LABORATORY AND FIELD TRIALS OF MIXTURES OF VARIOUS INSECT PATHOGENS AND INSECTICIDES AGAINST SOME FOREST INSECT PESTS

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INTRODUCTION

The simultaneous or sequential applications of insect pathogens and sub-lethal doses of selected insecticides is a promising approach to the integrated control of forest insect pests. In such an approach, the insecticide is used primarily as a means of stressing the insect to render it more susceptible to microbial infection. On the other hand, insects suffering from chronic or sub-lethal infection by pathogenic microorganisms should likewise be more susceptible to low doses of insecticide. Whichever way one views the process, in the final analysis the ultimate outcome of the method should be the reduction of tree defoliation with substantially reduced risk to environmental pollution.

In 1972, a series of experiments was started to test 1) the compatibility of various insecticides with insect pathogens, 2) the practicality for field use of various microbial formulations, 3) mortality responses of several insect species to Bacillus thuringiensis (BT) mixed with insecticides, 4) Benzyl cinnamate (an ultra violet chemical filter used in suntan lotions) as a microbial sunlight protectant, and 5) mist-blower application of mixtures of viruses or bacteria with fenitrothion or a synthetic pyrethroid, SBP-1382 XY against Choristoneura fumiferana. It is intended in this report to summarize the results and to discuss briefly their implications.

MATERIALS AND METHODS

Studies on Microbial Formulations

Two commercial B. thuringiensis formulations, Thuricide® (International Minerals and Chemicals) and Dipel® wettable powder (Abbott Laboratories) were tested for stability when suspended in spray oils or in spray oil emulsions (See Table 1). An oil carrier for the microbials was being sought for two main reasons. Firstly, aerial applications of oil suspensions are generally more efficient in terms of coverage, reduced drift, and evaporation, than are water suspensions. Secondly, there are several chemical ultra violet filters which should be tested in microbial formulations but which are only soluble in oils. The main criteria used for determining stability were separation into layers, creaming, sedimentation, color and clumping. All observations were made on suspensions stored in the refrigerator for 12 hours.

A series of tests were designed to determine the inhibitory effect of various spray oils, emulsifiers and dilute emulsifiable concentrate of fenitrothion on the growth of B. thuringiensis. Nutrient agar in petri dishes was seeded with B. thuringiensis (Dipel) and small, sterile Whatman #1 filter paper discs soaked in the various agents or insecticide were placed in triplicate on the agar surface. Experiments were repeated three times. The cultures were incubated at 29°C overnight and the inhibition zones developing around the discs were measured.

Dosage - Mortality Responses to *B. thuringiensis*, Insecticides and Mixtures of BT and Insecticides

The next two series of tests involved dosage - mortality responses of the white-marked tussock moth, *Hemerocampa leucostigmata*, the fall webworm, *Hyphantria cunea*, and the gypsy moth, *Porthetria dispar* to BT or various insecticides or mixtures of BT and insecticides.

H. leucostigmata larvae used were reared from eggs collected from the field in Pictou County, Nova Scotia. *H. cunea* were collected as second to third instar larvae in Ottawa and *P. dispar* were second generation insects originating from eggs collected at Ormstown, Quebec in 1970 and reared on artificial diet. Eggs were surface sterilized with 1/750 aqueous solution of zephiran chloride or 10% formaldehyde prior to hatching.

A molasses formulation of Dipel (BT) was used in all tests. Foliage species used were *Picea glauca* (*H. leucostigmata*) and *Betula papyrifera* (*H. cunea* and *P. dispar*). SBP-1382 XY, a stabilized synthetic pyrethroid was obtained from S.B. Penick Co., U.S.A., as a 24% emulsifiable concentrate. Fenitrothion 80% EC was obtained from Sumitomo Co., Japan. Matacil 99% technical grade (Chemagro) was used to make an emulsifiable concentrate which was then diluted with water for testing.

Foliage was dipped in various concentrations of BT or insecticide, dried, and fed to larvae. Daily mortality checks were made over a 5-day period and wet mounts of all dead larvae were examined microscopically for the presence of pathogens. All experiments were repeated three times with 40-50 larvae per replicate. Frass deposited by larvae was dried and weighed to give an estimate of the effect of the various treatments on feeding.

H. leucostigmata larvae were also sprayed in a spray tower with various dosages of SBP-1382. Experiments were replicated 3-6 times with 40-50 larvae each.

As a prelude to aerial application of pox and nuclear polyhedrosis virus combined with fenitrothion against C. fumiferana, experiments were conducted to study the dosage mortality responses of this species under simulated aerial spray conditions to each virus, to the insecticide, and to each virus combined with insecticide. Experiments were repeated 3-6 times with 40-50 larvae each.

In all combination tests, an effort was made to combine low (i.e. below 50%) doses of both pathogen and insecticide because additive effects are more likely to show up if dosages are relatively low.

Studies on Benzyl Cinnamate as Sunlight Protectant for B. thuringiensis and Insect Inclusion Virus

White spruce trees 4-6' high at Pembroke, Ontario and duplicate potted balsam fir (Abies balsamea) 2-3' high at Ottawa were sprayed to wetness with suspensions of B. thuringiensis or nuclear polyhedrosis virus in an emulsion containing 1% V/V of stove oil, 1% V/V Biofilm (an emulsifier obtained from Colloidal Products, U.S.A.) and 1% benzyl cinnamate (Fritzsche - D & O, U.S.A.). Benzyl cinnamate was mixed with the stove oil as the first step in preparing the emulsion. Dipel wettable powder (BT) containing 16,000 International Units of activity per milligram was added to the emulsion at the rate of 2.26 gm/100 ml. A pure concentrated suspension of H. leucostigmata nuclear polyhedra containing 10^9 polyhedral inclusion bodies per ml. was added to the emulsion to make

a final concentration of 10^7 polyhedra per ml. Lovo 192 (Fison's Overseas Products, Toronto) was added at 0.4% as an additional sticker-spreader. Weather records (Table 15) were kept for the Pembroke test on the site. Records for Ottawa are from DOT (Table 16).

The sprayed trees were exposed to direct sunlight for periods of from 1-28 days, after which, branches from them were colonized with C. fumiferana (BT treated) or H. leucostigmata (NPV treated). Experiments were repeated three times with 30-50 insects each. Insects were reared at 70-73°C and 70-80% relative humidity. All cadavers from the bioassay were diagnosed for cause of death. Mortality was checked daily for 5 days (BT treated) or 21 days (NPV treated) by phase contrast microscopy of squashed preparations.

Mist-blower Applications of Mixtures of Various Pathogens and Insecticides Against C. fumiferana

Following the above exploratory tests, mist-blower applications of pathogens mixed with a low dose of fenitrothion or SBP-1382 XY were carried out at Rankin, about 6 miles south of Pembroke, Ontario, against the spruce budworm. The spray formulations used in these tests are summarized in Table 17. Test trees were 20-30' high white spruce in an area of high budworm density. Fifteen spruce trees situated about 1.0 mile away served as controls. All experiments were replicated 3 times. Two 18" branch tip samples from middle and upper thirds of crowns were taken from each tree at the time of spray and at 3, 11, 18 and 21 days thereafter.

Details of larval development are found in Fig. 1 and cumulative meteorological conditions for each sample period are summarized in Table 19.

Data was recorded to give the following information: 1) incidence of pathogens among larvae and pupae, 2) incidence of parasites and associated species, and 3) relationship of treatment to pupation, adult emergence and defoliation. Studies on adult emergence were done in the laboratory with field collected pupae. Three 18" branches per tree from the upper, middle and lower thirds of crowns were used for estimating defoliation.

RESULTS AND DISCUSSION

Studies on Microbial Formulations

Results of the miscibility tests (Table 1) showed that Thuricide® slurry and Dipel wettable powder were relatively stable in Panosol and Superior Oil emulsions but not in the oils alone. Panosol (also Klearol and Aerotex), however, inhibited the growth of B. thuringiensis (Table 2). The emulsifiers Span 85, Biofilm, Tween 80 and Triton B 1956 had no inhibitory effect on bacterial growth (Table 3). Fenitrothion at a concentration of 0.01 oz/gallon in Aerotex-Atlox emulsion did not affect bacterial growth (Table 4) but growth was inhibited at 0.1 to 1.0 oz/gallon.

It was concluded from the above tests that Superior or Stove Oil, emulsified by Biofilm, would result in an emulsion stable enough for practical use and non-inhibiting to B. thuringiensis growth.

Dosage Mortality Tests

Dipel concentrations producing approximately 50% mortality among 3rd instar H. leucostigmata (Table 5), H. cunea (Table 6) and 2nd instar P. dispar (Table 7) were 10^{-2}, 10^{-4} and 10^{-3} dilutions, respectively. The incidence of NPV was low among H. cunea, slightly

higher among H. leucostigmata and high among P. dispar treated with high concentrations of Dipel. The fact that natural mortality from NPV among P. dispar was always higher in the treated than in the control group and that the incidence of NPV increased with increasing Dipel concentrations suggests that these two microorganisms are complementary in mortality effect. There was a general tendency towards reduction of frass, and thus feeding activity, as dosage increased.

Results of the foliage dipping tests showed that concentrations of 10^{-5}% and 10^{-4}% active ingredient of the stabilized pyrethroid, SBP-1382, killed 66.7% and 48% of 3rd instar H. leucostigmata and 2nd instar P. dispar, respectively (Tables 8 and 9). Feeding activity decreased as dosage increased (Table 9). SBP and fenitrothion at a concentration of 10^{-3}% active ingredient killed 80-99% of 3rd instar H. cunea while Matacil at 10^{-2}% caused only 60% mortality (Table 10). Feeding activity decreased in this species also as dosage increased.

Results of BT-insecticide combination experiments showed little truly additive effect by SBP, fenitrothion or Matacil (Table 11). SBP at 10^{-3} was slightly additive for H. cunea in that both total mortality from bacteria and incidence of bacterial septicemia increased from 74% to 86%. However, the combined mortality was only 6% above that for insecticide alone. In general, these insecticides at the dosages used appeared antagonistic to BT infection among H. cunea and P. dispar. This antagonism is reflected by feeding activity in that frass was always higher among insects fed the combination than among those fed BT alone. SBP at 10^{-5}% and Dipel at 10^{-2}% dilution were additive for H. leucostigmata.

Percentage mortality of H. leucostigmata sprayed in a Potter's tower showed that a dosage of 0.004 oz. SBP-1382 active ingredient per acre resulted in 29.3% mortality (Table 12) over 5 days. Tower tests on C. fumiferana gave 20.2% mortality with fenitrothion sprayed at 0.005 oz. active ingredient per acre (Table 13). No attempt was made here to calculate LD_{50} because the primary interest was to find sub-50% mortality dosage levels for combination tests with pathogens.

Percentage mortality from fenitrothion mixed with various pathogens and fed to spruce budworm are summarized in Table 14. Only the insecticide dosage producing the lowest mortality (38%) had a truly additive effect (69% mortality) when mixed with BT. Fenitrothion at the dosage tested appeared antagonistic to simultaneous NPV infection in that the incidence of virosis was always lower among larvae treated with virus-insecticide mixtures than among those treated with fenitrothion or NPV alone. Unfortunately, the mortality from NPV alone (85.3%) was too high to draw meaningful conclusions on combination effect. On the other hand, a fenitrothion dosage of 6.5×10^{-4} oz/acre and pox virus at 1.3 gm/acre were additive in combination.

Benzyl Cinnamate as Microbial Sunlight Protectant

Tables 15 and 16 show the meteorological conditions encountered by B. thuringiensis and the nuclear polyhedrosis virus of H. leucostigmata during various periods of exposure to natural sunlight. At Pembroke, temperature, relative humidity and rainfall were seasonally normal. The mean daily rate of solar radiation was relatively high for 1-day and 3-days exposure periods (976 and 605 cal/cm^2, respectively) and varied

between 471 and 541 cal/cm^2 for the other periods. Meteorological records during the Ottawa test were obtained from the Ottawa Weather Office, Department of Transports, and are shown in Table 16.

The data in Table 17 indicates that BT mixed with benzyl cinnamate was 43% active after 14 days and 28% active after 21 days weathering. NPV-benzyl cinnamate was 52% active after 7 days exposure but apparently lost activity thereafter. It has been known for some years that BT spores are sensitive to sunlight. Angus et al (1970) have shown that 72% of BT spores applied to white spruce in nature are inactivated after 24 hours and 85% after 72 hours exposure to sunlight. NPV of Trichoplusia ni lose about 80% of their viral activity after 17 days exposure (Jaques 1971) and NPV of Lambdina fiscellaria somniaria are about 90% inactivated after 35 hours of exposure to direct natural sunlight (Morris 1971). Thus, the present findings represent a considerable prolongation of the viability of BT and NPV under natural conditions.

Mist-blower Application Against C. fumiferana

Table 18 summarizes the formulations used in the mist-blower field trials at Rankin and Table 19 gives the meteorological conditions for each sample period. A weakness of the formulations (except Dipel + SBP and Dipel + fenitrothion) is the high pH which usually tends to reduce effectiveness of microbial insecticides. Temperature and humidity conditions were seasonal. Solar radiation and rainfall increased considerably with BT exposure time.

Larval Effects

It is obvious from the data in Table 20 that treatments, with or without sunlight protectant resulted in little or no mortality among larvae.

BT and SBP combined were additive in effect both in total mortality (14.7%) and in incidence of septicemia (46%).

Post-larval Effects

The effect of some of the treatments on pupal mortality was pronounced (Table 21). BT and NPV combined with benzyl cinnamate produced 100% pupal mortality. The effect was additive with but not without the protectant. Mortality from pox virus combined with BT but without protectant was 40% compared with 10% and 34% mortality from each pathogen alone. With the protectant the combined treatment was more than twice (83%) as effective.

Although the incidence of bacterial septicemia among cadavers was about 18% higher among insects sprayed with BT combined with SBP than among BT-alone treated ones, the total mortality was about 13% lower. Thus, the combination effect is only partially additive, the explanation of which is unknown at present. A partial combination effect is also observed among BT-fenitrothion treated insects where the incidence of septicemia was 57% higher than the sum of the incidences among insects treated with BT and insecticide separately.

When fenitrothion was applied 8 days after NPV, total pupal mortality was 67% with 92% of the cadavers virus-infected compared with 16% and 69%, respectively, when the insecticide was applied only 3 days after virus. It is likely that the low dose of insecticide effectively tipped the balance between survival and death at a time (8 days post virus) when the pupae were suffering from acute virosis.

The incidence of Dioryctria reniculella, an insect species commonly found associated with C. fumiferana, was as high as 47% among treated larvae and 31% among controls (Table 22). Pupal parasitism was generally low.

All treatments except fenitrothion alone caused substantial reductions in percent pupation (Table 23). The largest reductions were among insects treated with NPV alone or NPV-BT combinations.

The data in Table 24 show that total adult emergence was substantially reduced among insects treated with NPV alone, pox virus alone, pox virus + BT, SBP alone, BT + SBP or NPV + fenitrothion. Compared with controls, there were substantial reductions in female/male ratio among insects treated with BT alone, pox virus alone, BT + pox virus, BT + SBP, fenitrothion alone and BT + fenitrothion.

Studies on the effect of treatments on hatching are summarized in Table 25. Treatment by fenitrothion alone resulted in a progeny rate of 46 per female, precisely the same as found in a recent aerial test (Morris and Armstrong 1972). As in the aerial test, the lowest progeny rate (0.0) was found among insects sprayed with NPV plus fenitrothion.

In the final analysis, the success or failure of any defoliator control measure should be judged on the degree of protection from defoliation resulting from treatment. In the present studies, two combinations appeared to have afforded substantial protection viz BT-fenitrothion applied simultaneously and NPV and fenitrothion applied 8 days later. Reduction in percent defoliation were 50.7 and 46.2, respectively (Table 26). It will be recalled that the actual mortality among BT-fenitrothion treated larvae was relatively low (See Table 21). Thus the foliage protection must have resulted primarily from reduced feeding activity.

A somewhat stepped up field application of these two treatments combined with sunlight protectant and proprietary nutrient acidifiers to reduce pH of the spray suspensions is contemplated for 1973.

SUMMARY

A series of experiments were conducted during 1972 to determine 1) the compatibility of various insect microbial formulations with insecticides, 2) the mortality of several forest insect pest species to selected insecticides and pathogens separately and combined, 3) the effect of benzyl cinnamate (BC) as a sunlight protectant for insect pathogens and 4) the effect of mist-blower applications of viruses or bacteria mixed with fenitrothion or the pyrethroid, SBP-1382, on Choristoneura fumiferana. White spruce trees were sprayed with Bacillus thuringiensis (BT, Dipel) alone, nuclear polyhedrosis virus (NPV) alone, BT + NPV, BT + NPV + BC, pox virus alone, pox virus + BT, pox virus + BT + BC, SBP alone, SBP + BT, fenitrothion alone, fenitrothion + BT, pox virus + fenitrothion 8 days later, NPV + fenitrothion 3 days or 8 days later or water.

The results indicate that:

1. Superior or Stove Oil emulsified with Biofilm provide a stable and compatible emulsion carrier for commercial BT, and water suspensions of NPV or pox viruses.
2. Combinations of SBP, fenitrothion or Matacil and BT had no pronounced additive effect when tested against Hyphantria cunea or Porthetria dispar. On the other hand, a sub-lethal dose of fenitrothion producing 38% mortality among C. fumiferana when mixed with BT or pox virus (but not NPV) had truly additive effect in that both total mortality and incidences of pathogenesis were higher among combination treated insects than among insects treated with insecticide or pathogen alone. BT (Dipel) at 10^{-2} concentration and a 10^{-5}% SBP emulsifiable concentrate

also had an additive effect against Hemerocampa leucostigmata.

3. Benzyl cinnamate extended the activity of BT spores and NPV sprayed on trees for up to 21 days and 7 days respectively.

4. The mist-blower applications resulted in little or no mortality among spruce budworm larvae but the effects were pronounced during post-larval development. Pupal mortality was considerably increased by addition of benzyl cinnamate to some of the sprays. BT and SBP-1382 XY were partially additive in insecticidal effect. A sub-lethal dose of fenitrothion sequentially applied 8 days after virus application against larvae resulted in high pupal mortality and very low progeny rate. All treatment (except, fenitrothion alone) especially NPV or NPV + BT caused substantial reductions in pupation in the field. Most treatment reduced adult emergence and female/male sex ratio emergence from pupae. BT-fenitrothion applied simultaneously and NPV followed by fenitrothion 8 days later resulted in approximately 51% and 46% reduction in defoliation, respectively. These latter two treatments warrant further investigation by aerial application.

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TABLE 1

Miscibility of *Bacillus thuringiensis*[1] with Various Spray Oils and Emulsions

Oil Tested	Stability of Suspension[2]		
	In Emulsion		In Oil Alone[5]
	Thuricide Slurry[3]	Dipel WP[4]	Dipel WP
Stove	+	-	-
Diesel	+	+	-
Superior[6]	+	-	-
Panosol[6]	+	+	-
Aerotex	+	-	-
Klearol	+	-	-
Paraffin	-	-	-
Control (BT in water)	-	-	-

1 Thuricide (International Minerals and Chemicals) and Dipel wettable powder (Abbott Laboratories, Chicago).

2 General appearance after 12 hours in frig; + no separation of oil, light sediment, emulsion stable enough for practical use; (-) separation of oil and/or heavy sediment, emulsion not stable enough for practical use.

3 Thuricide 10%, Atlox 1%, Oil 1%, Water 88%.

4 Dipel 10%, Atlox 0.1%, Oil 10%, Water 80%.

5 Dipel WP 10% W/V.

6 Best overall stability.

TABLE 2

Inhibitory Effect of Various Spray Oils on the Growth of *Bacillus thuringiensis* (Dipel)[1] on Nutrient Agar Surface

Oils	Inhibition[2]	
	In Emulsion[3]	In Stock Oils
Controls (Untreated discs)	-	-
Controls (Deionized water)	-	-
Diesel	+	-
Stove	+	-
Paraffin	+	-
Klearol	+	+
Panosol	+	+
Superior	+	-
Aerotex	+	+

[1] Three replicates of 3 discs each.

[2] + and - indicate positive and negative inhibition, respectively.

[3] Atlox emulsifier 1%, oil 1% and water 98%.

TABLE 3

Inhibitory Effect of Various Emulsifiers on the Growth of Bacillus thuringiensis (Dipel) on Nutrient Agar Surface

Emulsifiers	Diameter of Inhibition Zones (cm)
Control (Untreated disc)	0.0
Control (Deionized water)	0.0
Span 85	0.0
Biofilm	0.0
Tween 80	0.0
Triton B 1956 L 530	0.0
Triton DF 20	2.6
Triton X 190	3.7
Triton 114	2.8
Triton 180	2.8
Triton 100	1.8
Atlox	2.2

[1] Each test 3 replicates of 3 discs each. Emulsifiers were diluted 1 percent in distilled water.

TABLE 4

Inhibitory Effect of Fenitrothion on the Growth of *Bacillus thuringiensis* on Nutrient Agar Surface

Treatment	Diameter of Inhibition Zone (cm)[1]
Control (Untreated disc)	0.0
Control (Deionized water)	0.0
1% Aerotex[2]	3.3
1% Atlox	3.0
Fenitrothion in Emulsion	
0.0001 oz./gallon	0.0
0.001 oz./gallon	0.0
0.01 oz./gallon	0.0
0.1 oz./gallon	2.1*
1.0 oz./gallon	3.0*

1 Mean of 5-6 replicates.

2 Emulsified by 1% Atlox.

* Dilution made with water. Inhibition here is attributable partly to Aerotex and Atlox at high concentrations.

TABLE 5

Corrected Percent Mortality[1] of 3rd. Instar
Hemerocampa leucostigmata Fed Foliage Dipped in
Various Concentrations of Bacillus thuringiensis (Dipel)

Number larvae Treated[2]	Dipel Concentration	Percent Mortality Total	BT+	NPV+	Frass/Larva (mg x 10^{-3}
147	Control (No treatment)	2.0	1.0	1.0	7.34
144	Controls (Water + Lovo)	0.0	0.0	0.0	6.94
150	10^{-5}	0.0	0.0	0.0	5.46
138	5×10^{-4}	0.0	0.0	0.0	0.44
148	10^{-4}	0.0	0.0	0.0	7.43
146	5×10^{-4}	5.0	5.0	1.0	3.15
137	10^{-3}	29.0	29.0	6.0	2.1
155	10^{-2}**	52.0	52.0	6.0	0.8

1 Cumulative over 5 days; corrected by Abbott's formula.

2 Numbers represent 3 replicates of 40-50 larvae each.

** Combined with SBP-1382 XY.

TABLE 6

Corrected Percent Mortality[1] of 3rd. Instar
Hyphantria cunea Drury Fed Foliage Dipped in
Various Concentrations of *Bacillus thuringiensis* (Dipel)

Number Larvae Treated[2]	Dipel Concentration	Percent Mortality			Frass/Larva (mg x 10^{-3})
		Total	BT	NPV+	
129	Control (Untreated)	3.0	0.0	3.0	4.2
91	Controls (Water + Lovo)	2.0	0.0	1.0	4.4
148	10^{-4}	52.6	52.6	0.0	0.7
135	2×10^{-4}**	73.2	73.2	0.0	0.2
142	10^{-3}	93.0	93.0	0.0	0.0
132	10^{-2}	94.0	94.0	0.0	0.0

1 Cumulative over 5 days; corrected by Abbott's formula.

2 Numbers represent 3 replicates of 40-50 larvae each except Water-Lovo treatment - 2 replicates.

** Combined with SBP-1382 XY, Fenitrothion or Matacil.

TABLE 7

Corrected Percent Mortality[1] of 2nd. Instar Porthetria dispar (L.) Fed Foliage Dipped in Various Concentrations of Bacillus thuringiensis (Dipel)

Number Larvae Treated[2]	Dipel Concentration	Percent Mortality			Frass/Larva (mg x 10^{-3})
		Total	BT+	NPV	
291	Controls (Untreated)	1.3	0.9	0.8	8.93
276	Controls (Water + Lovo)	2.7	2.7	2.5	7.97
134	10^{-5}	0.0	0.0	0.0	-
139	2×10^{-5}	0.0	0.0	0.0	-
137	10^{-4}	4.8	4.0	4.0	-
111	2×10^{-4}	17.0	17.0	11.0	9.90
132	10^{-3}**	53.4	47.0	13.0	0.0
147	10^{-2}	97.0	95.0	16.0	0.0

1 Cumulative over 5 days; corrected by Abbott's formula.

2 Three or six replicates of 40-50 insects each except Dipel 2×10^{-4} - 3 replicates of 30-40 each.

** Combined with SBP-1382 XY.

TABLE 8

Corrected Percent Mortality of 3rd. Instar
Hemerocampa leucostigmata Fed Foliage Dipped
in Various Concentrations of SBP-1382 XY

Number Larvae Treated[1]	SBP Concentration Percent Active Ingredient	Percent Mortality[2]
133	Control (Untreated)	16.0
142	Control (Water + Lovo)	11.9
138	10^{-5}	66.7
148	10^{-4}	67.9
148	10^{-3}	97.6

1 Three replicates of 40-50 larvae each.

2 Cumulative over 5 days; corrected by Abbott's formula.

TABLE 9

Corrected Percent Mortality[1] of 2nd. Instar *Porthetria dispar* (L.) Fed Foliage Dipped in Various Concentrations of SBP-1382 XY

Number Larvae Treated[2]	Insecticide Concentration Percent Active Ingredient	Percent Mortality	Frass/Larva (mg x 1,000)
291	Controls (water)	1.3	2.6
133	10^{-5}**	22.0	1.4
147	10^{-4}**	48.0	0.5
148	10^{-3}	80.0	0.0
151	10^{-2}	100.0	0.0
149	10^{-1}	100.0	0.0

[1] Cumulative over 5 days; corrected by Abbott's formula.

[2] Three replicates of 40-50 insects each.

** Combined with BT.

TABLE 10

Corrected Percent Mortality[1] of 3rd. Instar Hyphantria cunea Drury Fed Foliage Dipped in SBP-1382, Fenitrothion and Matacil

Number Larvae Treated[2]	Insecticide Concentration Percent Active Ingredient			Percent Mortality			Frass/Larva (mg x 10^{-3})
	SBP	Fenitrothion	Matacil	SBP	Fenitrothion	Matacil	
110	10^{-5}			10.0			4.5
135	10^{-4}			19.0			2.2
139	10^{-3}**			80.0			0.0
148		10^{-6}			6.0		5.6
115		10^{-5}			5.0		3.8
127		10^{-4}**			31.0		1.7
139		10^{-3}			99.0		0.0
145			10^{-5}			5.0	7.8
135			10^{-4}			5.0	6.7
135			10^{-3}			8.0	7.0
132			10^{-2}**			60.0	5.3

[1] Cumulative over 5 days; corrected by Abbott's formula.

[2] Three replicates of 30-50 larvae each.

** Combined with BT.

TABLE 11

Corrected Percent Mortality of *Hyphantria cunea*, *Porthetria dispar* and *Hemerocampa leucostigmata* Fed Foliage Dipped in Mixtures of Chemical Insecticides and Bacillus thuringiensis (Dipel)[1]

Insect Species/Instar	Dipel[2] Concn.	Insecticide Concentrate Percent Active Ingredient	Number of[3] Insects/ Combined Treatment	M_I	M_B(BT+)	M_{I+B}(BT+)	Frass/Larva (mg x 10^{-3})
Hyphantria cunea/III	2×10^{-4}	SBP/10^{-4}	130	19	74(74)	35(33)	1.5
	2×10^{-4}	SBP/10^{-3}	133	80	74(74)	86(86)	0.0
	2×10^{-4}	Fen./10^{-5}	148	5	74(74)	12(12)	2.1
	2×10^{-4}	Fen./10^{-4}	136	31	74(74)	20(19)	1.8
	2×10^{-4}	Mat./10^{-3}	155	8	74(74)	42(10)	1.0
	2×10^{-4}	Mat./10^{-2}	144	60	74(74)	81(1)	0.3
Porthetria dispar/II	2×10^{-3}	SBP/10^{-5}	151	22	54(47)	12(9)	1.5
	2×10^{-3}	SBP/10^{-4}	146	48	54(47)	28(25)	0.9
Hemerocampa leucostigmata/III	10^{-2}	SBP/10^{-5}	148	67	52(52)	46(45)	-
	10^{-2}	SBP/10^{-4}	146	68	52(52)	76(71)	-

1 Cumulative over 5 days; M_I - mortality from insecticide alone; M_B - mortality from BT alone; M_{I+B} - combined mortality. Mortality corrected by Abbott's formula.

2 Dipel molasses formulation obtained from Abbott Laboratories, Chicago.

3 Numbers represent 3 replicates of 40-55 insects each.

TABLE 12

Corrected Percent Mortality of 3rd. Instar *Hemerocampa leucostigmata* Sprayed on Artificial Diet with Various Concentrations of SBP-1382 XY[1]

Number Insects Treated[2]	Dosage Oz./U.S. gallon	Percent Mortality[3]
300	Controls (Untreated)	11.3
141	0.005	0.0
132	0.01	0.0
125	0.02	0.6
129	0.04	29.3
144	0.05	62.5
148	0.08	87.0
299	0.10	97.7

1 Spray rate 0.1 gallon per acre.

2 Three to six replicates of 40-50 larvae each.

3 Cumulative over 5 days; corrected by Abbott's formula.

TABLE 13

Corrected Percent Mortality of 4th. Instar *Choristoneura fumiferana* Fed Foliage Sprayed with Various Dosages of Fenitrothion[1] Emulsion

Number Insects Treated[2]	Dosage Oz./U.S. gallon	Percent Mortality[3]
280	Controls (Unsprayed)	5.0
148	Controls (Emulsion Only)	0.0
269	0.001	20.2
127	0.002	49.5
121	0.004	60.0
132	0.006	67.3
147	0.008	78.4
286	0.01	92.6

1 Insects sprayed on foliage at 0.5 U.S. gallons per acre.

2 Three to six replicates of 40-50 larvae each.

3 Cumulative over 5 days; corrected by Abbott's formula.

TABLE 14

Corrected Percent Mortality of 4th. Instar Choristoneura fumiferana Fed Foliage or Diet Sprayed with Mixtures of Various Pathogens and Fenitrothion

Number Insects Treated (Combined Test)[1]	Spray Dosage/Acre[2] Insecticide	Pathogen	M_I[3]	$M_P(+)$	$M_{I+P}(+)$
123	-	BT. 0.83	-	32.5(13)	-
119	6.5×10^{-4}	BT. 0.83	39.0	32.5(13)	10(4)
125	1.3×10^{-3}	BT. 0.83	48.0	32.5(13)	10(6)
116	2.6×10^{-3}	BT. 0.83	38.0	32.5(13)	69(37)
139	3.9×10^{-3}	BT. 0.83	69.0	32.5(13)	76(14)
119	5.2×10^{-3}	BT. 0.83	76.0	32.5(13)	61(32)
121	6.5×10^{-3}	BT. 0.83	90.0	32.5(13)	67(29)
103	-	NPV 3.25	-	85.7(82.3)	-
112	6.5×10^{-5}	NPV 3.25	0.0	85.7(82.3)	67.0(63.7)
79	6.5×10^{-4}	NPV 3.25	47.3	85.7(82.3)	85.7(77.0)
99	2.6×10^{-3}	NPV 3.25	67.0	85.7(82.3)	89.1(29.7)
105	-	Pox 1.30	-	22.3(13)	-
115	6.5×10^{-5}	Pox 1.30	0.0	22.3(13)	38.8(2.4)
104	6.5×10^{-4}	Pox 1.30	43.5	22.3(13)	51.7(29.4)
114	2.6×10^{-3}	Pox 1.30	63.5	22.3(13)	71.8(0)

1 Three replicates of 30-50 insects each.

2 Fenitrothion and BT dosage in oz./acre; NPV and Pox Viruses in gm/acre.

3 M_I, $M_P(+)$, $M_{I+P}(+)$ refers to mortality from insecticide, pathogen (positive diagnosis) and combined mortality, respectively.

TABLE 15

Cumulative Meteorological Conditions -
Rankin Sunlight Protectant Test 1972

Inclusive Dates	Temperature (°C)		Relative Humidity (%)		Solar Radiation (cal/cm^2)	Rainfall (inches)
	Mean Max	Mean Min	Mean Max	Mean Min		
June 20 - July 18	25.0	13.9	75.1	39.1	13,201	0.16
June 27 - July 18	26.7	13.9	75.4	34.7	11,025	0.06
July 4 - July 18	27.2	14.4	75.9	32.3	7,579	0.08
July 11 - July 18	27.8	16.7	75.4	39.5	3,727	0.13
July 15 - July 18	28.9	15.6	75.3	39.5	1,814	0.09
July 17 - July 18	28.9	17.2	76.0	33.0	976	0.03

TABLE 16

Cumulative Meteorological Conditions[1] - Ottawa
Sunlight Protectant Test 1972

Inclusive Dates	Temperature (°C) Mean Max/Mean Min	Relative Humidity (%) Mean Max/Mean Min	Rainfall (inches) Totals	Rainfall (inches) Daily Av.	Duration of Bright Sunlight (hrs.) Totals	Duration of Bright Sunlight (hrs.) Daily Av.
June 16 - July 14	23.0 / 13.0	88.0 / 52.2	7.28	0.26	185.3	6.62
June 23 - July 14	23.0 / 14.0	88.6 / 51.4	5.00	0.24	138.2	6.58
June 30 - July 14	24.4 / 13.3	86.4 / 46.3	4.06	0.29	109.1	7.79
July 7 - July 14	27.0 / 15.0	86.3 / 48.0	3.85	0.55	61.7	8.81
July 11 - July 14	28.5 / 16.5	89.0 / 55.3	3.24	1.08	25.0	8.30
July 13 - July 14	27.5 / 18.5	90.0 / 65.0	1.73	-	1.5	-

[1] Data from Ottawa Weather Office, DOT.

TABLE 17

Protective Action of Benzyl Cinnamate Against Weathering of *Bacillus thuringiensis* and Nuclear Polyhedrosis Virus

Treatment C.f.[1]	Treatment H.l.[1]	Exposure to Sunlight (days)	Number Insects in Bioassay[2] C.f.	Number Insects in Bioassay[2] H.l.	Corrected Percent Mortality[3] C.f. Total	Corrected Percent Mortality[3] C.f. BT+	Corrected Percent Mortality[3] H.l. Total	Corrected Percent Mortality[3] H.l. NPV+
BT Alone(C)[4]	NPV Alone(C)[4]	0	90	174	100.0	100.0	98.9	57.0
BT Alone	NPV Alone	1	98	161	96.4	96.4	70.5	36.1
BT + BC	NPV + BC	1	91	177	100.0	100.0	80.3	20.0
BT Alone	NPV Alone	3	90	166	88.1	88.1	30.0	8.3
BT + BC	NPV + BC	3	90	181	88.1	88.1	76.7	26.7
BT Alone	NPV Alone	7	92	227	58.3	58.3	93.0	47.9
BT + BC	NPV + BC	7	90	186	77.4	72.7	98.6	52.1
BT Alone	NPV Alone	14	90	193	58.0	47.6	0.0	0.0
BT + BC	NPV + BC	14	90	178	63.1	42.9	29.4	0.0
BT Alone	NPV Alone	21	90	165	13.2	8.4	0.0	0.0
BT + BC	NPV + BC	21	84	183	32.2	27.5	0.0	0.0
BT Alone	NPV Alone	28	90	238	14.4	8.4	0.0	0.0
BT + BC	NPV + BC	28	90	180	12.0	2.5	14.5	0.0

1 *Choristoneura fumiferana* and *Hemerocampa leucostigmata*. Tests carried out at Pembroke and Ottawa, respectively.

2 Three to five replicates of 30-50 insects each.

3 Corrected by Abbott's formula.

4 Treated foliage or plants unexposed to sunlight.

TABLE 18

Summary of Formulations Used in Mistblower Field Trials, Rankin 1972

Spray Formulation	Ingredients per 100 ml. Final Spray[1]										
	Stove Oil	Biofilm[2]	Lovo 1923[3]	Dipel WP	NPV Concn.	Pox Virus Concn.	Water	Benzyl Cinnamate	SBP-1382 XY EC[4]	Fenitrothion EC[4]	pH
Dipel Alone	1.0	1.0	0.4	2.26			97.6				8.3
NPV[5] Alone	1.0	1.0	0.4		6.2		91.2				8.5
Dipel + NPV	1.0	1.0	0.4	2.26	6.2		91.4				8.3
Dipel + NPV + BC[6]	1.0	1.0	0.4	2.26	6.2		90.4	1.0			8.3
Pox Virus[5] Alone	1.0	1.0	0.4			3.2	84.4				8.3
Dipel + Pox Virus	1.0	1.0	0.4	2.26		3.2	94.4				8.3
Dipel + Pox Virus + BC	1.0	1.0	0.4	2.26		3.2	93.4	1.0			8.3
SBP Alone			0.4						10^{-3}		6.4
SBP + Dipel			0.4	2.26					10^{-3}		8.1
Fenitrothion Alone			0.4							10^{-3}	6.4
Fenitrothion + Dipel				2.26						10^{-3}	7.5
Controls (Water)											

1 Dipel in gm., all other ingredients in ml. Dipel obtained from Abbott Laboratories, Chicago.

2 Emulsifier.

3 Sticker-spreader obtained from Fisons (Overseas) Products.

4 SBP 24 percent emulsifiable concentrate obtained from S.B. Pennick, N.Y., Fenitrothion 80 percent emulsifiable concentrate obtained from Sumitomo, Japan.

5 Viruses obtained from Insect Pathology Research Institute, Sault Ste. Marie, as water suspensions.

6 Benzyl Cinnamate obtained from Fritzsche - D & O Limited, Toronto.

TABLE 19

Cumulative Meteorological Conditions -
Rankin Mistblower Test 1972

Inclusive Dates	Temperature (°C)		Relative Humidity (%)		Solar Radiation (cal/cm^2)	Rainfall (inches)
	Mean Max	Mean Min	Mean Max	Mean Min		
May 29 - May 31	22.8	10.6	77.5	42.3	1,057	1.1
May 29 - June 8	22.2	10.0	77.2	36.5	4,801	1.79
May 29 - June 15	21.7	9.4	76.5	36.3	7,895	2.22
May 29 - June 22	21.7	10.0	76.8	37.8	11,335	4.34
May 29 - July 21	23.9	12.2	75.8	37.9	24,955	7.02

TABLE 20

Incidence of Pathogens Among Larvae - Mistblower Tests, Rankin 1972

Treatment	Total Larvae Collected	Percent[1] Mortality	Percent Cadavers Infected with			
			BT	NPV	Pox	Totals[2]
BT Alone	599	13.4(4.3)	18.3	0.0	0.0	18.3
NPV Alone	635	8.6(0.0)	0.0	25.0	0.0	25.0
BT + NPV	554	16.4(7.6)	25.7	3.3	0.0	29.0
BT + NPV + Protectant	382	19.2(10.7)	18.3	4.8	0.0	23.1
Pox Virus Alone	506	13.5(4.4)	0.0	1.3	1.3	1.3
Pox Virus + BT	399	19.2(10.7)	26.3	0.0	0.0	26.3
Pox Virus + BT + Protectant	536	11.1(1.8)	43.3	0.0	0.0	43.3
SBP Alone	453	8.1(0.0)	0.0	0.0	0.0	0.0
SBP + BT	519	22.8(14.7)	46.4	0.0	0.0	46.4
Fenitrothion Alone	285	24.4(16.5)	0.0	0.0	0.0	0.0
Fenitrothion + BT	195	18.1(8.4)	32.6	0.0	0.0	32.6
Pox Virus + Fenitrothion (S+8)	530	8.9(0.4)	0.0	0.0	0.0	0.0
NPV + Fenitrothion (S+3)	280	14.9(6.0)	0.0	19.5	0.0	19.5
NPV + Fenitrothion (S+8)	328	13.5(4.4)	0.0	20.5	0.0	20.5
Controls	786	9.5	0.0	5.0	0.0	5.0

[1] Mortality corrected by Abbott's formula in brackets.

[2] Infection by introduced pathogen(s). Corrected for incidence of NPV among controls.

TABLE 21

Incidence of Pathogens Among Pupae - Mistblower Tests,
Rankin 1972

Treatment	Total Pupae Collected	Percent[1] Mortality	Percent Cadavers Infected with BT	NPV	Y	Totals
BT Alone	400	46.5(10.2)	16.1	0.0	0.0	16.1
NPV Alone	150	87.3(78.7)	0.0	46.6	0.0	46.6
BT + NPV	137	41.6(2.0)	10.5	28.0	0.0	38.5
BT + NPV + Protectant	90	100.0(100)	13.3	56.7	0.0	70.0
Pox Virus Alone	228	60.5(33.7)	0.0	3.6	18.4	22.0
Pox Virus + BT	239	64.4(40.4)	64.9	0.0	1.9	66.8
Pox Virus + BT + Protectant	167	89.8(82.9)	34.0	0.0	0.0	34.0
SBP Alone	262	73.7(55.9)	0.0	0.0	0.0	0.0
SBP + BT	274	66.1(42.9)	23.8	0.0	0.0	23.8
Fenitrothion Alone	300	42.0(2.7)	1.6	0.0	0.0	1.6
Fenitrothion + BT	216	38.0(0.0)	74.4	0.0	0.0	74.4
Pox Virus + Fenitrothion (S+8)	295	46.7(10.6)	0.0	0.0	0.7	0.7
NPV + Fenitrothion (S+3)	26	50.0(16.1)	0.0	69.2	0.0	69.2
NPV + Fenitrothion (S+8)	103	80.6(67.4)	0.0	91.6	0.0	91.6
Controls	678	40.4	0.0	0.7	0.0	0.7

[1] Mortality corrected by Abbott's formula in brackets.

TABLE ??

Cumulative Incidence of Parasites and Associated Species - Mistblower Tests - Rankin 1972

Treatments	Associated[1] Species(%)	Pupal Parasitism(%)
Pre-spray[2]	7.8	-
Controls[3]	31.0	1.4
BT Alone	12.0	0.7
NPV Alone	11.1	0.4
BT + NPV	4.2	0.8
BT + NPV + Protectant	8.7	1.1
Pox Virus Alone	7.6	1.2
Pox Virus + BT	11.8	1.8
Pox Virus + BT + Protectant	13.1	1.8
SBP Alone	22.6	2.4
SBP + BT	12.7	0.6
Fenitrothion Alone	17.9	1.1
Fenitrothion + BT	46.9	0.8
Pox Virus + Fenitrothion (S+8)	11.1	1.1
NPV + Fenitrothion (S+3)	28.8	0.3
NPV + Fenitrothion (S+8)	28.0	0.3

1 Dioryctria reniculella Grate (spruce cone moth).

2 No larval parasites found; total of 78 samples from 39 trees.

3 Cumulative for 8 trees.

TABLE 23

Cumulative Effects of Treatments on Pupation in the Field - Mistblower Tests, Rankin 1972

Treatments	Percent Pupation
BT alone	21.4
NPV alone	2.5
BT + NPV	3.7
BT + NPV + Protectant	4.3
Pox Virus alone	14.2
Pox Virus + BT	20.8
Pox Virus + BT + Protectant	15.1
SBP alone	19.2
SBP + BT	25.4
Fenitrothion alone	38.3
Fenitrothion + BT	16.4
Pox Virus + Fenitrothion (S+8)	27.3
NPV + Fenitrothion (S+3)	13.3
NPV + Fenitrothion (S+8)	11.5
Controls	33.0

TABLE 24

Adult Emergence from Field-Collected Pupae
Mistblower Test - Rankin 1972

Treatments	Total Pupae		Percent Emergence		
	Females	Males	Females	Males	Total
BT alone	201	158	30.0	55.0	46.5
NPV alone	77	83	15.5	12.1	15.6
BT + NPV	58	84	51.7	57.1	62.4
BT + NPV + Protectant	40	51	62.5	62.8	75.0
Pox Virus alone	96	95	24.0	41.1	34.7
Pox Virus + BT	119	90	13.5	35.6	26.1
Pox Virus + BT + Protectant	70	72	44.3	43.1	53.0
SBP alone	99	122	13.1	17.2	16.2
SBP + BT	85	119	10.6	27.7	21.4
Fenitrothion alone	145	104	33.8	50.0	46.5
Fenitrothion + BT	107	111	56.1	56.8	63.4
Pox Virus + Fenitrothion (S+8)	115	125	47.8	37.6	45.1
NPV + Fenitrothion (S+3)	4*	1	-	-	-
NPV + Fenitrothion (S+8)	39	54	25.6	11.1	19.5
Controls	267	243	53.0	47.0	50.0

* Only 5 pupae could be found on the 3 trees.

TABLE 25

Effects of Treatments on Hatching - Mistblower Tests, Rankin 1972

Treatments	Number Larvae per Emerged Female
BT Alone	28
NPV Alone	9
BT + NPV	20
BT + NPV + Protectant	11
Pox Virus Alone	16
Pox Virus + BT	16
Pox Virus + Protectant	12
SBP Alone	13
SBP + BT	24
Fenitrothion Alone	46
Fenitrothion + BT	37
Pox Virus + Fenitrothion (S+8)	51
NPV + Fenitrothion (S+3)[1]	-
NPV + Fenitrothion (S+8)	0
Controls[2]	25

[1] Too few pupae collected for rearing test.

[2] Pupae collected from 8 white spruce trees.

TABLE 26

Defoliation[1] for Mistblower Tests - Rankin 1972

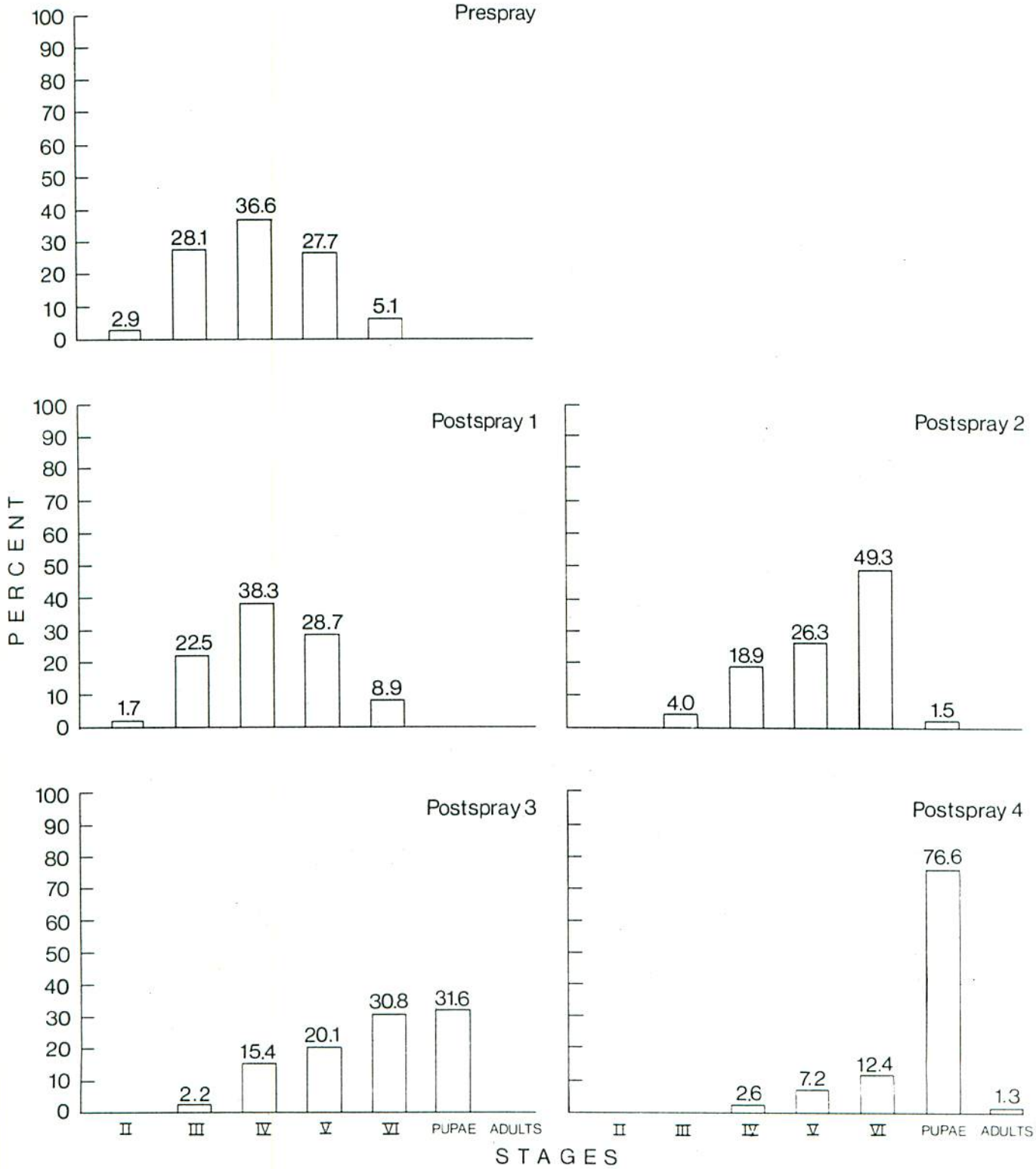
Treatment	Percent Defoliation	Percent Reduction[2] in Defoliation
BT Alone	66.5	13.5
NPV Alone	97.4	0.0
BT + NPV	90.9	0.0
BT + NPV + Benzyl Cinnamate	59.0	21.0
Pox Virus Alone	88.4	0.0
Pox Virus + BT	64.9	15.1
Pox Virus + BT + Benzyl Cinnamate	80.7	0.0
SBP Alone	51.2	28.8
SBP + BT	56.2	23.8
Fenitrothion Alone	35.1	44.9
Fenitrothion + BT	29.3	50.7
Pox Virus + Fenitrothion (S+8)	71.1	8.9
NPV + Fenitrothion (S+3)	43.9	36.1
NPV + Fenitrothion (S+8)	33.8	46.2
Controls[3]	80.0	

1 Determined from 3-18" branch samples from upper, middle and lower crowns. All trees are white spruce.

2 Difference between control and test figures.

3 Determined from 15 white spruces.

Larval Development-Mistblower
Tests Rankin 1972



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