FIELD TESTS WITH A HIGHLY CONCENTRATED *BACILLUS THURINGIENSIS* FORMULA AGAINST SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.) (LEPIDOPTERA: TORTRICIDAE)

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Field tests were conducted in 1980, 1981, and 1982 with a new *Bacillus thuringiensis* formula called Futura. Tests were conducted with a Grumman AgCat aircraft on 40 ha forest blocks and with a DC-4G aircraft on larger forested areas. Futura which is able to disperse the required 20×10^9 I.U./ha for spruce budworm control in 2.5 L/ha proved to be physically and bacteriologically stable and easy to mix. Also it assured a deposit of up to 80% emitted volume, a larval mortality in the range of 70 to 90%, and a foliage protection of about 70%. Because of its low-volume dispersion property and other qualities it is a most valuable alternative to chemical insecticides for spruce budworm control.

Introduction

Investigations with *Bacillus thuringiensis* carried out during the past 10 years, have shown that this entomopathogen can be used effectively for the operational control of spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) (Smirnoff 1978, Smirnoff 1979, Smirnoff 1980a). This work led to the development of a compact, efficient, and economical *B. thuringiensis* suspension which could be dispersed at the dosage of 20×10^9 I.U. in a final volume of 2.5 L/ha (Smirnoff 1981). This preparation, called Futura, was tested during calibration tests (Smirnoff 1980b, c) and field tested in 1980, 1981, 1982, and 1983 (Smirnoff *et al.* 1982, Smirnoff and Valéro 1983).

Materials and Methods

All stands were composed of 70% balsam fir, *Abies balsamea* (L.) Mill., with white spruce, *Picea glauca* (Moench) Voss, trembling aspen, *Populus tremuloides* Michx., and white birch, *Betula papyrifera* Marsh., as remaining species. These stands were 55 to 65 years old and considered adequate for the requirements of pulp and paper industries.

The tests were conducted in the Chicoutimi region of Quebec on 40 ha blocks of forest severly infested with spruce budworm. Three blocks (one each year) of 3 500 ha, were treated by the Quebec Department of Energy and Resources in the Rivière-du-Loup region of Quebec. Three sample plots, consisting of transects with 5 marked trees, one chain apart, were established in each 40 ha block and eight in a nearby untreated area. Nine sample plots were established in the 3 500 ha treated blocks and eight in a nearby untreated area.

Samples were taken from the upper part of the median section of trees. To assess larval mortality, two samples were made before, and four after treatment in each of the sample plots. Post-treatment samples were collected 10 days after spraying, at peak L_6 and again at 90% adult emergence. For determining defoliation, the methods of Fettes (1950) and Dorais and Hardy (1976) were used. Also, assessment of the current year growth mass was carried out. Tree mortality, efficiency of treatment sequences, loss of wood volume, and stand improvement were assessed in 0.04 ha sample plots established in each treated block and in the untreated area.

The 40 ha blocks were treated with a Grumman AgCat aircraft equipped with a boom and nozzle spray system composed of flat fan (T8004) nozzles. The 3 500 ha blocks were done either with a DC-4G or a Super Constellation L-749 aircraft equipped with a boom and nozzle spray system composed of 110 open type nozzles evenly distributed on each boom in 1981 and 1982, and close to fuselage in 1983 to improve deposit. The spray system on both aircrafts had been calibrated extensively to optimize deposit[1]/. For the three years the dispersions were carried out from June 3 to 15, temperature varied between 1°C and 14°C, relative humidity between 60 and 100%, and winds were always below 10 km/h.

The method of Smirnoff (1980b, 1982) was used to assess deposit in number of viable spores per square centimetre. This implies distribution of containers with peptonized water at 30 m intervals along a transect perpendicular to flight lines.

Concurrently, biochemical analyses were conducted on residual pupae from blocks treated with *B. thuringiensis* and from the untreated area to determine any residual effect of *B. thuringiensis* on the spruce budworm populations (Smirnoff 1983). Measurements were also made on residual pupae from blocks treated with chemical insecticides to obtain comparative values.

Formulas used:
1.- Futura [2]/: 15% *B. thuringiensis* primary powder (80 000 International Units/mg); 44.8% Sorbo (an aqueous 70% sorbitol solution); 33.7% water; 6.5% inert ingredients; and 1/1 600 Chevron sticker.

[1]/Smirnoff and Valéro, submitted, Environment Canada, Canadian Forestry Service Res. Notes.
[2]/Biochem Products, Montchanin, Delaware, USA

Each hectare was sprayed with 1.5 L of the above preparation diluted with 1 L of non-chlorinated water and 1.56 mL of Chevron sticker.
2.- Thuricide 48B [3]/: 2 parts Thuricide 48B; 1 part non-chlorinated water; and 1/1600 Chevron sticker.
3.- Thuricide 48LV [3]/: 50 parts Thuricide 48LV; 20 parts Sorbo; 30 parts non-chlorinated water; and 1/1600 Chevron sticker.

Results

Field tests

1980. In 1980 spruce budworm populations in the region of the field station, Chicoutimi, Quebec, were rather low and tests aimed mainly at comparing Futura with the Thuricide + sorbitol formula used at 4.7 L/ha in large field tests. Results revealed that deposit with Futura was good with 348 756 viable spores per square centimetre over a theoretical emission of 445 000 spores per square centimetre meaning that 80% of emitted number of spores were deposited at ground level. Larval mortality was 72.2% and defoliation 7.45%. These figures compared advantageously with results given by the Thuricide + sorbitol formula and were of course much better than those obtained in untreated plots. Futura dispersed well, provoked a suitable larval mortality, and protected foliage sufficiently. The next step was to test it in severely infested stands supporting various degrees of defoliation.

1981. In 1981 three series of field tests were carried out. The first treated 7 x 40 ha plots with Futura at 2.5 L/ha (20×10^9 I.U./ha) using a Grumman AgCat aircraft. The second test treated 895 ha with Futura at 2.35 L/ha (18.8×10^9 I.U./ ha) by means of a Super Constellation L-749 aircraft. The third test, carried out simultaneously with the first test as a compagnion, involved treatments using Thuricide 32B/sorbo/water/chitinase/Chevron (20×10^9 I.U./ha in a final volume of 4.7 L/ha) by means of a Grumman AgCat aircraft. Results are summarized in Table 1. They show that Futura dispersed by the Grumman AgCat was able to yield a good larval mortality and foliage protection. Larval mortality was 72.0% 10 days after treatment dispersed at 2.5 L/ha compared to 70.3% with Thuricide + sorbitol, dispersed at 4.7 L/ha. Defoliation with Futura averaged 44.1%, but some of the blocks were in a desperate situation after several years of severe defoliation. Thuricide + sorbitol yielded a 67.0% defoliation for a stand in an acceptable condition (Table 1).

Dispersion of Futura by means of the Super Constellation aircraft at 2.5 L/ha yielded a 75.0 to 96.9% larval mortality for a pretreatment population averaging 27.5 larvae per 45 cm with a peak at 46.2 larvae per 45 cm (Table 1). Defoliation was 25 to 40% in plots with 10 to 20 larvae per 45 cm and 50 to 60% in plots with 25 to 35 larvae per 45 cm.

Finally, foliage potential (buds formed in the fall) increased in all treated plots and decreased in untreated plots (Table 1). At the end of the 1981 field season it was concluded that Futura was able to kill spruce budworm and protect foliage with half of the volume of the B. thuringiensis formulas used precedingly, 2.5 L/ha instead of 4.7 L/ha.

1982. Five 40 ha blocks were treated with Futura (20×10^9 I.U. in 2.5 L/ha); one 40 ha block with Thuricide 48B (20×10^9 I.U. in 2.5 L/ha); another 40 ha block with Futura (30×10^9 I.U. in 3.75 L/ha); and one more with Thuricide 48LV (30×10^9 I.U. in 4.7 L/ha). The aim was to determine the efficiency of Futura in different conditions of stand deterioration and population levels and compared it with a higher dosage of an other formula. Results are summarized in Table 2. Also, a 3 500 ha block was treated with Futura by means of a DC-4G aircraft.

The number of B. thuringiensis viable spores initially emitted was 445 000 per square centimetre for a dosage of 20×10^9 I.U./ha and 667 500 per square centimetre for a dosage of 30×10^9 I.U./ha. In blocks treated with Futura at 2.5 L/ha for a dosage of 20×10^9 I.U., the number of viable spores deposited varied from 269 830 to 406 821 spores per square centimetre and averaged 351 848 per square centimetre. Deposit was 268 862 spores per square centimetre with Thuricide 48B. In blocks treated with a dosage of 30×10^9 I.U./ha, deposit was 520 300 viable spores per square centimetre for Futura (3.75 L/ha) and for Thuricide 48LV (4.7 L/ha), 608 388 spores per square centimetre. Deposit with Futura dispersed with a DC-4G was 222 400 viable spores per square centimetre (Table 2). Deposit varied from 60 to 90% of the emitted number of spores during treatment with the Grumman AgCat aircraft.

Larval mortality established 10 days after treatment averaged 91.5% in blocks treated at 20×10^9 I.U./ha compared with 95.3% in blocks treated at 30×10^9 I.U./ha showing that higher dosage has little effect on spruce budworm mortality. Defoliation of current year shoots averaged 13.4% in blocks treated at 20×10^9 I.U./ha and was 24.7% in blocks treated at 30×10^9 I.U./ha. In untreated areas defoliation was 100% (Table 2).

The current year growth mass also indicates the valuable protection resulting from the use of Futura at 20×10^9 I.U./ha. For each 100 cm branch, 49.0 g of current year shoots were protected while only 1.2 g remained in untreated plots (Table 2). Results using the DC-4G were satisfactory (Table 2). Also, calibration tests conducted in the fall of 1982 with the DC-4G aircraft revealed that an increase of 50% in deposit of B. thuringiensis can be obtained by distributing the 110 nozzles on the DC-4G close to fuselage, 55 on each side. [4]/

[3]/ Zoecon Inc., a subsidiray of Sandoz Inc., Palo Alto, California, USA

[4]/Smirnoff and Valéro, submitted Environment Canada, Canadian Forestry Service, Bi-Mon. Res. Notes.

Table 1. Results of field tests with Bacillus thuringiensis against the spruce budworm in 1981

Formulas	Dosages x 10^9 I.U./ha	Volume L/ha	Deposit viable spores/ cm^2	Pretreatment Populations		Current year growth defoliation %	Mass of current year growth/100 cm branch g	Buds formed (for the next year) %
				Larvae/ 45 cm branch tip	Larvae/ bud			
			Grumman AgCat					
Futura	20.0	2.5	283 000	24.9	—	34.7	26.5	93.3
Futura	20.0	2.5	342 850	30.1	—	58.8	17.1	84.5
Futura	20.0	2.5	381 600	17.2	—	38.9	18.6	70.5
Average-Futura	20.0	2.5	335 816	24.0	—	44.1	20.7	82.8
Thuricide 32B/ Sorbo/Water	20.0	4.7	413 000	22.2	—	67.0	17.3	85.9
Untreated [a]	—	—	—	25.2	—	96.4	4.8	39.4
			Super constellation L-749					
Futura [b]	18.8	2.35	295 000	10-20	—	25-40	15.1	76.9
Untreated [c]	—	—	—	29.5	—	98.7	0.7	6.6

a/ Average for 8 sample plots; b/ Average for 9 sample plots; c/ Average for 5 sample plots.

Table 2. Results of field tests with Bacillus thuringiensis against the spruce budworm in 1982

Formulas	Dosages x 10^9 I.U./ha	Volume L/ha	Deposit viable spores/ cm^2	Pretreatment Populations		Current year growth defoliation %	Mass of current year growth/100 cm branch g	Buds formed (for the next year) %
				Larvae/ 45 cm branch tip	Larvae/ bud			
			Grumman AgCat					
Futura	20.0	2.5	336 380	28.0	0.14	21.9	33.4	77.8
Futura	20.0	2.5	384 780	17.3	0.13	12.5	46.6	77.9
Futura	20.0	2.5	269 830	20.2	0.19	19.6	42.9	77.8
Futura	20.0	2.5	406 821	24.8	0.37	12.9	67.4	81.1
Futura	20.0	2.5	361 427	12.2	0.13	10.3	54.7	85.3
Average-Futura	—	—	351 848	20.5	0.19	13.4	49.0	79.9
Thuricide 48B	20.0	2.5	268 862	11.8	0.09	8.8	67.4	68.4
Futura	30.0	3.75	520 300	22.7	0.26	22.7	41.2	91.1
Thuricide 48LV	30.0	4.7	608 388	36.8	0.24	26.7	48.3	84.1
Untreated [a]	—	—	—	25.7	0.24	100.0	1.2	4.6
			DC-4G: Four engines					
Futura [b]	20.0	2.55	222 400	31.8	0.19	24.9	43.7	80.1
Untreated [c]	—	—	—	30.1	0.28	100.0	0.6	10.2

a/ Average for 8 sample plots; b/ Average for 9 sample plots; c/ Average for 5 sample plots.

1983. The field tests were aimed at determining the theoretical minimum dosage that can be used with Futura. Thus, Futura was dispersed at 2.50, 2.14, 1.43, and 0.71 L/ha (35.0, 30.0, 20.0, and 10.0 ounces/acre). Also, a new device called "pyramid" was developed to determine spreading of B. thuringiensis dispersion in a tree for each dosage tested and for each flight. Petri dishes with nutrient agar medium and containers with peptonized water were placed at different levels and depth in the tree crown and under it. Some 2 000 analyses were carried out and preliminary results reveal: there is no relation between the number of viable spores per square centimetre and the number of droplets per square centimetre; evaluation of the number of viable spores dispersed is the only valuable figure for deposit assessment; deposit at different levels in the tree varied with height of dispersion (flight altitude).

Results of field tests are just being analysed and some of them are reported in Table 3. It can only be said at this time that it seems possible to reduce the volume of Futura provided that purer B. thuringiensis concentrates be obtained, i.e. concentrates with less inert material. This would permit droplets with a larger number of spores, but still provide the 20 x 10^9 I.U./ha required for spruce budworm control.

Also, a 3 500 ha block was treated with Futura at 2.5 L/ha by means of a DC-4G aircraft. Although pretreatment population was high, 50.6 larvae per 45 cm or 0.31 larvae per bud, larval mortality was 94.1%, treatment efficiency (Abbott 1925) 78.9%, and defoliation between 40-50%.

Results of Long-Term Studies

Observations carried out during the last 5 years in blocks treated with different treatment sequences, in the B. thuringiensis treated blocks, and in the untreated area, revealed an average of 255 dead trees per hectare in treated blocks and 706 dead trees per hectare in the untreated area. Thus B. thuringiensis treatments saved 451 stems per hectare. Tree mortality in treated blocks was 12% and it was 40% in the untreated area. This represents the protection of some 29 metric tons of foliage per hectare (12.91 tons per acre) over a 5 year period. Studies continue to determine protection in terms of volume of wood per hectare.

Results of the biochemical analyses conducted during the past 8 years revealed that residual pupae of C. fumiferana surviving in B. thuringiensis treated areas have greatly reduce potential activity compared with that of pupae from untreated areas, as shown by their weight, calcium level, and total protein content as well as by their hydroxybutyrate dehydrogenase (αHBDH) and phosphatase activities. B. thuringiensis has a residual effect on the surviving C. fumiferana populations, involving a marked decline in the energy potential which is seen in practice as a reduction in the reproduction ability of the residual pest population (Smirnoff 1976, 1983). Conversely, the weights of calcium and total proteins of pupae from areas treated with Fenitrothion (organophosphate) are higher than in untreated pupae. Because αHBDH is of great importance during metamorphosis and adult development, this higher level is significant. It thus appears that the organophosphate treatments encourage the survival of a

Table 3. Results of field tests with Bacillus thuringiensis against the spruce budworm in 1983

Formulas	Dosages x 10^9 I.U./ha	Volume L/ha	Deposit viable spores/ cm^2	Pretreatment Populations		Current year growth defoliation %	Mass of current year growth/100 cm branch g	Buds formed (for the next year) %
				Larvae/ 45 cm branch tip	Larvae/ bud			
			Grumman AgCat					
Futura	20.0	2.50	349 841	12.3	0.08	32.8	36.0	84.2
Futura	17.0	2.14	142 000	21.1	0.24	55.9	18.6	78.5
Futura	16.3	1.43	143 456	19.9	0.19	54.8	12.4	73.4
Futura	8.0	0.71	135 475	18.8	0.19	49.8	22.1	79.7
Untreated a/	—	—	—	20.9	0.28	93.1	2.7	33.2

a/ Average for 8 sample plots.

vigorous C. fumiferana residual population with a high potential activity; in addition, such treatments ensures that more foliage is available for the survivors the following year. Moreover, the emergence of very vigorous populations of C. fumiferana could contribute to the formation of permanent spruce budworm epidemics in areas repeatedly treated with chemical insecticides, as suggested by the epidemiological studies conducted by Blais (1974).

Discussion and Conclusion

The results showed that the high concentrated and low volume B. thuringiensis formula which provides a dispersion of 20 x 10^9 I.U. in a final volume of 2.5 L/ha, assures good control of C. fumiferana populations and adequate foliage protection. The low-volume required, 2.5 L/ha, meets objectives imposed on cost of material and treatment, because this suspension can be successfully dispersed by means of large capacity aircrafts. Futura formula requires little mixing at the airport, is transported in a concentrated form, and dispersed in a final volume lower than that of the twice yearly dispersions of chemical insecticides presently used against spruce budworm. The conservative sorbitol allows storage of the ready to use Futura, including water, for several months on field sites. Sorbitol is a technical sugar and its osmotic pressure is higher than that of bacterial spores preventing fermentation and contamination. Under laboratory conditions, sorbitol keeps Futura's physical properties at a temperature of -22° C for several months. In 1983, Futura was dispersed in flight temperatures as low as -7° C and results showed that its physical properties were not affected under these field conditions. Also, B. thuringiensis should no longer be considered as a possible way of budworm control, but as an operational method with the same "ready to use" property as all other insecticides presently used against spruce budworm. An efficient control of C. fumiferana can be obtained with 20 x 10^9 I.U./ha. Therefore why use 30 x 10^9 I.U./ha (10 x 10^9 I.U. more) to obtain 10.4% less protection? Is it not a costly waste of material? This was confirmed by B. thuringiensis core tests conducted within CANUSA. These tests also showed that dispersion of B. thuringiensis at 30 x 10^9 I.U./ha is not more efficient than that of 20 x 10^9 I.U./ha (F.B. Lewis, personal communication). B. thuringiensis will be used by foresters for the operational control of C. fumiferana if its use remains operationally and economically valid and realistic. As with any method of control, the successful use of B. thuringiensis relies on the strict respect of technological recommendations (Smirnoff 1980d), which means use of the recommended formulas, dosages, final volume per hectare, and proper calibration of the spray system of the aircraft.

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