Not for publication

SUSCEPTIBILITY OF BRITISH COLUMBIA FOREST INSECTS TO MIXTURES OF *BACILLUS THURINGIENSIS* AND LOW DOSES OF CHEMICAL PESTICIDES

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Summary

Experiments were started in 1968 to test the susceptibility of some forest insects of British Columbia to mixtures of *Bacillus thuringiensis* and chemical pesticides.

Preliminary to the main tests, petroleum ether, ethyl acetate, propanol, ethyl ether, chloroform and water extracts of several coniferous and broad leafed forest-insect host tree foliage were examined for their inhibitory effects on development of the pathogen. The results showed that petroleum ether, ethyl acetate and ethyl ether extracts of foliage of conifers inhibited bacterial growth. Several pure terpenes extracted from coniferous trees by Dr. von Rudloff (N.R.C., Saskatoon) were also tested and found to inhibit bacterial development. There was a drastic reduction in the production of spores and crystals by terpene-treated bacteria.

Other preliminary laboratory tests involved the study of the effects of some chemical insecticides on the physiological characteristics of *B. thuringiensis*. In general, the chemical insecticides had no appreciable effect on the bacterial physiology.

Tests with mixtures of the bacteria and Zectran, phosphamidon, pyrethrum, malathion and piperonyl butoxide (Butacide) against *Halisidota argentata*, *Malacosoma disstria*, *Malacosoma pluviale*, *Alceris variana*, *Hyphantria cunea*, *Orgyia pseudotsugata* and *Melanolophia imitata* showed that small or sub-lethal doses of the insecticides generally enhanced the susceptibility of these insects to the bacteria.

Susceptibility of British Columbia Forest Insects to Mixtures of *Bacillus thuringiensis* and Low Doses of Chemical Pesticides

INTRODUCTION

The concept of the integrated control of insect pests stresses the development of procedures where biological and chemical controls are used compatibly. While biotic control agents may provide a significant degree of control, they may not always be sufficient to keep the pests at tolerable levels. In these circumstances, artificial (chemical) control should be used to its fullest advantage to augment the natural control phenomenon. The use of mixtures of the insect pathogen, *Bacillus thuringiensis* and sub-lethal amounts of chemical pesticides is a promising approach to the integrated control of lepidopterous defoliators. Such an approach could, in effect, reduce considerably the quantity of poisonous chemicals introduced into the forest ecosystem.

Before applying mixtures of bacteria and chemical pesticides, it is essential to know whether the latter exerts any deleterious effects on its partner and whether the foliage on which the target insects feed contains antibiotic agents, which could inhibit development of the bacteria in the insect gut. This report contains results of experiments to determine the effects of foliage extracts and chemical insecticides on the bacterial physiology and the susceptibility of some forest insects to mixtures of bacteria and chemical insecticides.

ANTIBIOTIC EFFECTS OF FOLIAGE EXTRACTS

Five grams of foliage each, were collected from several forest trees in May, June and July[1/], quick-frozen by addition of liquid nitrogen and briskly ground to a fine powder. Twenty-five ml cold ethyl acetate was added and thoroughly mixed with the cold powder and 5 ml aliquots quickly transferred to 10 ml Virtis vacuum vials. The aliquots were quick frozen again by immersing the vials in liquid nitrogen. The vials were then transferred to a freeze drier (Virtis Freeze-Mobile model) and dried for 60 min. The freeze-dried material was sealed under 30 micron original vacuum and transferred to a cold room at -45°C for storage.

In the extraction procedure that followed, 5 ml of petroleum ether was thoroughly mixed with the vial contents, and centrifuged at 7000 rpm for 15 min. The supernatant was removed and the procedure repeated on the residue and subsequent residues after adding 2 ml, respectively, of ethyl acetate, 50 per cent propanol, ethyl ether, chloroform, and water.

Nutrient agar plates were seeded with a commercially prepared *Bacillus thuringiensis*. Five mm diameter filter paper discs were soaked in each extract and placed on the seeded plate. The diameters of inhibition zones developing around the discs were measured after three days of incubation at 28° - 29°C.

The results are summarized in Table I. With the possible exception of the crab apple, the broad-leafed trees known to contain relatively

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[1/] Only a single tree of each species was used.

little inhibition. Most of the conifers, on the other hand, known to contain relatively large amounts of terpenes produced relatively large inhibition zones. The antibacterial substances in the petroleum ether extracts of foliage samples generally increased over the three-month period but antibacterial substances in the ethyl acetate and ethyl ether extracts decreased as the season progressed. These observations may be of some importance in the timing of application of bacterial insecticide.

EFFECT OF TERPENES ON THE BACTERIAL DEVELOPMENT

The above data suggested that terpenes may be partly responsible for antibiotic effect of the foliage extracts. Accordingly, several samples of 95 to 98 per cent pure terpenes, sent to us by Dr. E. von Rudloff, N.R.C., Saskatoon, were tested for their inhibitory effect on _B. thuringiensis_. The filter paper disc method as described above was used. The average diameter of three inhibition zones was calculated for each test. Smears were taken of colonies growing inside the inhibition zones and the total number of vegetative cells, spores and crystals were counted in 10-20 randomly chosen microscopic fields.

The results in Table 2 show that nearly all of the 24 terpenes tested had some antibiotic properties, especially - Pinene, Pulegone, Menthone, 4 - Teripineol, - Terpineol, Citral, Citronellal, Citronellol and Linalool. According to Dr. von Rudloff (personal communication) all of these terpenes, except Citral and Citronellal occur in _Pinus_ and _Picea_ leaf oils. Citral is prepared from lemongrass oil and was obtained from a commercial source. Citronellal has been identified in the leaf oil of the common juniper.

There was generally a drastic reduction in the production of spores and crystals by terpene-treated bacteria (Table 3). Bornyl acetate, Citral and Citronellal seriously affected crystal production but not spore production.

EFFECT OF SOME CHEMICAL INSECTICIDES ON THE PHYSIOLOGICAL PROPERTIES OF *B. THURINGIENSIS*

A 1/1000 dilution of Thuricide 90TSH950T was mixed with various insecticides for 60 min on a magnetic stirrer. A nutrient agar plate was streaked from this suspension and a resultant bacterial colony was propagated in nutrient broth. This broth culture was used in biochemical tests. All biochemical tests were repeated with spores and crystals taken directly from the stirred mixture of bacteria and insecticides. The physiological reactions studied (Table 4) were limited to the characteristics of greatest importance in the taxonomy of *B. thuringiensis*. Viable counts were done by the standard pour plate method.

The results (Table 4) showed that neither of the controls conformed strictly to the physiological characterization of *B. thuringiensis* var. *galleriae* as outlined by de Barjac and Bonnefoi (J. Invertebrate Pathol. 11, 1968, pp. 335-347). This is not entirely surprising, however, for commercially formulated products. Gas was produced in media that were inoculated directly from the mixtures of bacteria and insecticides but not in media inoculated from an isolated colony. The production of gas by *B. thuringiensis* is most uncharacteristic and it can only be assumed that the commercial product contained some component or combination of components that changed the normal physiological pattern of the vegetative bacteria.

In general, there was little difference in physiological reactions between control bacteria and bacteria mixed with chemical insecticides. The only possible exception was piperonyl butoxide effect on the bacterial fermentation of melezitose, levulose, sucrose and esculin. None of the chemical insecticides tested affected viability of the spores. The general conclusion was that the chemical insecticides had no appreciable effect on the bacterial physiology.

SUSCEPTIBILITY OF FOREST INSECTS TO MIXTURES OF *B. THURINGIENSIS* AND LOW DOSES OF CHEMICAL INSECTICIDES

Between 50 and 105 field collected larvae were used in each test. Small branches of the species' primary tree host were dipped in mixtures of various concentrations of *B. thuringiensis* and chemical pesticides. Larvae were placed directly on the air-dried branches and reared in an insectary at room temperature (22° - 23°C) or in growth chambers at constant temperatures of 10°, 22° or 28°C for seven days. Dead larvae were diagnosed daily for vegetative cells of the bacteria with a phase contrast microscope. The frass collected from each test was dried, cleaned and weighed.

Preliminary field trials were conducted against the western tent caterpillar, *Malacosoma pluviale*, on Sydney Island and the green-striped forest looper, *Melanolophia imitata*, at Port Alice.

Halisidota argentata

Results of the test against the silver spotted tiger moth are summarized in Table 5. The most effective combination was thuricide 90TS at 10^{-1} with pyrethrum at 10^{-3}. They separately produced 24 and 57 per cent mortality, respectively, after seven days as compared with 94 per cent mortality when combined. This effectiveness was also reflected by the percentages of bacteria - positive cadavers (22 and 9 as against 80). The nine per cent _B. t._ positive resulted from contamination. The amount of frass collected decreased as the concentrations of the insecticides increased, indicating reduced feeding activity. The population from which the larvae were collected contained some nuclear polyhedrosis and microsporidia.

Malacosoma disstria

The forest tent caterpillar was highly susceptible to all concentrations of thuricide or chemical pesticides used (Table 6). In the present tests, a sub-lethal dose was not found. However, in general, the addition of both phosphamidon and malathion to Thuricide increased the total mortality over mortality from Thuricide alone. A corresponding increase in bacteria-containing cadavers was noted only at Thuricide 1/100. There was no measurable feeding activity in larvae fed foliage treated with combinations of bacteria and phosphamidon or malathion at 2 X 10^{-3} dilution. Both nuclear polyhedrosis virus and microsporidia were found in these larvae.

Acleris variana

The test with the black-headed budworm was limited by the number of larvae available. A sub-lethal dose of phosphamidon was not found (Table 7). However, when the lower dosage of Thuricide was mixed with phosphamidon, the percentage of cadavers containing vegetative bacteria was markedly increased. At the higher Thuricide dosage, such an increase only occurred with the lower phosphamidon dosage. Presumably, phosphamidon at

10^{-4} was high enough to kill all larvae before the ingested spores could develop to vegetative cells. The population from which these larvae were collected showed a relatively high incidence of polyhedrosis virus.

Hyphantria cunea

Results of the experiments with the fall webworm are summarized in Table 8. This species is extremely susceptible to Thuricide. Since mortality at seven days post-treatment was complete (100%) in all tests, the mortality comparisons were made at three days post-treatment instead of the usual seven. The addition of malathion, phosphadmidon, pyrethrum or piperonyl butoxide (10^{-6}) increased considerably the susceptibility of the larvae to Thuricide (1/100 dilution) when test larvae were reared at 22°C. This augmentative effect was pronounced even at chemical insecticide dosages that produced no mortality at all. The occurrence of vegetative cells in cadavers and frass accumulation followed a pattern similar to that of *H. argentata*. When larvae were reared at 28°C mortality from the mixtures were similar to or lower than mortality from bacteria alone. In general, the higher temperature did not produce any higher mortality among insects treated with chemical pesticides alone but it did produce higher mortality among insects treated with bacteria alone. The reasons for the reduced mortality among some insects treated with mixtures at 28°C is unknown at the present stage of investigation.

It should be pointed out that the population from which these webworms were collected contained larvae with nuclear polyhedrosis and granulosis viruses and microsporidia. The larvae in these tests were not diagnosed for granulosis virus. This virus was diagnosed in several larvae from the test population by Dr. J. Weiser, Simon Fraser University.

Orgyia pseudotsugata

In nearly all cases, addition of small or sub-lethal doses of chemical insecticides increased both the total larval mortality and the numbers of cadavers containing vegetative bacteria (Table 9). The augmentative effect was most pronounced with malathion-bacteria mixtures. The chemical pesticides alone generally caused only minor reductions in feeding activity but mixtures of chemicals and bacteria cause large reductions.

Elevated temperature did not affect mortality from malathion alone but there was a considerable increase in total mortality, number of cadavers with vegetative cells and feeding activity among larvae fed malathion mixed with bacteria (Table 10). The Lt_{50}s were correspondingly reduced.

Melanolophia imitata

The green-striped forest looper was generally highly resistant to the bacteria alone, to the chemical pesticides alone or to mixtures of the two (Table 11).

At the highest Thuricide concentration (1/100), only 22 per cent of the dead larvae contained vegetative cells. However, the bacteria was highly effective in reducing feeding activity. While the chemical pesticides had little or no effect on feeding, mixtures of chemicals and bacteria profoundly reduced feeding activity. The Port Alice population from which these larvae were collected apparently contained relatively high incidences of microsporidia and polyhedrosis virus.

The addition of low doses of malathion, pyrethrum and phosphamidon increased appreciably the susceptibility of the insects to bacteria only at the 28°C temperature (Table 12). There were, simultaneously, marked reductions in feeding activity.

It appears that the chemicals tested would have no practical value in enhancing the insecticidal effect of the bacteria against this species although they would help to reduce its feeding activity.

In the summer of 1969, a field test with a mixture of piperonyl butoxide (Butacide) and B. thuringiensis was carried out against M. imitata at Port Alice, B. C. The results, summarized in Table 13, show that the larval population was reduced to a greater extent on trees sprayed with a mixture of the two insecticides than on trees sprayed with either alone. However, the overall control was not high enough to warrant larger scale trials. Combinations of other chemical insecticides and bacteria should be investigated.

Malacosoma pluviale

Results of the tests to determine sub-lethal doses of the pesticides showed unusually large changes in mortalities between Zectran 10^{-4} and 10^{-5}, phosphamidon 10^{-5} and 10^{-6}, pyrethrum 10^{-3} and 10^{-4}, malathion 10^{-4} and 10^{-5} and piperonyl butoxide 10^{-3} and 10^{-4} (Table 14). Further testing within these limits is indicated.

The addition of small doses of all the chemical pesticides tested enhanced the susceptibility of this species to Thuricide at 1/100 dilution (Table 14). Although the total mortalities from the mixtures were not always higher than mortality from Thuricide alone, the numbers of bacteria-positive cadavers were always higher. A Thuricide 90TS-H-950T, which unfortunately arrived after the tests were completed with S7-150, proved to be considerably more pathogenic for this species (Table 14). The field population from which test insects were collected contained a relatively high incidence of nuclear polyhedrosis virus.

Immediately before a small field trial on Sydney Island with Thuricide S7-150 mixed with phosphamidon, fourth to sixth instar larvae from the test trees were tested in the laboratory for susceptibility to the insecticides. The results (Table 15) showed a considerable enhancement of the susceptibility of the insect to bacteria by phosphamidon at 0.25%.

In the field tests which followed, two cone-shaped frass collectors were placed within each of seven highly infested 30 Ft red alder trees. Each of six of the trees were sprayed with 500 ml of 0.4 per cent Lovo 192, or different concentrations of phosphamidon or Thuricide, or mixtures of both agents, using a back pack power sprayer. The seventh tree was left unsprayed. Frass was collected, dried, cleaned and weighed and hygrothermograph records kept over a 14-day period. The weight of frass was used as the criterion for measuring the insecticidal effectiveness of each application.

The results (Table 16) show a consistent reduction in the weight of frass collected from trees sprayed with Thuricide mixed with 0.25 per cent phosphamidon, indicating a continuous decrease in feeding activity. These preliminary data suggest that mixtures of these two insecticides constitutes a more effective insecticide than either alone in controlling the western tent caterpillar. The results provide a basis for more sophisticated future pilot field tests in the integrated control of this species.

Table 1

Inhibitory effect of different extracts of foliage from various plants on the development of <u>Bacillus thuringiensis</u> var. <u>galleriae</u>[1/]

Plant species	Diameters (mm)[2/] of inhibition zones produced by extracts						
	Date collected	Petroleum ether	Ethyl acetate	50% Propanol	Ethyl ether	Chloroform	Water
Control (solvents only)		0	0	0	0	0	0
<u>Alnus rubra</u> Bong.	5/21/68	0	0.3	0	0	0	0
(Red alder)	6/18/68	0	2.0	0	0	0	0
	7/23/68	0	3.0	0	1.5	0	0
<u>Pseudotsuga menziesii</u>	5/21/68	4.0	1.5	3.0	3.0	2.0	0
(Mirb.) Franco	6/19/68	3.5	2.0	1.5	2.5	1.0	0
(Douglas fir)	7/24/68	3.0	2.0	2.0	1.5	0.5	0
<u>Larix occidentalis</u>	5/17/68	2	1.5	1.5	1.5	1.0	0.5
Nutt.	6/18/68	1.5	1.5	2.0	2.0	0.5	0
(Western larch)	7/24/68	1.5	1.5	1.5	2.0	0.5	0
<u>Crataegus douglasii</u>	5/17/68	0	0	0	0	1.3	0
Lindl.	6/18/68	0	0	0	0	0	0
(Black hawthorn)	7/23/68	0	0	0	0	0	0
<u>Pinus contorta</u> Dougl.	5/12/68	3.0	2.0	2.0	2.0	0	0
var. <u>latifolia</u> Engelm.	6/18/68	2.0	1.5	2.0	1.5	0	0
(Lodgepole pine)	7/24/68	1.5	0.5	0.5	0	0	0
<u>Populus tremuloides</u>	5/17/68	0	0	0.5	0	0	0
Michx.	6/18/68	0	0	0	2.0	2.0	0
(White poplar)	7/24/68	0	0	0.5	0	0	0
<u>Malus fusca</u> (Raf.)	5/21/68	0	5.6	0	4.3	0	0
Schneid.	6/18/68	0	6.0	0	6.0	0	0
(Pacific crab apple)	7/24/68	0	3.0	0	3.5	0	0
<u>Quercus garryana</u> Dougl.	5/17/68	0	1.0	0	1.0	1.0	0
(Garry oak)	6/18/68	0	0.5	1.0	1.0	0	0
	7/23/68	0	0	0	0	0	0
<u>Abies grandis</u> (Dougl.)	5/21/68	1.0	0	0	1.0	0	0
Lindl.	6/19/68	1.5	2.0	0.5	2.0	0.5	0
(Grand fir)	7/24/68	1.0	1.5	1.0	2.0	0.5	0
<u>Tsuga heterophylla</u> (Raf.)	5/21/68	1.0	0	0	1.0	0	0
Sarg.	6/19/68	1.0	0	0	1.0	0	0
(Western hemlock)	7/23/68	1.0	0	0	2.0	0	0
<u>Picea engelmanni</u> Parry	6/18/68	2.0	3.0	1.5	2.5	0	0
(Engelmann spruce)	7/24/68	1.5	2.0	1.5	1.0	1.0	0

1/ Thuricide S6-175 obtained from Bioferm, Wasco, California.

2/ Average of three inhibition zones.

Table 2

Inhibitory effect of terpenes[1] on the development of

Bacillus thuringiensis var. *galleriae*[2]

Terpenes	Diameters (mm) of inhibition zones at various concentrations[3]		
	Undiluted	10^{-1}	10^{-2}
Cyclic monoterpeness			
- Pinene	15	0	0
Pulegone	6	0	0
Menthone 2:1 mixture of isomers	6	0	0
4 - Terpineol	6	3	0
- Terpineol	4	2	0
d - Carvone	3	0	0
Menthol	2.5	0	0
dl - Piperitone	2.5	0	0
1:8 Cineole	2	0	0
l - Bornyl acetate	2	0	0
L - Menthyl acetate	2	0	0
L - Limonene	1.5	0	0
d - Fenchone	1.5	0	0
dl - Camphor	0	0	0
dl - Borneol 2:1 mixture of isomers	0	0	0
Aliphatic (acyclic) monoterpenes			
Citral A + B 2:1 mixture	15	14	3
Citronellol	8	4	0
Linalool	6	0	0
Geraniol	4	1.5	0
Citronellal	4	0	0
Geraniol + Nurol: 2:1	3	3	2
Linalyl acetate	2	0	0
Cyclic sesquiterterpenes			
Cedrene	3	2	0.5
Cedral	0	0	0

1/ Obtained from Dr. E. von Rudloff, N. R. C., Sask. Samples were 95 to 98 per cent pure terpenes.

2/ Thuricide S6-175 obtained from Bioferm, Wasco, Calif.

3/ All dilutions were made with petroleum ether. Figures are for average diameter in three tests.

Table 3

Effect of terpenes on the production of spores and crystals by *Bacillus thuringiensis*[1/] grown on nutrient agar for three days

Terpenes[2/]	Total counts[3/]	Per cent spores[4/]	Per cent crystals[4/]
Control (untreated bacteria)	1536	42	60
Pulegone	201	0.5	1.5
Menthone 2:1 mixture of isomers	219	24.7	4
4 - Terpineol	415	14	8.4
- Terpineol	156	4.5	6.4
Menthol	218	28.4	1.3
dl - Piperitone	327	11.6	1
1:8 Cineole	206	5.3	1.9
l - Bornyl acetate	388	59	18
L - Menthyl acetate	273	18.3	8.8
d - Limolene	200	21	25
d - Fenchone	286	13.9	1.7
Citral A + B 2:1 mixture	514	38.5	0
Linalool	346	17.9	4.9
Citronellal	860	58.7	5.2
Linalyl acetate	123	11.3	0

1/ Thuricide S6-175 obtained from Bioferm, Wasco, Calif.

2/ All terpenes tested undiluted except Citral/100.

3/ Total vegetative cells, spores and crystals on 10-20 randomly selected microscopic fields.

4/ Per cent of total counts.

Table 4

Changes in vegetative cell metabolism of commercial *Bacillus thuringiensis* var. *galleriae* spores[1/] mixed with various chemical insecticides[2/] for 60 min.

Tests	Control[3/] Bacteria only		Zectran 10^{-4}		Phosphamidon 10^{-5}		Pyrethrum 10^{-3}		Malathion 10^{-5}		Piperonyl butoxide 10^{-3}	
	I[4/]	II	I	II	I	II	I	II	I	II	I	II
Maltose	AG	A	AG	A	AG	A	AG	A	AG	A	AG	A
Saccharose	AG	A	AG	A	AG	A	AG	A	AG	A	AG	A
Galactose	AG	–	AG	–	AG	–	AG	–	A	–	AG	–
Melezitose	AG	A±	AG	–	A±	–	G	–	–	–	A	–
Cellobiose	AG	A±	AG	–	AG	–	AG	–	AG	A	AG	A
Levulose	AG	A	AG	A	AG	A	AG	A	AG	A	A	A
Salicin	AG	A	AG	A	AG	A	AG	A	AG	A	AG	A
Sucrose	AG	A	AG	A	AG	A	AG	A	AG	A	A	A
Mannose	AG	A	AG	A	AG	A	AG	A	AG	A	AG	A
Esculin	AG±	–	AG	–	AG±	–	AG	–	–	–	–	–
Acetylmethyl-carbinol	–	–	–	–	–	–	–	–	–	–	–	–
Urease	±	±	+	–	–	–	–	–	+	–	+	–
Pellicle	–	–	–	–	–	–	–	–	–	–	–	–
Lecithinase	+	+	+	+	+	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Pigment	–	–	–	–	–	–	–	–	–	–	–	–
Viable counts	1.62×10^{10}		1.96×10^{10}		1.58×10^{10}		1.49×10^{10}		1.65×10^{10}		1.67×10^{10}	

1/ Thuricide TH90TS-H950T obtained from Bioferm, Wasco. Calif.

2/ Zectran 22%; Phosphamidon 90%; Pyrethrum 1.2% (with 12.6% piperonyl butoxide); Malathion 5 lb Imperial gallon; Piperonyl butoxide (Butacide).

3/ Controls repeated three times; test suspensions repeated twice.

4/ I - inoculation made direct from mixtures of chemical insecticides and commercial thuricide. II - inoculation made from colony isolated from mixtures of chemical insecticides and commercial thuricide. A - acid; G - gas production.

Table 5

Susceptibility of the silver spotted tiger moth, Halisidota argentata[1] to mixtures of Bacillus thuringiensis[2] and sub-lethal amounts of pyrethrum[3]

Treatment	No. treated	Cumulative per cent mortality[4]		Lt_{50} (days)[5]	Frass index	Cumulative per cent mortality from other pathogens[6]
		Total	B.t.+			
Control (Lovo 0.4%)	50	2	0	-	9	0
90TS/10	49	24	22	-	5	0
90TS/100	50	4	4	-	10	0
90TS/1000	50	0	0	-	12	0
D -01802/10	48	46	46	-	0	0
D -01802/100	46	17	15	-	8	0
D -01802/1000	48	0	0	-	14	0
Pyrethrum 10^{-3}	47	57	9	7	2	13 M
+90TS/10	50	94	80	4	0	0
+90TS/100	50	2	2	-	5	0
+90TS/1000	50	0	0	-	9	0
+D -01802/10	50	84	72	5	0	0
+D -01802/100	48	2	0	-	3	0
+D -01802/1000	50	8	4	-	7	0
Pyrethrum 10^{-4}	49	0	0	-	0	0
+90TS/10	51	39	33	-	2	6 MP
+90TS/100	51	4	4	-	8	0
+90TS/1000	50	0	0	-	12	0
+D -01802/10	52	35	35	-	4	0
+D -01802/100	47	11	6	-	9	4 M
+D -01802/1000	50	0	0	-	17	0
Pyrethrum 10^{-5}	50	0	0	0	9	0
+90TS/10	48	40	33	-	3	0
+90TS/100	46	2	2	-	9	0
+90TS/1000	50	0	0	0	15	0
+D -01802/10	50	38	26	-	4	0
+D -01802/100	50	4	4	-	7	0
+D -01802/1000	50	2	2	-	13	0

1/ Third instar larvae fed on Douglas fir.

2/ Thuricide 90TS and Thuricide D -01802 obtained from Bioferm, Wasco, Calif.

3/ Pyrethrum 1.2% (with piperonyl butoxide 12.6%).

4/ Mortality adjusted for mortality in controls by Abbott's formula.

5/ Number of days to 50 per cent mortality within the 7-day test period.

6/ P = Polyhedrosis virus; M = microsporidia.

Table 6

Susceptibility of Malacosoma disstria[1/] to mixtures of Bacillus thuringiensis[2/] and chemical insecticides[3/]

Treatment	No. treated	Cumulative per cent mortality[4/] Total	B.t.+	Lt_{50} (days)[5/]	Frass index	Cumulative per cent mortality from other pathogens[6/]
Control (Lovo 0.4%)	74	12	3	-	77	8 MP
Thuricide/10	95	91	84	3	3.6	3 P
Thuricide/100	98	75	57	5	7.6	0
Thuricide/1000	90	56	27	6	10.0	7 P
Phosphamidon 10^{-4}	96	100	5	2	0	6 MP
+Th/10	90	100	83	2	0	0
+Th/100	98	100	78	2	0	2 MP
+Th/1000	100	100	67	2	0	2 MP
Phosphamidon 5 X 10^{-5}	94	100	3	2	0	1 P
+Th/10	97	100	77	2	0	1 P
+Th/100	100	97	81	2	0	1 M
+Th/1000	97	99	44	3	0	3 P
Malathion 2 X 10^{-3}	105	100	1	1	0	4 M
+Th/10	98	100	79	1	0	0
+Th/100	95	100	63	1	0	7 MP
+Th/1000	99	100	20	1	0	5 MP
Malathion 2 X 10^{-4}	92	5	0	-	28	2 MP
+Th/10	95	95	88	3	0	3 P
+Th/100	103	76	57	5	8	0
+Th/1000	104	45	24	-	22	3 P

1/ Third instar larvae fed on trembling aspen.

2/ Thuricide S7-150 obtained from Bioferm, Wasco, Calif.

3/ Phosphamidon 90%; Malathion 5 lb/Imp. gallon.

4/ Corrected for mortality in controls by Abbott's formula.

5/ Number of days to 50 per cent mortality.

6/ P = polyhedrosis virus; M = microsporidia.

Table 7

Susceptibility of the black-headed budworm, <u>Acleris</u> <u>variana</u>[1/], to mixture of <u>Bacillus</u> <u>thuringiensis</u>[2/] and phosphamidon[3/]

Treatment	No. treated	Cumulative per cent mortality		Lt_{50} (days)	Frass index	Cumulative per cent mortality from other pathogens[4/]
		Total	<u>B</u>.<u>t</u>.+			
Control (Lovo 0.4%)	17	0	0	-	18	0
Thuricide/100	20	80	65	5	0	0
Thuricide/1000	25	20	8	-	0	4 P
Phos. 1 X 10^{-4}	54	100	2	2	-	11 P
+Th/100	51	100	53	3	-	4 P
+Th/1000	47	100	64	3	0	13 P
Phos. 5 X 10^{-5}	45	100	0	3	0	16 MP
+Th/100	36	100	83	3	0	3 P
+Th/1000	28	100	54	2	0	7 P

1/ Third to fifth instar larvae on western hemlock.

2/ Thuricide D -01802 obtained from Bioferm, Wasco, Calif.

3/ Phosphamidon 90%.

4/ P = Polyhedrosis virus; M = microsporidia.

Table 8

Susceptibility of the fall webworm, *Hyphantria cunea*[1/], to mixtures of *Bacillus thuringiensis*[2/] and sub-lethal amounts of chemical pesticides[3/]

Treatment	No. treated		Cumulative per cent mortality[4/] Total		Cumulative per cent mortality[4/] *B.t.*+		Lt_{50} (days)		Frass index[5/]		Cumulative per cent mortality from other pathogens[6/]	
	22°C	28°C	22°C	28°C	22°C	28°C	22°C	28°C	22°C	28°C	22°C	28°C
Control (Lovo 0.4%)	47	46	0	0	0	0	-	-	25	26	0	0
Thuricide/100	77	53	70	96	69	96	3	2	0	0	4 MP	0
Thuricide/1000	45	55	60	73	60	73	3	3	0	0	7 MP	0
Malathion 10^{-5}	52	42	0	0	0	0	-	-	12	6	0	0
+ Th/100	50	51	94	82	92	82	2	2	0	0	0	0
+ Th/1000	43	46	21	33	21	30	7	4	0	0	0	0
Malathion 10^{-6}	47	44	4	2	0	0	0	-	3	0	0	0
+ Th/100	51	51	80	88	76	88	3	2	0	0	2 P	0
+ Th/1000	49	46	69	39	63	39	3	4	0	0	4 M	0
Phosphamidon 10^{-4}	41	47	27	64	0	13	-	3	10	3	0	0
+ Th/100	48	47	94	96	92	94	2	3	0	0	0	2 P
+ Th/1000	47	49	62	78	53	72	3	3	0	0	0	0
Phosphamidon 10^{-5}	38	47	0	2	0	-	-	-	18	3	0	2 P
+ Th/100	47	45	89	89	87	89	2	3	0	0	0	0
+ Th/1000	49	44	40	52	34	48	4	3	0	0	0	0
Piperonyl Butoxide 10^{-5}	42	47	57	0	24	0	2	-	2	4	0	0
+ Th/100	45	50	67	96	60	96	3	2	0	0	0	0
+ Th/1000	47	47	38	85	38	83	3	2	0	0	0	0

... contd.

Table 8 (continued)

Treatment	No. treated		Cumulative per cent mortality[4/] Total		Cumulative per cent mortality[4/] B.t.+		Lt_{50} (days)		Frass index[5/]		Cumulative per cent mortality from other pathogens[6/]	
	22°C	28°C	22°C	28°C	22°C	28°C	22°C	28°C	22°C	28°C	22°C	28°C
Piperonyl Butoxide 10^{-6}	33	37	79	24	21	14	2	-	5	5	0	0
+ Th/100	50	51	90	63	88	61	2	3	0	0	0	0
+ Th/1000	47	47	28	19	26	17	5	4	1	0	0	0
Pyrethrum 10^{-5}	39	48	0	0	0	0	-	-	13	19	0	0
+ Th/100	43	48	91	100	91	100	2	2	0	0	0	0
+ Th/1000	48	49	85	57	85	57	2	3	0	0	0	0
Pyrethrum 10^{-6}	43	48	2	0	0	0	-	-	2	19	0	0
+ Th/100	51	48	76	100	76	100	2	2	0	0	0	0
+ Th/1000	47	44	28	75	23	68	5	3	0	0	0	0

1/ Third instar larvae fed on Red Alder.

2/ Thuricide 90TSH950T obtained from Bioferm, Wasco, Calif.

3/ Phosphamidon 90%; Pyrethrum 1.2% (with piperonyl butoxide 12.6%); Malathion 5 lb/Imp. gallon; Piperonyl butoxide (Butacide).

4/ Mortality at three days after placing larvae on treated foliage; mortality was total at 7 days. The field population from which these larvae were collected contained graunlosis virus.

5/ Frass taken seven days after placing larvae on foliage.

6/ P = nuclear polyhedrosis virus; M = microsporidia.

Table 9

Susceptibility of the Douglas fir tussock moth, Orgyia pseudotsugata[1/], to mixtures of Bacillus thuringiensis[2/] and sub-lethal amounts of chemical pesticides[3/]

Treatment	No. treated	Cumulative per cent mortality[4/] Total	B.t.+	Lt_{50} (days)	Frass index	Cumulative per cent mortality from other pathogens[5/]
Control (Lovo 0.4%)	50	10	0	-	86	0
Thuricide/100	48	82	82	5	8	0
Thuricide/1000	48	51	48	6	20	2 P
Malathion 10^{-5}	46	28	0	-	60	4 P
+ Th/100	48	96	92	5	2	2 P
+ Th/1000	49	84	57	5	8	0
Malathion 10^{-6}	50	0	0	-	76	0
+ Th/100	51	93	92	5	5	0
+ Th/1000	49	56	46	7	16	0
Zectran 10^{-4}	50	36	0	-	42	0
+ Th/100	47	86	72	4	3	2 P
+ Th/1000	49	71	54	5	18	6 MP
Zectran 10^{-5}	48	0	0	-	73	0
+ Th/100	49	93	92	4	2	0
+ Th/1000	46	49	46	7	16	0
Pyrethrum 10^{-3}	48	0	0	-	81	0
+ Th/100	49	91	90	5	4	0
+ Th/1000	47	71	55	6	9	4 MP
Pyrethrum 10^{-4}	46	8	0	-	107	0
+ Th/100	50	93	88	5	5	0
+ Th/1000	48	38	33	-	39	0
Piperonyl 10^{-3}	46	52	0	6	29	0
+ Th/100	49	89	74	5	2	2 M
+ Th/1000	49	78	44	5	4	4 P
Piperonyl 10^{-4}	49	4	0	-	58	0
+ Th/100	47	98	85	4	1	0
+ Th/1000	49	71	32	6	10	2 P

1/ Third instar larvae fed on Douglas fir.

2/ Thuricide 90TSH950T obtained from Bioferm, Wasco, Calif.

3/ Zectran 22%; Phosphamidon 90%; Pyrethrum 1.2% (with piperonyl butoxide 12.6%); Malathion 5 lb/Imp. gallon; Piperonyl butoxide (Butacide)

4/ Adjusted for mortality in controls by Abbott's formula.

5/ P = polyhedrosis virus; M = microsporidia.

Table 10

Effect of temperature on the susceptibility of the Douglas-fir tussock moth, <u>Orgyia pseudotsugata</u>[1/], to mixtures of <u>Bacillus thuringiensis</u>[2/] and sub-lethal amounts of Malathion[3/]

Treatment	No. treated			Cumulative per cent mortality[4/] Total			Cumulative per cent mortality[4/] B.t.+			Lt_{50} (days)			Frass index			Cumulative per cent mortality from other pathogens[5/]		
	52°	70°	82°	52°	70°	82°	52°	70°	82°	52°	70°	82°	52°	70°	82°	52°	70°	82°
Control (Lovo 0.4%)	48	48	45	16	4	7	0	0	0	-	-	-	33	173	200	2P	0	0
Thuricide/100	53	49	49	29	90	100	9	82	94	-	4	3	0.9	6	4	0	4P	0
/1000	53	46	47	0	57	72	0	43	70	-	6	4	6	30	19	0	2P	2P
Malathion 10^{-6}	48	50	51	0	0	0	0	0	0	-	-	-	25	148	231	0	2P	0
+Th/100	50	47	49	10	96	100	6	85	94	-	5	4	3	4	2	0	0	0
+Th/1000	49	49	45	10	33	55	4	28	51	-	-	7	8	54	56	6M	0	0

1/ Third and fourth instar larvae fed on Douglas fir.

2/ Thuricide 90TSH950T obtained from Bioferm, Wasco, Calif.

3/ Malathion 5 lb/Imp. gallon.

4/ Adjusted for mortality in controls by Abbott's formula.

5/ P = polyhedrosis virus; M = microsporidia.

Table 11

Susceptibility of the green-striped forest looper, Melanolophia imitata[1] to mixtures of Bacillus thuringiensis[2] and sub-lethal amounts of chemical insecticides[3]

Treatment	No. treated	Cumulative per cent mortality[4] Total	Cumulative per cent mortality[4] B.t.+	Lt_{50} (days)	Frass index[5]	Cumulative per cent mortality from other pathogens[6]
Lovo 0.4%	22	14	0	-	68	0
Thuricide/100	50	72	22	5	1	22 MP
Thuricide/1000	48	73	10	6	3	27 MP
Thuricide/10,000	44	5	0	-	98	9 M
Zectran 10^{-4}	48	0	0	-	70	0
+ Th/100	46	55	20	7	3	2 P
+ Th/1000	46	27	24	-	3	0
+ Th/10,000	47	3	3	-	10	0
Zectran 10^{-5}	50	0	0	-	80	0
+ Th/100	47	65	17	6	1	11 MP
+ Th/1000	48	24	4	-	4	13 MP
+ Th/10,000	50	12	2	-	8	2 M
Malathion 10^{-5}	47	6	0	-	68	2 MP
+ Th/100	47	53	28	7	3	0
+ Th/1000	48	20	17	-	4	0
+ Th/10,000	48	30	8	-	7	4 MP
Malathion 10^{-6}	48	0	0	-	62	0
+ Th/100	45	72	33	6	1	18 MP
+ Th/1000	58	40	12	-	3	3 M
+ Th/10,000	42	0	0	-	7	2 M
Piperonyl Butoxide 10^{-3}	45	67	0	5	8	20 MPF
+ Th/100	48	80	25	5	1	12 MP
+ Th/1000	52	86	37	5	1	8 MPF
+ Th/10,000	44	94	5	3	2	32 MPF
Piperonyl Butoxide 10^{-4}	50	30	0	-	45	4 M
+ Th/100	41	72	15	5	1	7 P
+ Th/1000	51	64	12	6	2	6 MP
+ Th/10,000	51	74	4	3	1	28 MP

1/ Second to fourth instars fed on western hemlock

2/ Thuricide 90TSH950T obtained from Bioferm, Wasco, Calif.

3/ Zectran 22%; Phosphamidon 90%; Pyrethrum 1.2% (with piperonyl butoxide 12.6%); Malathion 5 lb/Imp. gallon; Piperonyl butoxide (Butacide).

4/ Seven days after placing larvae on treated foliage; mortality-adjusted for mortality in controls by Abbott's formula.

5/ Grams per larva X 1000

6/ P = polyhedrosis virus; M = microsporidia; F = fungus

Table 12

Effect of temperature on the susceptibility of the green-striped forest looper, *Melanolophia imitata*, to mixtures of *Bacillus thuringiensis* and sub-lethal amounts of chemical pesticides

Treatment	No. treated			Cumulative per cent mortality Total			Cumulative per cent mortality B.t.+			Lt_{50} (days)			Frass index			Cumulative per cent mortality from other pathogens		
	10°	22°	28°	10°	22°	28°	10°	22°	28°	10°	22°	28°	10°	22°	28°	10°	22°	28°
Control (Lovo 0.4%)	26	23	22	8	23	5	0	0	0	-	-	-	6	47	49	0	5M	0
Thuricide/1000	50	49	43	26	49	46	8	10	28	-	-	-	1	3	7	0	12P	0
Malathion 10^{-6}	47	46	46	1	0	33	0	0	0	-	-	-	30	82	28	0	0	0
Mal. 10^{-6} + Th/1000	47	47	46	28	12	91	2	12	70	-	-	6	77	5	7	4MP	2P	7MP
Pyrethrum 10^{-4}	50	49	45	20	0	29	0	0	11	-	-	-	16	84	82	6M	2P	0
+Th/1000	51	48	48	60	60	61	10	15	29	6	4	5	0.3	6	3	4MP	8P	0
Phosphamidon 10^{-4}	46	47	46	13	12	47	0	0	13	-	-	7	28	-	34	4M	2M	2M
+Th/1000	51	45	50	18	36	89	2	4	26	-	7	3	1	4	1	2P	2P	2P

Table 13

Larval counts in field trials with mixtures of *Bacillus thuringiensis*[1] and piperonyl butoxide (Butacide) against the green-striped forest looper, *Melanolophia imitata*

Time of larval counts	Treatments											
	Control (Lovo 0.4%)			Piperonyl butoxide only			Thuricide/100 only			Piperonyl butoxide with Thuricide/100		
	Alive	Dead	B.t.+	Alive	Dead	B.t.+	Alive	Dead	B.t.+	Alive	Dead	B.t.+
Pre-spray totals[2]/av.	100/8.3	0	0	118/9.8	0	0	143/11.9	0	0	197/16.4	0	0
Post-spray S + 1	48	0	0	67	0	0	82	0	0	73	1	1
S + 2	70	0	0	37	0	0	47	3	2	37	1	1
S + 3	51	0	0	50	0	0	53	1	1	58	0	0
S + 5	47	0	0	57	0	0	54	2	2	88	6	5
S + 6	63	0	0	66	0	0	51	3	3	94	5	5
S + 7	32	0	0	42	0	0	40	2	2	47	10	10
S + 8	40	0	0	48	0	0	26	4	4	71	4	1
Totals	351	0	0	367	0	0	353	15	14	468	27	23
Average	4.2	-	-	4.3	-	-	4.2	-	-	5.6	-	-
Larval reduction[3]	4.1	-	-	5.5	-	-	7.7	-	-	10.8	-	-

[1] Thuricide 90TSH950T obtained from Bioferm, Wasco, Calif. Mean Max /mean min. temperature and per cent relative humidity were 67/54 and 89/57, respectively.

[2] Totals for 12-18" branch tips from 6-30' trees per treatment; S+1 etc. = 1 day etc. after application of spray; no collections were made on S+4 because of rain.

[3] Expressed as the difference between the average pre-spray counts and the average post-spray counts.

Table 14

Susceptibility of the western tent caterpillar, Malacosoma pluviale[1/], to mixtures of Bacillus thuringiensis[2/] and sub-lethal amounts of chemical pesticides[3/]

Treatment	No. treated	Cumulative per cent mortality[4/] Total	B.t.+	Lt_{50} (days)	Frass index	Cumulative per cent mortality from other pathogens[5/]
Control (Lovo 0.4%)	50	0	0	-	40	0
Zectran 10^{-3}	53	100	0	1	0	0
10^{-4}	47	89	0	5	0	0
10^{-5}	45	18	0	-	0	0
10^{-6}	47	2	0	-	14	0
Phosphamidon 10^{-5}	49	74	0	4	0	0
10^{-6}	50	4	0	-	28	0
10^{-7}	49	2	0	-	24	0
Pyrethrum 10^{-3}	51	63	0	6	0	0
10^{-4}	49	10	0	-	0	0
10^{-5}	49	8	0	-	17	0
Malathion 10^{-3}	50	100	0	1	0	0
10^{-4}	47	98	0	2	0	0
10^{-5}	52	46	0	-	0	0
10^{-7}	107	24	0	-	39	0
Piperonyl butoxide 10^{-3}	49	84	0	4	0	0
10^{-4}	49	14	0	-	27	0
10^{-5}	45	9	0	-	36	0
10^{-6}	48	4	0	-	35	0
Thuricide/100	102	86	55	5	0	2 P
Thuricide/1000	95	85	41	6	0	8 P
Zectran 10^{-6} + Th/100	95	84	78	4	0	20 P
+ Th/1000	98	60	41	5	0	0
Phos. 10^{-6} + Th/100	99	80	70	6	0	4 P
+ Th/1000	89	61	46	7	0	39 P
Pyrethrum 10^{-5} + Th/100	98	100	75	3	0	0
+ Th/1000	98	75	53	7	0	0

... contd.

Table 14 (continued)

Treatment		No. treated	Cumulative per cent mortality[4] Total	B.t.+	Lt_{50} (days)	Frass index	Cumulative per cent mortality from other pathogens[5]
Malathion 10^{-7}	+ Th/100	99	91	51	5	0	0
	+ Th/1000	93	76	19	7	0	0
Piperonyl butoxide 10^{-6}	+ Th/100	95	95	59	5	0	4 P
	+ Th/1000	91	96	48	5	0	0
Th90Ts-4-950T/10		99	100	88	2	0	1 P
	/100	92	100	90	2	0	1 P
	/1000	96	100	81	2	0	1 P

1/ Second and third instar larvae fed on Black Hawthorn.

2/ Thuricide S7-150 obtained from Bioferm, Wasco, Calif.

3/ Zectran 22%; Phosphamidon 90%; Pyrethrum 1.2% (with piperonyl butoxide 21.6%); Malathion 5 lb/Imp. gal; Piperonyl butoxide (Butacide)

4/ Adjusted for mortality in controls by Abbott's formula.

5/ P = polyhedrosis.

Table 15

Results of pre-spray laboratory tests of the susceptibility of Sydney Island population of *Malacomosa pluviale*[1/] to mixtures of *Bacillus thuringiensis*[2/] and phosphamidon[3/]

Treatment	No. treated	Cumulative per cent mortality Total	*B.t.*+	Lt_{50} (days)	Frass index	Cumulative per cent mortality from other pathogens[4/]
Control (Lovo 0.4%)	35	83	20	4	67	34 P
Thuricide /20	48	92	56	3	0	2 P
Phosphamidon 0.125%	44	98	2	2	0	9 P
+ Th/20	52	96	63	2	0	10 P
Phosphamidon 0.25%	51	100	10	2	0	8 P
+ Th/20	48	100	96	2	0	8 P

1/ Fourth, fifth and sixth instar larvae fed on red alder.

2/ Thuricide S7-186 obtained from Bioferm, Wasco, Calif.

3/ Phosphamidon 90%.

4/ P = Polyhedrosis.

Table 16

Results of field trials with mixtures of *Bacillus thuringiensis*[1] and phosphamidon against the western tent caterpillar, *Malacosoma pluviale*[2]

Treatment	Average[3] weight (grams) of frass collected following application of insecticides[4]				
	S + 2	S + 6	S + 9	S + 14	Totals
Control (untreated)	0.32	0.40	0.29	0.30	1.31
Per cent of total	24	31	22	23	
Control (Lovo 0.4%)	0.65	0.60	0.84	0.44	2.53
Per cent of total	26	24	32	17	
Thuricide /20	0.30	1.00	0.05	0.06	1.41
Per cent of total	21	71	4	4	
Phos. 0.125%	0.42	0.11	0.07	0	0.61
Per cent of total	69	18	11	0	
Phos. 0.125% + Th/20	1.26	0.62	1.00	0.41	3.29
Per cent of total	38	19	30	12	
Phos. 0.25%	0.33	0.13	0.08	0.15	0.69
Per cent of total	48	19	12	22	
Phos. 0.25% + Th/20	0.21	0.14	0.04	0.03	0.42
Per cent of total	50	33	10	7	

1/ Thuricide S7-186 obtained from Bioferm, Wasco, Calif.

2/ Larvae were in the 4th to 6th instars. Mean max/mean min temperature and mean max/mean min per cent relative humidity were 67/46 and 97/44, respectively, during the test period.

3/ Average of 2 collections from each 30' tree.

4/ S + 2 etc. = 2 days etc. after application of spray. Phosphamidon 90%.