Tests of *Bacillus thuringiensis* var. *thuringiensis* Berliner and *B. cereus* Frankland and Frankland on Larvae of *Choristoneura fumiferana* (Clemens)[1]

By W. A. SMIRNOFF

Forest Research Laboratory, P. O. Box 35, Sillery, Quebec, P.Q.

Introduction

The susceptibility of the spruce budworm, *Choristoneura fumiferana* (Clemens), under laboratory conditions, to commercial preparations of *Bacillus thuringiensis* var. *thuringiensis* Berliner was reported by Angus *et al.* in 1961. This bacterium was recently tested, under field conditions, both as dusts and sprays. The results of these tests are presented here with comments on the effect of the environment and other factors.

Materials and Methods

Thuricide[2] (containing 30 x 10⁹ viable spores per gram) was tested against *C. fumiferana* larvae, in various concentrations in water with 4 per cent latex added, or emulsified in fuel oil with Span 80.[3]

Experiments were conducted in the forest and in an outdoor laboratory in the vicinity of St. David, Chicoutimi district, Quebec, from 1959 to 1961. In the laboratory, tests were carried out at an average temperature of 20°C in aerated boxes of transparent plastic or in glass containers. Balsam fir foliage was sprayed with the pathogenic material by means of a small hand-atomizer. After the foliage had dried, it was fed to larvae for a period of 96 hours after which the larvae were transferred to unsprayed foliage. Each test consisted of 15 to 20 specimens of the same instar collected in the same locality. The tests were replicated four to six times and mortality determined daily. All dead specimens were examined microscopically the same day.

Dead insects were crushed in a drop of water and examined with a dark field condenser and phase-contrast objectives. When necessary, diagnosis was verified by examining smears stained with nigrosin or other stains. After examination, the dead insects were classified according to the following categories: (1) those containing bacterial cells; (2) those containing bacterial cells and spores of microsporidia; (3) those containing spores of microsporidia only; (4) those killed by fungi, virus, parasites or other causes. Dead larvae containing cells of *Bacillus*, were considered as having died from *Bacillus* infection even though their bodies contained other species of microorganisms.

When a bacterial culture was to be isolated from the abdominal cavity of a dead insect, the insect was first surface-sterilized in one-per-cent merthiolate solution or 0.4 per cent of a quaternary ammonium compound[4] (Martignoni and Milstead, 1960). After washing, a loopful of the material was streaked on nutrient agar. To determine the number of crystals and spores and to obtain information on the growth of bacteria, smears of cultures were examined by the method of dark-field colour refraction (Smirnoff, 1961).

Spraying and dusting in the forest was done with a Micro Hart Sprayer and Duster. The sample plots were ¼ acre in area and all fir trees 10 to 15 years old were treated. After treatment, the branches and larvae were enclosed in bags

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TABLE I

Pathogenicity of various strains of *B. thuringiensis* and *B. cereus* for larvae of *Choristoneura fumiferana* (Clem.) (Laboratory, average temp. 21.5°C.; 1120 larvae in tests.)

Treatment with strain	Larval instar	Period of mortality (days)	Percentage of dead larvae and pupae	Percentage of dead larvae and pupae containing		
				Bacteria	Microsporidia	Others[1]
B. thuringiensis						
Thuricide	II-III	7	100	51	3	46
	V-VI	6	100	55	15	30
Rohm and Haas Bakthane	II-III	16	49	32	3	14
	V-VI	12	68	40	9	19
from *F. jocosa*	II-III	5	100	77	10	13
	V-VI	8	80	42	10	28
from *P. erichsonii*	II-III	6	68	39	3	26
	V-VI	8	46	27	11	8
from *N. gibbosa*	II-III	8	98	40	11	47
	V-VI	10	70	42	9	19
B. cereus						
from *T. irregularis*	II-III	10	100	60	10	30
	V-VI	10	93	74	4	15
Control	II-III	18	25	0	12	13
	V-VI	14	21	0	16	5

Capsule virus, fungi, parasites and unknown.

made of nylon mesh and removed for analysis 24 days later during the period of moth emergence.

Every week for a period of four weeks, four to six whole trees and several branches 18 inches in length were cut and examined carefully. Dead insects were examined individually under the microscope and living insects taken to the field insectary for rearing. All the larvae that died in rearing were also examined microscopically.

In addition, various strains of *B. thuringiensis* and *Bacillus cereus* Frankland and Frankland were tested in the laboratory. The bacteria were grown on nutrient agar after isolation from commercial preparations of *B. thuringiensis* Thuricide, Bakthane[5], and isolations from the following insects: *Feralia jocosa* Guenée, *Nadata gibbosa* J. E. Smith, *Pristiphora erichsonii* (Hartig) and *Trichiocampus irregularis* (Dyar).

Results

Natural infection

Under forest conditions, natural mortality of the population did not exceed 26 per cent. Of this, 6 per cent was due to microsporidia (*Perezia fumiferanae* Thomson); 1 to 2 per cent to virus disease (capsule virus), and 0.5 to 1 per cent to fungus disease (*Beauveria* sp. and *Isaria* sp.). The remainder were killed by entomophagous parasites or died from unknown causes. However, in control rearings, in the laboratory, total mortality increased gradually with the temperature as a result of greater infection by microsporidia (Table III).

TABLE II

Pathogenicity of Thuricide in water or oil suspension for IV-V-VI instar larvae of *Choristoneura fumiferana* (Clem.) (2200 larvae in the tests.)

Concentration of Thuricide grams/gal.	Period of mortality (days)	Percentage of dead larvae and pupae[1]	Percentage of dead larvae and pupae containing		
			Bacteria	Microsporidia	Others[2]
Water suspension					
0.4g.	7	71	17	15	39
8.0g.	9	87	51	10	26
20.0g.	8	100	49	27	24
40.0g.	9	97	54	14	29
200.0g.	8	100	58	16	26
Oil suspension					
0.4g.	9	80	20	11	49
8.0g.	10	90	16	18	56
20.0g.	9	82	46	2	34
40.0g.	7	96	42	5	49
200.0g.	8	98	46	18	34
Control					
Water and latex (4%)	17	38	0	13	25
Oil	11	40	0	11	29

[1]All insects that reached moth stage were deleted from totals.
[2]Capsule virus, fungi, parasites and unknown.

About 16 per cent of the moths captured in the forest contained spores of microsporidia within their bodies. This would seem to indicate that in their natural habitat, a large number of larvae are able to complete their development in spite of being infected with this sporozoan parasite.

Effect of B. thuringiensis and B. cereus

B. thuringiensis Thuricide produced 100 per cent mortality, with 50 per cent of the specimens carrying bacterial cells; the strain isolated from the Rohm and Haas product was about half as effective under the same conditions for the same species of insect. This was also true for strains isolated from *Nadata gibbosa* and *Pristiphora erichsonii*, but the strains isolated from *Feralia jocosa* were much more pathogenic, especially so for larvae in the earlier instars (Table I).

The high mortality among larvae of any instar indicates the pathogenicity of *B. cereus* and a large number of bacterial cells was observed in dead larvae and pupae. *B. cereus* was discovered in the bodies of many dead moths but *B. thuringiensis* was not.

Role of B. thuringiensis crystals in C. fumiferana larvae

Heimpel and Angus (1959) showed that some species of lepidopterous larvae become paralysed soon after ingesting *B. thuringiensis* var. *sotto*. In the present study, it was found that spores and crystals of *B. thuringiensis*, applied as concentrated aqueous suspensions or as dried powders, did not cause the immediate death of *C. fumiferana* larvae. In all cases, the death of larvae occurred in three or more days due to typical septicaemia.

This was investigated further in the following test. Three separate fir branches all bearing larvae, were dusted thickly with each of the following materials: *Thuricide* (containing spores as well as crystals); *B. thuringiensis* SO-69 (containing chiefly spores); *B. cereus* isolated from *Trichiocampus irregularis*

TABLE III

The influence of temperature on V-VI instar larvae of *Choristoneura fumiferana* (Clem.) infected with *B. thuringiensis* and *B. cereus* (1200 larvae in the test.)

Treatment	Temper-ature (°C.)	Period of mortality (days)	Percentage of dead larvae and pupae[1]	Percentage of dead larvae and pupae containing		
				Bacteria	Microsporidia	Others[2]
Laboratory Trials						
B. thuringiensis	35°	3	100	81	3	16
Thuricide	28°	4	100	80	0	20
	19.2°	6	100	50	0	50
B. cereus	35°	5	86	64	0	22
strain from	28°	5	100	82	0	18
T. irregularis	19.2°	7	100	74	10	16
Control	35°	8	94	0	36	58
	28°	20	55	0	28	27
	19.2°	16	38	0	10	28
Field trials						
B. thuringiensis Thuricide	13.2°	10	67	36	7	24
B. cereus strain from T. irregularis	13.2°	9	88	72	4	12
Control	13.2°	20	26	0	6	20

[1]All insects that reached moth stage were deleted from totals.
[2]Capsule virus, fungi, parasites and unknown.

(containing spores only). In each case, mortality began only after a period of three to four days. Furthermore, feeding tests made with water suspension of *B. thuringiensis* crystals purified at 80 per cent did not cause any mortality. This suggests that the crystals are not essential to the pathogenicity of *B. thuringiensis* for *C. fumiferana*.

Influence of temperature

Tests with *B. thuringiensis* showed that the incubation time and amount of mortality vary with temperature; temperatures below 14°C delayed the development of infection. Tests with *B. thuringiensis* and *B. cereus* at temperatures of 35°, 28°, and 19°C showed that mortality occurs two to three days sooner and is accompanied by an increase in the number of larvae killed by bacteria at 28° and 35°C. Table III shows that the death rate was lower at fluctuating temperatures which averaged 13.2°C than for larvae kept at higher temperatures. However, mortality of larvae infected with *B. cereus* was greater at the lower temperature. It may thus be an advantage to use the isolated strain of *B. cereus* since lower temperatures are more common under field conditions. The different results obtained in 1960 and 1961 in field plots may be explained by the fact that the average temperature in 1961 was 3.0°C lower, during the first ten days after dusting or spraying, than during the same period in 1960. Mortality in 1961 was lower even though a higher dosage of Thuricide was used (Table IV).

Mortality in control rearings at 35°C reached an average of 94 per cent after eight days. Microscopic analysis showed that 36 per cent of these larvae had died due to a microsporidial disease and 58 per cent by unknown causes (probably

TABLE IV

Mortality of larvae and pupae 8 and 24 days after spraying and dusting forest plots with various quantities of Thuricide per gallon of water or oil suspension.
(80-1000 g. per gallon, 10 gallons per acre)

Grams per acre	Percentage of dead larvae and pupae		Percentage of dead larvae and pupae containing					
			Bacteria		Microsporidia		Others[1]	
	8 days	24 days	8 days	24 days	8 days	24 days	8 days	24 days
	1960 — Average Temperature: 15.9°C.							
80 (water)	0	14	0	14	0	0	0	0
(oil)	0	44	0	11	0	0	0	33
800 (water)	45	84	33	31	0	15	12	38
(oil)	25	54	14	36	4	0	7	18
800 (dust)	80	100	73	85	0	0	7	15
Control	0	20	0	0	0	4	0	16
	1961 — Average Temperature: 12.9°C.							
1000 (water)	21	63	19	32	2	5	0	26
800 (dust)	28	60	26	41	1	0	1	19
Control	0	14	0	0	0	5	0	9

[1]Capsule virus, fungi, parasites and unknown.

high temperature). According to evidence obtained from control rearings, an increase in temperature increases mortality from microsporidia.

Tests with various dosages of B. thuringiensis (Thuricide)

In the laboratory: Up to 70 per cent of *C. fumiferana* larvae died when fed needles sprayed with aqueous suspensions of 0.4 g. of Thuricide per gallon of water (4 per cent latex added); however, only about 17 per cent of the dead larvae contained vegetative cells of *B. thuringiensis*. Increasing the concentration to 20-40 g. Thuricide per gallon increased the proportion of larvae containing the bacteria at death to about 55 per cent. Further increases in the quantity of Thuricide did not increase this percentage even when 200 g. of Thuricide per gallon of water were used (Table II).

An equivalent amount of Thuricide emulsified in fuel oil with Span 80 gave similar results, but the number of dead specimens containing bacteria was generally less (Table II).

In the forest: In 1960, aqueous suspensions of Thuricide were found more effective than oil emulsions, whether in the field or in the laboratory. Best results were obtained after spraying with aqueous suspensions of 80 g. Thuricide per gallon of water, in amounts of 10 gallons per acre. Particularly good results were obtained by dusting at the rate of 800 g. per acre. In eight days, the larval mortality reached 80 per cent, practically all larvae having died from *Bacillus* infection (Table IV). However, during experiments carried out in 1961, spraying and dusting did not give comparable results, probably due to cool temperatures during the first days after spraying.

It was found that the longer *C. fumiferana* larvae feed on needles contaminated with *B. thuringiensis*, and the higher the concentration of *Bacillus*, the

TABLE V
Larval mortality after various periods of exposure on crowns of Balsam fir trees
dusted with Thuricide

Treatment (per crown)	Larvae transferred after (hours)	Period of mortality (days)	Percentage of dead larvae and pupae	Percentage of dead larvae and pupae containing		
				Bacteria	Microsporidia	Others[1]
Dust: 1g.	7[2]	12	49	5	15	29
	22	13	50	11	16	27
	48	8	69	19	6	44
Dust: 6g.	7	5	63	31	16	16
	22	10	100	62	0	36
	48	8	100	80	10	10
Dust: 1g.	7[3]	15	44	0	15	29
	22	14	42	0	13	29
Dust: 6g.	7[3]	12	41	11	11	19
	22	12	63	16	16	31

[1]Capsule virus, fungi, parasites and unknown.
[2]The crown dusted with larvae *in situ* which were left to feed for the time mentioned and then transferred to clean foliage
[3]Five days after dusting the crown, it was colonized with larvae which were left to feed for the time mentioned and then transferred to clean foliage.

sooner the onset of mortality.   The importance of time and the concentration of the spray was tested by spraying and dusting tree needles infested with *C. fumiferana* larvae.   Table V shows that the mortality of the larvae permitted to feed on treated foliage for seven hours and then transferred to untreated branches was both less and slower than with larvae left on treated foliage for a period of 48 hours.   It was further noted that the effectiveness of foliage sprayed or dusted with *B. thuringiensis* diminished with time especially when accompanied by rain. It is possible that after several days of contact between the pathogenic material and the surface of tree needles on which it has been deposited, inactivation of the former takes place.   It has been suggested that the spores on foliage germinate and subsequently die (Stephens, 1957).   It is also possible that some volatile substance released by the foliage of balsam fir may inactivate the spores, as this has been shown by Gukasian (1958) for *B. thuringiensis* var. *dendrolimus*.   It was found that foliage dusted with 6 g. of Thuricide and then washed with water from a sprayer, lost a fairly large part of the insecticide since mortality in larvae feeding on such needles decreased by 40 per cent.   Other tests showed that the inoculum on crowns dusted with 1 g. and 6 g. Thuricide, even though protected from rain, does not remain effective after five days (Table V).

In the forest, high dosages of Thuricide dusted or sprayed upon branches had no effect on larvae feeding on the foliage after 20 days of weathering since not a single case of *Bacillus* infection was noted.

Discussion and Comments

The data in Tables I and III indicate that some strains of *B. cereus* are as effective as *B. thuringiensis*.   Data in Table III show further that *B. cereus* is more effective than *B. thuringiensis* at low temperatures.   Since *B. cereus* does not produce crystals, this suggests that crystals may not be essential to cause

mortality.   Such a suggestion is strengthened by the observation that all infected larvae die of a typical septicaemia, usually after three or four days.

It is encouraging that a number of bacterial strains were found to be pathogenic for *C. fumiferana* particularly since there is a possibility of selecting an adapted strain.   Thus, it may be feasible to select strains to fit a particular set of ecological conditions.

The field results indicate that laboratory studies of pathogenicity cannot give a complete picture of the effectiveness of a particular microorganism since field conditions profoundly affect the development of diseases.   The most important factors seem to be temperature and precipitation.   An interesting problem is the gradual loss of pathogenicity of sprayed foliage protected from rain.

The results also indicate that the presence of other pathogens, in this case a microsporidian, enormously complicates the assessment of results.   It is not known to what extent the two microorganisms inhibit or complement each other. This question deserves additional study.

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