

The count of egg masses carried out in August and September revealed that in more than 50% of the sample plots in the treated area, the number of egg masses was between 258 and 653 for 500 sq. ft of foliage, pointing to a low infestation for 1973. In the control area, the number of egg masses was between 952 and 2897 pointing to a severe infestation for 1973.

Larvae were weighed and measured periodically in treated and control plots. Results showed that larvae from treated plots were lighter and shorter than those from the control plots (Table 1). For example, measurements of weight and length of larvae carried out on June 22, 14 days after spraying, revealed a 57% decrease in the weight and 27% decrease in the length of larvae in treated plots (Table 1). Weight of male pupae from treated plots was 34.8 mg and females 42.8 mg while in the control area male pupae weighed 39.8 mg and females 48.7 mg. Further evidence of the perturbations observed in larvae infected by B. t. was shown by measurements and weight of frass. The average weight of frass per day of drop-tables was 6.9 grams in the treated area and 11.0 grams in controls while the average diameter of frass was 0.7 mm in the treated area and 1.1 mm in controls.

Defoliation

By using the new method proposed this year it was established that 47% of the foliage in the treated area and 12% in control area remained on trees. However, accuracy of the method is still questionable.

Biochemical assessment

Biochemical analyses were conducted on larvae and pupae to determine changes in the metabolism and the physiological conditions of larvae and pupae infected by B. thuringiensis. The criteria studied were the quantities of certain metabolites and total lipids. The activity of the transaminases (GOT) and (GPT) did not vary, but that of the dehydrogenases was greatly reduced (ICDH from 603 to 186 mU/ml) and that of the phosphatases increased (alkaline phosphatase from 200 to 490 mU/ml) (Table II). Furthermore, the infection provoked a strong decrease, from 4.8 to 2.7%, in the lipid reserves of the organism and a strong elevation, of the amount of chloride in the hemolymph. A strong increase of transaminase activity took place in infected pupae (GOT - up to 32, mU/g) while that of the dehydrogenase increased from 38.8 to 11.2 mU/g, and the activity of the phosphatases was also superior (phosphatase alkaline: 185 mU/g as compared to 120 mU/g in the control). A strong cholinesterase activity was observed in the healthy pupae (49.0 mU/g) whereas it diminished to 17.5 mU/g in infected pupae. The amount of chloride was the same in infected healthy pupae, while the amount of the total lipids decreased by half during the infection (from 5.3 to 2.9%).

DISCUSSION AND CONCLUSIONS

In the area treated with B.t. + Chitinase in 1971, the number of

eggs masses indicated a low infestation for 1973. In the areas where the spray deposit was higher than 0.4 gal/acre and the number of B.t. colonies per cm^2 of agar plate was higher than 77, average mortality was 88.2%. In the remaining 30% of the stand, average mortality was 64%.

It was the first time that TBM aircraft were used for treatment with B.t. It was found that the boom and nozzles type of equipment was less efficient than the Micronair system on Stearman plane used in the 1971 operation (Smirnoff et al. in press). The Micronair permitted the emission of smaller droplets. In fact, with the Micronair 100% of the emitted volume was in the droplet range under 200 µm while with the boom and nozzles only 60% of the emitted volume was in this droplet category. The remaining 40% of the total volume was in droplet size categories between 200 µm and 450 µm (Fig. 1).

Although it proved effective, the formulation is still expensive. The price of Thuricide is high and the adjuvant, Polyglycol 400 costs $2.50 per gallon US. To be economical, the price of the adjuvant should not exceed 50 to 60 cents per gallon. Investigations are now being conducted to find a substitute.

The formulation Thuricide + chitinase proved to be much more effective than Thuricide alone. This was shown in 1971 and again in 1972 by Dr. Dimond, University of Maine, who conducted a series of tests with Thuricide alone and Thuricide + chitinase. He reported that: "Only the formulations with the chitinase additive gave satisfactory spruce budworm

mortality and foliage protection".

The B.t. + chitinase formulation provoked a rapid septicemia. Infected larvae lost their appetite, shrank, and lost weight before dying which resulted in less damage to trees; foliage protection was evident, particularly at top of trees. Best results were obtained when larvae were treated at the peak of third instar.

Results obtained with the proposed method for the estimation of foliage protection, 47% in the treated and 12% in the control area, differ from those obtained through visual estimates namely 70% and 10% for treated and control areas respectively. We feel that the proposed method may be more accurate than the visual estimates. Observer bias, the position of the tree crown, degree of visibility, etc... could affect the accuracy of visual estimates. Comparisons were made to evaluate foliage protection resulting from spraying with a chemical insecticide, and with B.t. By the proposed method, it was established that the insecticide fenitrothion gave 44% protection while B.t. gave 47% protection. The method of estimating defoliation is however new and will need to be improved.

Since commercial chitinase is sold at about $200 a gram, its cost was prohibitive even for a small quantity. However, we developed a semi-industrial technique for the production of the enzyme chitinase from chicken gastric juices. We established that by using this method the cost of one gram of chitinase was reduced to approximately $8. Upon our request, specialists from Canada Packers established the price at $13 a gram (between 8 - 13 cents per acre).

The spraying rate that was used (2 gal/acre) could possibly be reduced but only by using spraying equipment that give droplets ranging from 50 to 200 μ.

Treatment with B.t. + chitinase appeared to have no effect on insect species other than the spruce budworm. Dipterous and hymenopterous parasites were unaffected. Laboratory experiments conducted in 1971 and 1972 with Thuricide HPC + chitinase formulation revealed that this mixture had no effect on birds or mice. It should be remembered that the enzyme chitinase is present in free form in the gastric juices of all insectivorous animals. Measurements of the number of particles in the air in the experimental area prior to and at various periods after treatment, revealed that the quantity of particles remained about the same, 5×10^4 particles per cm^3 of air. This indicated that no air pollution resulted from the mass application of bacteria by means of aircraft.

The experiment was conducted on a 10,000-acre area with the express purpose determining the practicability of using the B.t. + chitinase formulation for the control of the spruce budworm on a large scale. In this respect, research is being continued with the objective of decreasing the cost of the final formulation and of its dispersion.

RECOMMENDATIONS

The use of *B.t.* + chitinase formulation may be used in areas where treatments with chemical insecticides are undesirable.

Spray systems should be modified to produce smaller droplets (100 - 200 µm) and a more uniform coverage.

Evaporation in atmosphere should be reduced to a minimum by modifying the formulation (particularly the adjuvant). The minimum effective deposit should be 0.5 gal/acre.

The industrial production of chitinase should be undertaken to reduce costs.

ACKNOWLEDGMENTS

Sincere thanks are expressed to the Deputy Minister and staff, Department of Lands and Forests, Quebec; to the Chemical Control Research Institute, Ottawa, particularly, Mr. A.P. Randall and W. Haliburton; to Drs. R.F. Anderson and M. Rogoff, International Minerals Corporation, Chicago, Illinois, U.S.A., for their technical assistance.

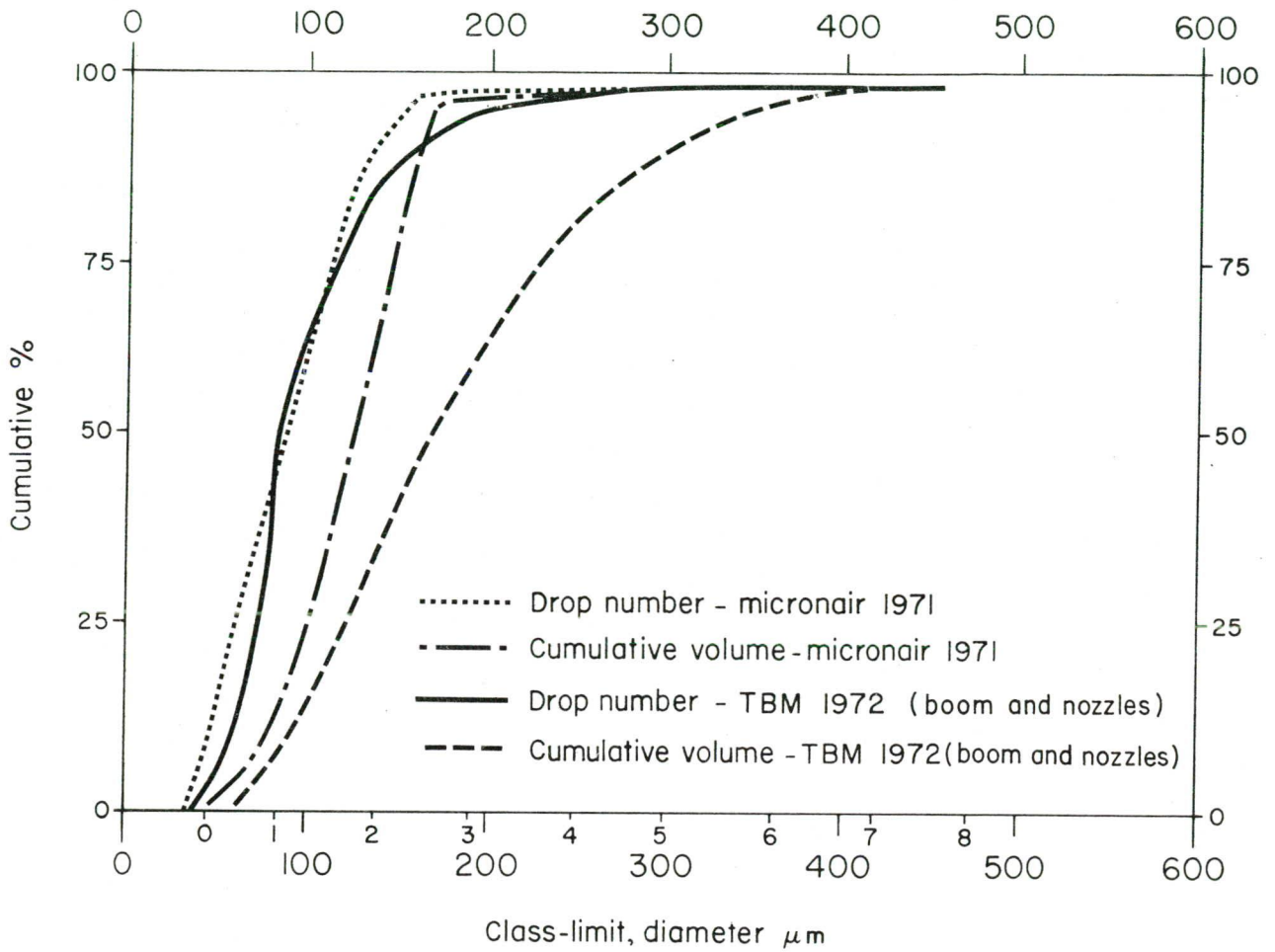

Figure 1. Comparison between spray deposit of Bacillus thuringiensis formulation with Micronair and with boom and nozzles showing drop size vs cumulative frequency and volume established by the reading of sample cards.

TABLE I

Results of the aerial application of Thuricide HPC + chitinase over 10,000 acres of spruce budworm infested balsam fir forest - Temiscouata 1972

% larval mortality				% dead larvae in the buds		Weight and length of living larvae collected at time indicated							
						6/15/72		6/22/72		6/28/72		7/12/72 (pupae)	
Total sprayed area .0045-1.3237 gal/acre	Lower spray deposit areas, 30% of the territory .0045-.3477 gal/acre	Higher spray deposit areas 70% of the territory .4037-1.3237 gal/acre	Control	Treated area	Control	Weight gm	Length mm	Weight gm	Length mm	Weight gm	Length mm	Weight ♂ gm	Length ♀ mm
						Treated areas							
73.1	64.0	88.2	48.5	27.2	8.9	.0054	6.56	.0176	10.22	.0497	13.91	.0348	.0421
(51-93)	(51-76)	(84.93)	(39-53)	(4-43)	(3-18)								
						Control							
						.0073	6.92	.0410	13.94	.0579	14.16	.0398	.0487

TABLE II

Enzyme activity[1] and amount of certain metabolites in the hemolymph of spruce budworm larvae and pupae, infected with Bacillus thuringiensis

		GOT[2]	ICDH[3]	GLDH[4]	ALKALINE PHOSPHATASE	ACID PHOSPHATASE	CHOLINESTERASE	ALDOLASE	GLYCEROL mg %	CHLORIDE mEq/L or Kg	TOTAL LIPIDS % FRESH WEIGHT
HEMOLYMPH OF LARVAE	THURICIDE + CHITINASE	170.0	186.0	210.0	490.0	50.0	NIL	8.0	4.40	100.5	2.70
	CONTROL	190.0	603.0	100.0	200.0	NIL	NIL	12.0	7.20	36.0	4.80
PUPAE	THURICIDE + CHITINASE	32.0	11.2	NIL	185.0	NIL	17.5	4.0	6.40	32.0	2.90
	CONTROL	NIL	38.8	12.0	120.0	NIL	49.0	8.0	10.90	32.0	5.30

[1] Results are in mμ/ml in the hemolymph and mμ/g in pupae

$$1 \text{ m}\mu/\text{ml} = \frac{1 \text{ m}\mu \text{ converted mole substract}}{1 \text{ min} \times 1 \text{ ml}}$$

[2] GOT : glutamic oxaloacetic transaminase
[3] ICDH: isocitrate dehydrogenase
[4] GLDH: glutamic dehydrogenase

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