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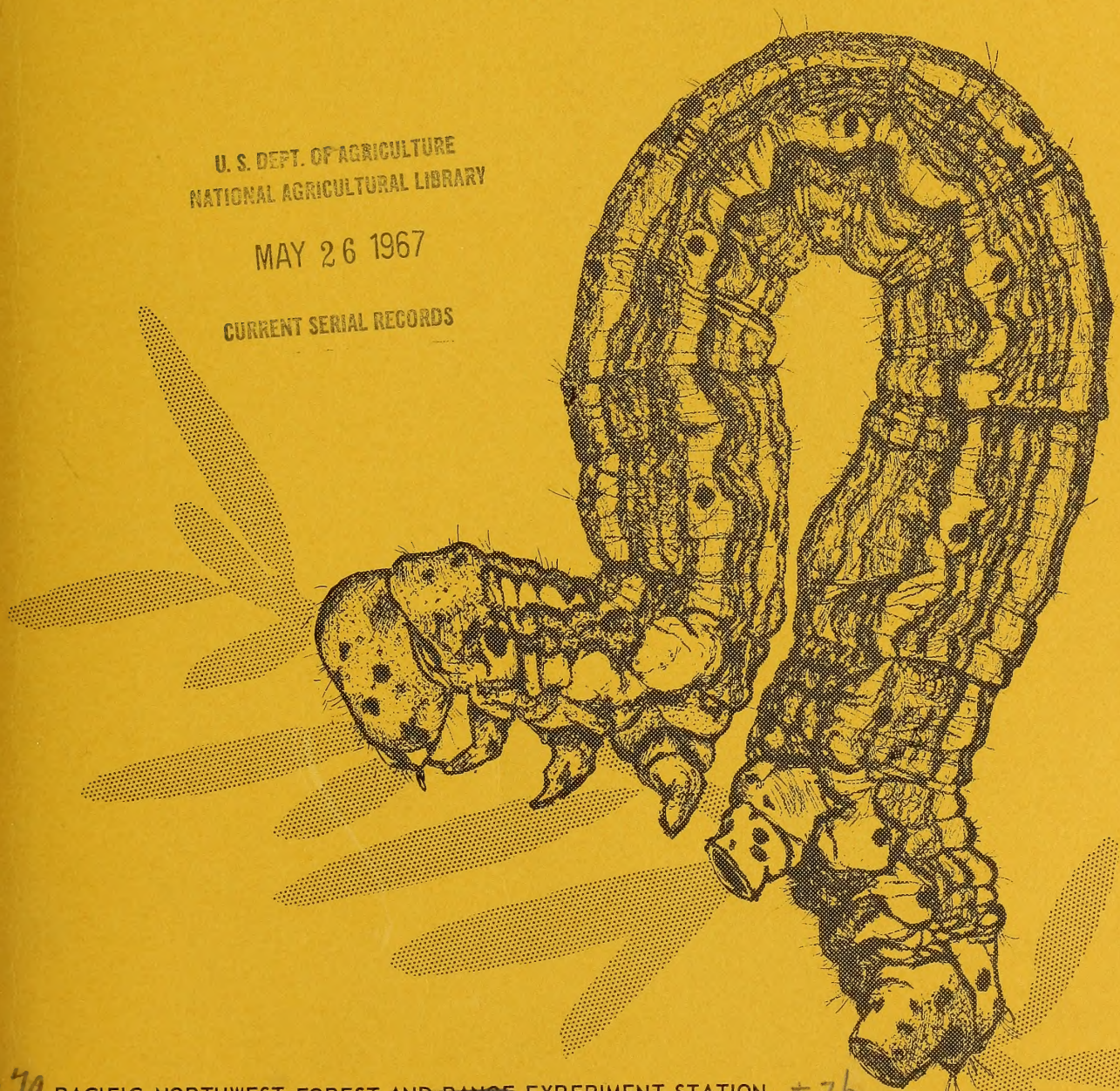
field testing of *Bacillus thuringiensis* for control of western hemlock looper

by v.m. carolin & c.g. thompson



PACIFIC NORTHWEST FOREST ~~AND RANGE~~ EXPERIMENT STATION
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CONTENTS

INTRODUCTION

The western hemlock looper, *Lambdina fiscellaria lugubrosa* (Hulst), periodically causes severe damage in coastal forests of Oregon, Washington, and British Columbia. Heavy feeding by this insect can kill hemlock trees in 1 year. Because outbreaks develop rapidly, direct control is usually needed the year after discovery of tree damage to prevent wholesale destruction of valuable timber. Aerial spraying with DDT in oil was proven an effective control measure in 1945 (Anonymous 1946, pp. 46-53). Since then, the method has been used successfully for looper control in the Northwest.

Potential hazard of DDT to other resources in coastal areas--big game, salmon, oysters, clams, and dairying--has led to increasing caution in its use and a search for alternative control measures. Microbial insecticides, such as viruses and bacteria, are possible substitutes. One of these, *Bacillus thuringiensis* Berliner, demonstrated by Steinhaus (1951) to be effective in control of the alfalfa caterpillar, has shown considerable promise for control of other agricultural insects. Results of field trials in Canada during 1960 on the spruce budworm (*Choristoneura fumiferana* (Clemens)) and black-headed budworm (*Acleris variana* (Fernald)) appeared to justify further study of the potential usefulness of *B. thuringiensis* preparations in the control of forest defoliators (Prebble 1961). Since then, results of combined field and laboratory tests have been reported by Smirnoff (1963) for spruce budworm, Lewis and Connola (1966) for gypsy moth (*Porthetria dispar* L.), and a pilot test on spruce budworm by Klein and Lewis (1966).

An opportunity to field-test *B. thuringiensis* var. *thuringiensis*, in a commercial water-based formulation,[1/] was provided by an outbreak of hemlock looper in southwest Washington in 1963. Thuricide 90-T, which our laboratory tests proved toxic to the hemlock looper and four other western forest pests, was applied by helicopter in a large-scale test under operational conditions.

TEST CONDITIONS

The test was conducted in an uneven-aged stand of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) on Long Island, a body of land about 6 miles long and 1 to 2 miles wide in Willapa Bay. The bay and adjacent timberlands provided a buffer area against a large looper control operation on the mainland using chemical insecticides. The principal overstory was 60- to 100-year-old hemlock with old-growth western redcedar (*Thuja plicata* Donn) and Sitka spruce

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[1/] Trade name Thuricide 90-T, prepared by the Bioferm Corp., Wasco, Calif., and marketed by Stauffer Chemical Co.

(*Picea sitchensis* (Bong.) Carr.) interspersed. Intensity of looper infestation was extremely variable. Access to the island was chiefly by boat; some supplies were airlifted by helicopter.

A test area of 325 acres was selected in the southern portion of the island where looper populations were generally high. An unsprayed check area was about 2 miles to the north in a stand similar to that in the test area but having relatively low populations. Locating and sampling plots required the cutting of parallel lines through the underbrush, starting from existing trails. To expedite the tests, the Weyerhaeuser Co. postponed logging for 3 years.

Physical conditions--particularly accessibility and suitability of stands for sampling--limited the field test to a single rate of application. A minimum rate of 1 gallon of Thuricide plus 1 gallon of water per acre was indicated by results of field tests on gypsy moth in the Northeast in 1962, now reported by Lewis and Connola (1966). In May 1963, prior to our field test, preliminary tests in cooperation with Agricultural Research Service provided initial data for obtaining this rate of application at heights of flight of 75 and 150 feet. Preliminary tests also included two other rates of application--1 gallon of Thuricide plus 3 gallons of water and 2 gallons of Thuricide plus 2 gallons of water. Small trees were exposed to the three rates of application and hemlock looper larvae then reared on these trees. Because no real difference in mortalities was evident between the three applications, the decision was made to field-test at the minimum rate.

Field work began on June 12. Approximately a month was required to construct lines, install plots, and obtain preliminary estimates of looper populations on plot trees. Spraying was on July 19, timed with the peak of the third instar. The Thuricide and water were combined at the nearby Willapa Game Refuge headquarters and mixed mechanically during transport to the heliport on a jeep carrying two 30-gallon tanks. Calcofluor white, a fluorescent material, was added at the rate of 1 percent by volume to simplify assessment of spray distribution. A Bell G-2 helicopter, equipped with a 30-foot boom and 18 No. 4664 Tee Jet spray nozzles, delivered the spray at the computed rate of 2 gallons per acre on 60-foot swaths, using a pump pressure of about 40 pounds per square inch. Airspeed was 30 miles per hour and flight level about 150 feet above the tree tops (fig. 1). Mass median (m.m.d.) of droplets at the spray nozzles was 160 to 170 microns. Each spray load was 60 gallons.

Figure 1.--Helicopter spraying test area with water-based suspension of *Bacillus thuringiensis*.

During the test period, July and August, a fog or low overcast persisted most of each day; stand temperatures usually ranged from 55° to 65° F., reaching 70° to 72° on the few days with sunshine. Relative humidity was 60 to 70 percent in midafternoon, increasing to 90 to 100 percent at night.

OBJECTIVES

The broad objective of the field test was to determine whether economic control of the western hemlock looper with *B. thuringiensis* was immediately feasible. Specific objectives were to:

A. Determine when larval mortality commenced, reached a peak, and ended and how much mortality occurred.
B. Relate spray deposit and spray distribution to total looper mortality at the end of a 20-day period.
C. Test the frass-drop method as means for evaluating looper mortality.
D. Deduce indirect effects of spraying on larval development and biotic control agents.
E. Evaluate population trends and tree damage occurring 1 and 2 years after spraying.

ASSESSMENT OF LARVAL MORTALITY

Methods

Test and check areas were sampled by means of systematically located plots. Each plot consisted of three codominant hemlocks which were sampled for living larvae; a cloth-bottomed tray was placed under each tree to collect dead larvae and frass falling from trees (fig. 2). In the test area, 20 plots were located along five east-west lines, which were about 180 yards apart. In the check area, 10 plots were equally divided between two north-south lines about 150 yards apart. In both areas, plots were located at 150- to 160-yard intervals along lines.

Figure 2.--Sample tree at plot used to evaluate larval mortality from spraying with *B. thuringiensis*. Note dense underbrush.

Figure 3.--Ground tray used to collect dead larvae and frass falling from sample trees.

The pattern of larval mortality caused by *Bacillus* was determined by counting and collecting dead larvae found at intervals in ground trays and culturing these to determine presence or absence of *Bacillus*. Each ground tray was 16 x 20 inches outside, with an inside area of 2 square feet. Two sides of each tray were painted with deer repellent, to avoid damage by mammals (fig. 3). Trays in the test area were emptied the evening before spraying, then examined every 2 days until 20 days after spraying. Trays in the check area were cleaned the day of test-area spraying, then examined 7, 13, and 19 days later.

Quantitative estimates of larval mortality were obtained from periodic counts of larvae on foliage samples collected with a 35-foot pole-pruner equipped with a basket (fig. 4). At each sampling, five 18-inch branch tips were clipped from each plot tree; the total larvae on the 15 branch tips from the three trees represented the population estimate for the plot. In the test area, trees were sampled 3 to 4 days before spraying and 4, 7, 10, 13, 16, and 19 days after spraying. In the check area, trees were sampled 2 days before test-area spraying and 6, 12, and 18 days after spraying. The total mortality at individual plots was estimated by comparing numbers of larvae on 15 branch tips before test-area spraying and 19 days after spraying. In the test area, regression analysis was used to determine the significance of downward population trends, as indicated by periodic counts.

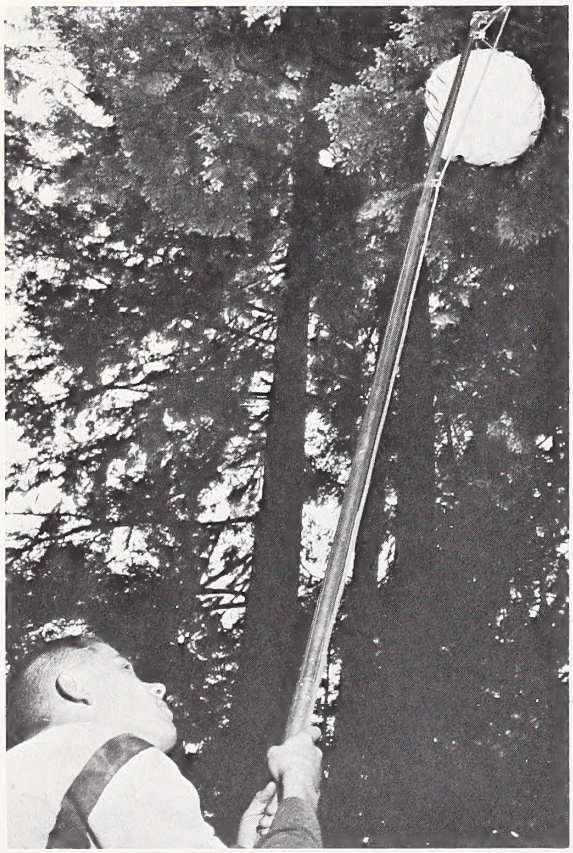

Figure 4.--Cutting 18-inch branch tips from sample trees to estimate numbers of living larvae.

Results

Pattern of Larval Mortality

Numerous distressed larvae were noticed on foliage samples 4 and 7 days after spraying, and the first dead larvae in ground trays were found 6 days after spraying. Mortality quickly reached a peak 8 days after spraying, with 20 larvae found in the trays, then decreased gradually until a sharp decline on the 16th day (fig. 5). Only 94 dead larvae were found in the trays over the 20-day period.

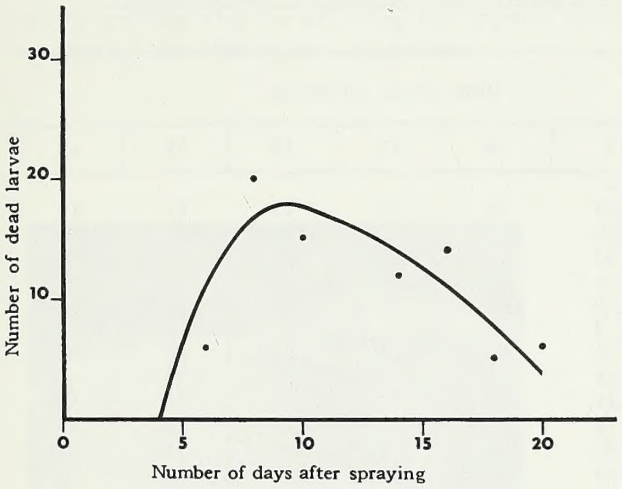

Figure 5.--Numbers of dead larvae found in ground trays during a 20-day period after spraying with *B. thuringiensis*.

An additional 47 dead larvae were found on branch tips removed by pole-pruner; approximately equal numbers were found in each of five samplings up to the 16th day after spraying. Dislodgment of dead larvae was apparently a gradual process.

Bacillus infections were found in varying percentages of larvae obtained 6 to 18 days after spraying. From the 8th to the 12th day after spraying, around 70 percent of the dead larvae were infected; in collections on the 14th and 16th days after spraying, around 50 percent were infected. On the 18th day after spraying, one out of five larvae found (20 percent) was infected with *Bacillus*. The decline in detectable *Bacillus* infections after the 12th day was also indicated by analysis of dead larvae found on tips cut by pole-pruner. Of 16 larvae collected 13 days after spraying, 50 percent were infected.

Amount of Larval Mortality

Numbers of larvae found on 18-inch branch tips before spraying and at intervals after spraying (table 1) indicated generally low and variable mortality in the treated area. Larval mortality 19 days after spraying ranged from 0 to 66 percent for the 20 plots, averaging 30 percent for the area. Six plots showed a range of 49 to 66 percent mortality; seven plots, 11 to 43 percent mortality; and seven plots, virtually no mortality. Numbers of larvae on 18-inch branch tips 33 days after spraying, about a week before pupation commenced, showed few further decreases, except at three plots (Nos. 2, 19, 20) where heavy

defoliation had occurred. In the check area, natural larval mortality 18 days after test-area spraying was about 10 percent, increasing to 14 percent at 33 days.

Table 1.--Numbers of western hemlock looper larvae on fifteen 18-inch hemlock tips per sample plot before and after aerial spraying with *B. thuringiensis*.

Plot No.	Days before spraying		Days after spraying						
	9-17	4	4	7	10	13	16	19	33
1	58	45	20	29	26	26	15	17	14
2	(370)[1/]	370	356	259	205	241	146	187	115
3	27	20	17	20	24	18	13	14	23
4	(35)[1/]	35	24	31	38	35	24	41	34
5	32	32	16	10	19	22	22	11	21
6	15	7	12	3	8	4	2	7	2
7	24	25	28	31	14	14	24	16	25
8	16	18	24	26	25	9	10	15	6
9	14	29	17	21	11	10	15	14	14
10	20	15	18	12	22	8	17	20	12
11	68	71	34	30	63	73	35	34	40
12	10	4	7	9	13	10	13	12	3
13	42	47	43	57	55	66	37	53	40
14	(65)[1/]	65	95	96	106	89	73	44	50
15	50	49	62	51	67	61	50	28	22
16	25	18	10	15	13	6	10	16	6
17	23	30	16	16	21	10	22	15	19
18	16	22	16	12	12	19	12	17	10
19	94	104	91	119	80	122	88	103	49
20	197	135	114	164	105	113	111	131	55
Total	1,201	1,141	1,020	1,011	927	956	739	795	560

[1/] Figures for 4 days before spraying inserted, because samples were not taken at 9-17 days.

Linear regression analyses performed for all plots showed that at only three plots (Nos. 1, 2, and 9) was there significance ($P = 0.05$) in the downward slope of the regression line. A regression analysis for the test area, pooling all plots, failed to show significance. Sampling error accompanying the use of the 18-inch branch tip was considerable and probably obscured small reductions in populations. The prespray population estimate was particularly important since it tended to anchor one end of the regression line. On six plots the prespray count was clearly shown by subsequent sampling to be low; regression analyses without the prespray counts showed the recalculated slopes of lines for two of these plots (Nos. 8 and 14) to border on significance.

RELATION OF
SPRAY DEPOSIT AND DISTRIBUTION TO MORTALITY

Methods

On the day of spraying, samples were taken from each 5-gallon drum to determine the viable spore concentration of the undiluted Thuricide. These were processed by diluting the spore suspension, culturing the spores, and counting the *Bacillus* colonies.

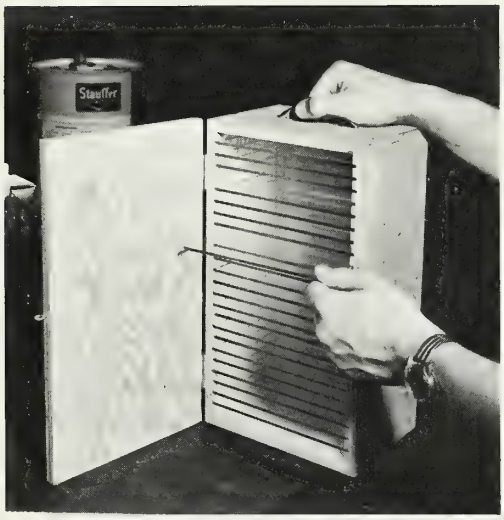

Figure 6.--Plywood case used to protect spray deposits while transporting glass plates in the field.

At each plot in the treated area, eight 5x7-inch glass plates were used to collect spray deposit, and eight 5x7-inch cards of a selected cover stock were placed to record spray distribution. To insure exposure of slides and cards to falling spray, two small clearings were made at each plot under existing openings in the forest canopy. Four plates and four cards were placed in an opening uphill and four of each in an opening downhill from the access line at each plot, on surfaces above the ground such as logs and stumps. After the spraying, plates were transported in specially made plywood cases (fig. 6) to Oregon State University, where number of viable spores was determined.[2/] Cards were brought immediately to our Portland headquarters and photographed under ultraviolet light to obtain a permanent record of numbers and sizes of spray particles. An indication of spray distribution in the lower crown of sample trees was obtained by examining foliage samples in a darkened room with a portable ultraviolet lamp.

The persistence of *Bacillus* in field deposits was analyzed at a field laboratory by two methods. In one, leaves of salal (*Gaultheria shallon* Pursh) were collected daily for 20 days following spraying. One-inch squares were cut from these leaves and washed with 10 milliliters of distilled water. After the washing was diluted 1:10, 1:100, and 1:1000, 0.1 milliliter of each dilution was

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[2/] Contract arrangement with Microbiology Department, Oregon State University.

Figure 7.--Rearing of larvae in glass jars on field-collected foliage to determine residual effects of spray deposits.

spread on agar. After incubation, *Bacillus* colonies were counted to estimate viable spore concentrations. In the second method, selected hemlock foliage, averaging 1.5 spray droplets per hemlock needle, was collected, first at 2-day intervals and then at 3-day intervals for up to 24 days after spraying, and fed to larvae collected from unsprayed areas. Larvae were reared at room temperature in glass jars (fig. 7), and percent mortality was recorded by number of days after spraying that foliage was collected.

Density of spray particles found on cards was compared with larval mortalities at the different plots. Density was based on number of particles counted under a microscope on a square inch in the center of each spray card photo. Data were examined for possible correlation; then, arbitrary density categories were set up for gross analysis, as follows:

Low, both uphill and downhill locations at a plot averaging less than 25 droplets per square inch.

Intermediate, one location averaging 50 to 100 and the other 37 or less droplets per square inch. Also, plots at which both locations averaged 25 to 50 droplets per square inch.

High, one location averaging more than 100 droplets per square inch, and the other location averaging 38 to 88 droplets per square inch.

Size of particles on spray cards was also determined and compared with particle size computed for the helicopter spray emission system. Particles on the center square inch of spray card photos were measured through a microscope with a calibrated eyepiece, and a spread factor was applied[3/] to convert to particle size at impact.

[3/] Spread factor for the batch of Thuricide and cover stock used was determined by Bohdan Maksymiuk, Pacific Northwest Forest and Range Experiment Station, Forestry Sciences Laboratory, Corvallis, Oregon.

Results

Analysis of *Bacillus* Deposit

Numbers of viable spores in the undiluted Thuricide formulation averaged 18×10^9 per milliliter, slightly more than half that claimed by the manufacturer--30×10^9 per milliliter. Numbers of viable spores on glass plates exposed to spraying varied greatly, both within and between plots, but appeared to be generally low. Average counts for individual plots ranged from 545 to 290,000 viable spores per 35 square inches of plate; 13 plots were in the range of 2,000 to 7,500 viable spores per plate, or 57 to 214 spores per square inch.

Deposits on salal leaves (fig. 8) showed very little loss in viable spore count during the first 4 days after spraying, even though a soaking rain occurred on the 3d day after spraying. Some decrease in spore count occurred at the end of 6 days, but the median-sized droplets still contained more than a lethal dose.

Figure 8.--Spray deposits on foliage of salal.

After 9 days, however, a sharp drop in spore count was found, and the average droplet on most leaves no longer contained a lethal dose. Beginning with the 9th day, great variation was found between individual samples but a few leaves still retained close to the original spore count. After 14 days, only an occasional leaf had droplets still containing lethal doses. The loss in spore count appeared due to a "weathering-off" of the spray material and was accompanied by a loss of fluorescence of the Calcofluor in the material.

Results from feeding sprayed foliage to larvae from an unsprayed area paralleled the recovery of viable spore deposits from the salal leaves. However, at room temperature, larvae fed rapidly, compared with field conditions,

and readily contacted the *Bacillus* deposit. Mortality was high for the first 6 days--much higher than that encountered in the field--then dropped off rapidly. Results of these tests were:

Days after spraying (Number)	Larvae per test run (Number)	Test runs (Number)	Larval mortality (Percent)
2	30	3	94
4	30	3	90
6	30	3	85
9	30	3	40
12	30	3	10
15	30	3	4
18	30	3	2
21	30	3	0
24	30	3	0

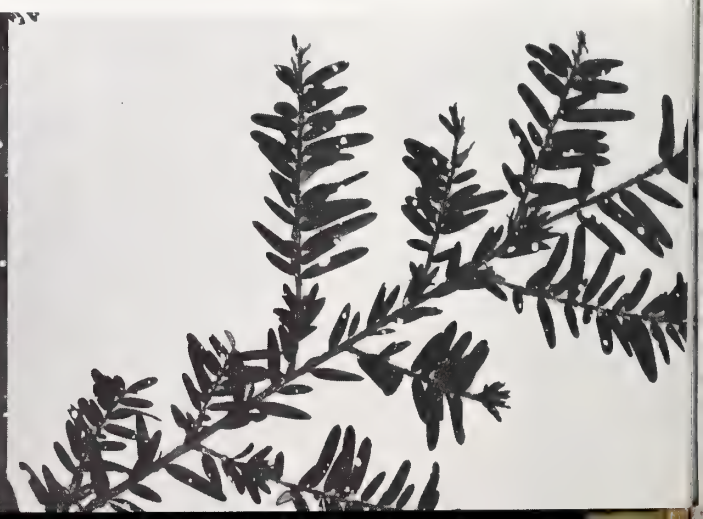
The importance of temperature as a factor affecting larval mortality through rate of feeding was clearly indicated, thus confirming results by Smirnoff (1963).

Relation Between Spray Distribution and Larval Mortality

Particle density, as recorded on deposit cards, appeared generally good, according to standards for use of DDT. Average number of particles per square inch was 41; averages for four-card groups ranged from 4 to 134 per square inch. All cards received some spray. However, distribution of spray particles on lower branches was actually quite poor, ranging from one droplet per 6 to 7 needles at a plot showing a significant larval reduction to one droplet per 500 to 1,000 needles at plots with no significant reduction. Examples of particle density on deposit cards (fig. 9) and on foliage (fig. 10) are shown for plot 2, where significant larval reductions occurred.

Figure 9.--Spray deposits on one-half of a deposit card placed at plot 2.

Figure 10.--Spray deposits on branchlets in lower crown of a tree at plot 2.



Particle density on deposit cards and larval mortality at the different plots were not correlated. A similar finding was reported by Maksymiuk (1963) in aerial spray tests for control of spruce budworm. Two of three plots showing statistically significant larval mortality were in the intermediate spray density category and one in the high density category. Two plots at which significance was approached after the prespray count was dropped were in the intermediate category. In the following tabulation, the three plots with significant mortality are indicated by an asterisk (*).

Spray particle density	Number of plots	Larval mortalities
Low	6	0, 0, 0, 11, 30, 66
Intermediate	11	0, 0, 0, 17, 23, 32, 36, 43, 49*, 52*, 52
High	3	0, 50, 62*

Size of spray particles on deposit cards averaged around 285 microns, ranging from 50 to 1,035 microns, after a computed spread factor of 2.03 was applied. Average particle size was thus about 100 microns greater than that calculated for the spray system. It is likely that the smaller particles were lost through drift and evaporation and, also, that some of the larger particles absorbed water while falling through the humid atmosphere. Peculiarities in the behavior of water-based sprays, when applied from the air, have been described by Fettes (1958).

Because of their size, most spray particles probably represented more than a lethal dose. It is reasoned that density of spray particles on the foliage in this test was far more important than number of viable spores deposited. Although spray distribution as recorded on cards appeared good, coverage of the lower crown was insufficient to cause substantial larval mortality.

TESTING FRASS DROP FOR ASSESSING MORTALITY

Methods

The frass-drop method was found effective by Morris (1949) in studying the larval population and ecology of the European spruce sawfly. Its use for estimating populations is based on determining: (1) the amount of frass falling on a tray during a known period of time and (2) the amount of frass produced by an average larva during the same period of time. Frass production by an individual larva (termed "frass yield" by Morris) varies with its size (larval instar) and with environmental conditions. Amount of frass is usually expressed by either volume or weight. The method is promising, but needs further field testing to prove its capabilities for estimating populations of specific defoliators.

Frass drop was sampled at each plot by three ground trays, the same ones used for collecting dead larvae. In the treated area, cumulations were removed from the trays 4, 8, 12, 16, and 20 days after spraying and in the check area, 7, 13, and 19 days. Frass production for the first period in the check area was adjusted to a 6-day base. Contents of each tray were kept separate, by collecting date, in small cardboard boxes with hinged lids. After ovendrying, debris was removed and frass weighed. Frass production for a plot was the sum of the ovendry weights, in grams, of frass from the three trays. The selection of weight in preference to volume as a measurement unit was purely arbitrary.

Larval mortality due to the spray application was assessed in three steps. The first was a comparison of trends in frass production between treated and check areas, with the trend in the check area assumed to be normal. The second was analysis of rate of frass production in the treated area by 4-day periods to determine whether the effect of the *Bacillus* was recognizable and when it occurred. Analysis required calculation of theoretical frass production, as though no mortality had occurred, starting with the fifth period when the *Bacillus* deposit was no longer viable and working back to the first period. Proportion of larvae in instars III, IV, and V was used to calculate a frass production index for each 4-day period. The small proportion of larvae in instar II was ignored. Weighting factors of 1, 3, and 9 were used for instars III, IV, and V, respectively, since frass yield in grams over unit periods of time approximately tripled between instars III and IV, and IV and V.[4/] The frass production index was thus the sum of $n_{III} \times 1 + n_{IV} \times 3 + n_{V} \times 9$, with n being the percent of larvae in a designated instar. A conversion factor between the frass production index and actual frass production was determined for the fifth 4-day period and applied to the preceding 4-day periods to obtain hypothetical frass production. Large differences between actual and hypothetical frass production for specific periods were to be considered real.

The third procedure was to identify individual plots, at which little increase in frass production occurred, by examining frass production in 4-day periods. These plots were compared with those identified by foliage sampling as showing significant reductions in larval numbers.

Results

Trends in frass production were strikingly different between check and treated areas. Frass production in the check area increased slowly during an 18-day period. In contrast, production in the treated area, starting at a similar

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[4/] Unpublished report on file at the Pacific Northwest Forest and Range Experiment Station, Portland, Oregon.

level to that in the check area, increased sharply 5 to 8 days after spraying, with steady increases thereafter. Trend lines are shown in figure 11, with frass production in the check area doubled to put it on a 20-plot basis.

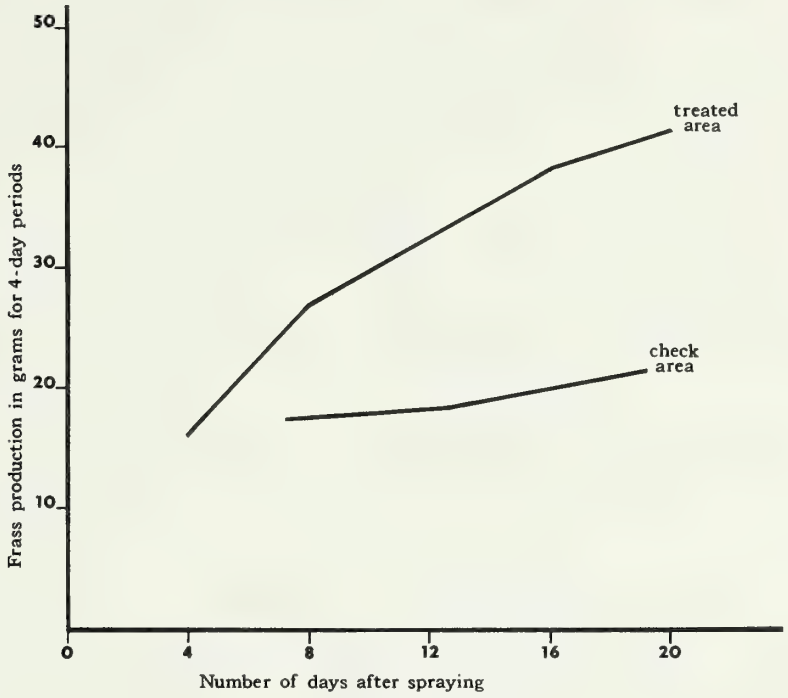

Figure 11.--Relative trends in frass production in check and treated areas during a 20-day period after spraying with *B. thuringiensis*.

Analysis by the frass production index method, however, showed that the strong increase in frass production in the treated area, 5 to 8 days after spraying , was more apparent than real. Actually, frass production 1 to 4 days after spraying was found to be abnormally low. Comparison of actual and theoretical frass weights (table 2) showed only small differences for the other 4-day periods. The initially low frass production undoubtedly reflected sublethal as well as lethal effects of the Thuricide; otherwise, the apparent increase in frass production at 5 to 8 days would have been small. Some evidence that the size of this increase was inversely related to spraying effectiveness was found by examining individual plot data. At 7 plots, shown by foliage sampling to have little or no larval mortality, increase in frass production at 5 to 8 days was 40 percent; at 13 plots showing some mortality, the increase was 90 percent.

Four plots were identified as showing little or no increase in frass production 5 to 8 days after spraying and only small subsequent increases. All of

Table 2.--Proportions of instars and comparison of theoretical and actual frass amounts produced during a 20-day period after spraying with *B. thuringiensis*.

Days after spraying	Percent of larvae in instar					Frass production index	Conversion factor	Frass production	
	I	II	III	IV	V			Theoretical	Actual
								Grams	
1 - 4	0	5	65	29	1	161	0.102	16.4	11.7
5 - 8	0	5	49	45	1	193	.102	19.7	20.9
9 - 12	0	3	46	47	4	223	.102	22.7	24.7
13 - 16	0	2	35	58	5	254	.102	25.9	29.6
17 - 20	0	1	25	61	13	325	.102	33.1	33.1

these were designated by foliage sampling as showing some larval mortality. The sequence of frass production for these four plots is shown below:

Plot No.	Days after spraying				
	0-4	5-8	9-12	13-16	17-20
	(Grams)				
8	0.20	0.28	0.23	0.33	0.38
9	.50	.34	.46	.78	.91
17	.52	.54	1.09	1.00	1.20
18	.42	.34	.72	.74	.80

Plot 9 was also identified by foliage sampling as showing a significant reduction in larval numbers; at plot 8, the downward trend showed borderline significance after the prespray count was dropped. Thus, results of the frass-drop method are confirmed for two of the four plots. However, the method failed to identify two other plots at which significant downward trends were shown by the foliage-sampling method.

INDIRECT EFFECTS OF SPRAYING

Methods

Collections of larvae for analysis of development were obtained at intervals during the test period when plot trees were sampled by pole-pruner. Larvae were preserved in alcohol and later identified as to instar by body markings and head width. Standards for recognition of instars were developed the previous year.[5/]

Effects of the Thuricide spraying on rate of larval development were deduced by comparing larval development on similar dates between the test and check areas. In the test area, the proportion of larvae in the different instars on a given date was based on half of the plots along four access lines and all plots on a fifth line where populations were low. Up to 50 larvae per plot were examined for each collecting date. Development between lines was compared, then an average for the area obtained. In the check area, all larvae obtained on a given date were examined for instar, since populations were lower than those in the test area and half as many plots were used.

Findings from analysis of frass production were also considered in the interpretation of effects on rate of larval development.

Collections of looper larvae to record incidence of insect parasites and disease were made 10 and 40 days after spraying in both the treated and check areas and were reared in the laboratory. Larvae were mostly obtained by pole-pruning sample trees, but some were hand collected from lower branches. In addition, a collection of pupae was made in October from selected plots in the test area by beating lower branches and small trees. In the following year, a collection of larvae, timed according to the second collection in 1963, and a collection of pupae were made in the test area.

Possible beneficial effects of the spraying were assessed by (1) comparing insect parasitism and disease activity between treated and check areas and (2) comparing insect parasitism and disease activity between 1963 and 1964 in the treated area. Possible adverse effects of *Bacillus* toxins on dipterous parasites, through infection of the looper host, were explored during rearing of collections by observing success of pupation of parasite maggots and emergence of adult flies. Of two principal fly parasites, one overwintered as a puparium and the other as a maggot. Techniques for successfully overwintering these parasites had previously been developed to ensure a sound basis for detecting abnormal pupation and emergence patterns. Pupation and emergence patterns of hymenopterous parasites reared were also observed, although toxins produced by the *Bacillus* are presumed to have little or no effect on these insects.

[5/] Unpublished report on file at the Pacific Northwest Forest and Range Experiment Station, Portland, Oregon.

Results

Effects on Larval Development

Retardation of larval feeding for 4 days after spraying, shown by frass-drop studies, affected larval development for this short period. Subsequent development in the treated area appeared normal and slightly accelerated. Although development in the check area was ahead of that in the treated area prior to spraying, development in the two areas on August 20-21 was almost identical. On August 29, when the second collection for parasites was made, pupation was just commencing in both areas. Table 3 compares development in the two areas.

Table 3.--Proportions of instars by date, in check and treated areas

Area and collection date	Percent of larvae in instar				
	I	II	III	IV	V
Check area:					
July 17	1	16	67	16	0
July 25	1	4	37	55	3
July 31	0	1	28	64	7
August 6	0	0	6	54	40
August 20	0	0	7	24	69
Treated area:					
July 15-16	0	24	69	7	0
July 26	0	5	49	45	1
August 1	0	2	44	50	4
August 7	0	1	25	61	13
August 21	0	0	9	25	66

Effects on Insect Parasites and Disease

There was no evidence that the Thuricide spraying affected the abundance of insect parasites and disease, either the year of spraying or the following year.

During the year of spraying, apparent mortalities inflicted by parasites and disease were higher in the check area than the treated area, but levels of parasite and disease activity were not determined the year before spraying. The same species of insect parasites were recorded in both areas. Polyhedrosis virus, an important control agent of the western hemlock looper, was absent from the treated area but common in the check area. Death from unknown causes was high during rearing of larvae from both the treated area and the check area and in both early and late larval collections.

Comparison of parasite and disease incidence between 1963 and 1964 in the treated area showed no consistent changes. In 1964, parasitization of large larvae increased somewhat; parasitization of pupae decreased. Identifiable disease, none of it polyhedrosis virus, strongly increased, and death from unknown causes decreased. The total effect of identifiable disease and unexplained mortality changed from 42 percent in 1963 to 55 percent in 1964. Data on incidence of parasites and disease are shown in table 4.

Table 4.--Summary of mortality caused by biotic control agents on western hemlock looper in treated and check areas, 1963-64

Year	Stage collected	Treated area					Check area			
		Loopers reared	Insect parasites	Insect disease	Insect predation	Unknown causes	Loopers reared	Insect parasites	Insect disease	Unknown causes
		Number	Percent				Number	Percent		
1963	Larvae[1/]	162	8	0	--	31	138	13	0	41
	Larvae[2/]	277	11	0	--	42	136	47	5	26
	Pupae	240	27	1	16	--	--	--	--	--
1964	Larvae[2/]	180	19	[3/]43	--	12	--	--	--	--
	Pupae	92	14	2	13	--	--	--	--	--

[1/] Larvae in instars III and IV, collected 10 days after spraying.

[2/] Larvae in instar V, and pupation commencing.

[3/] Consisted of pathogenic fungus disease and secondary bacterial infection.

No evidence was found of indirect effects of the spraying on tachinid flies from a toxin produced by the *Bacillus*. Maggots of *Chaetophlepsis nasellensis* Rein., mostly from the collection 10 days after spraying, pupated readily. After puparia were overwintered, adults emerged from 13 of 14 nonparasitized puparia. Maggots of *Omotoma* sp., obtained chiefly from the collection 40 days after spraying, overwintered successfully in pulverized duff, and all formed puparia; 70 percent produced adults. On the other hand, cocoons of a hymenopterous parasite, *Apanteles* sp., produced very few adults. Because of the small numbers involved, no difference between treated and check areas was evident.

In short, none of the results provided any clues as to possible side effects of the *Bacillus* on insect parasites and disease under forest conditions.

TRENDS 1 AND 2 YEARS AFTER SPRAYING

Methods

After the 1963 spraying, trend in looper populations and the course of tree damage in the test area were followed for 2 years. Tree recovery in relation to residual looper populations was of particular interest. Tree reaction to heavy defoliation is sometimes delayed; Kinghorn (1954) documented occurrence of tree mortality up to 4 years after the collapse of an outbreak.

Population trend for the period 1963-64 was determined by sampling larvae in 1964 at the peak of the second, fourth, and fifth instars and comparing estimates with those obtained for the same developmental periods in 1963. Foliage-sampling techniques were the same for both years. However, after the first sampling in 1964, number of plots was reduced from 20 to 15. Three plots were dropped because populations had been very low since 1963 and two, because plot trees were logged. Sampling effort in 1965 was guided by observations on the effect of weather on flight and mating in the fall of 1964. As a result, sampling of larvae was restricted to the peak of the fifth instar and intensity of sampling increased. By clipping and examining 18-inch branch tips from lower limbs for 30 minutes at each of 15 plots, two men sampled twice as many branch tips as in 1963 and 1964 when samples were taken higher on the tree.

As a check on population trends of larvae, pupae were sampled each of the 3 years on a time basis. At selected plots, small trees and lower branches of large trees were cut and beaten over a mat, and the dislodged sound pupae were counted. This method was used because foliage-sampling techniques are not applicable; larvae leave the foliage and pupate in a variety of places.

Initial tree damage in the test area was determined in late September 1963 by examining all plot trees with field glasses. Further examinations were made in February and June 1964 and May and October 1965. During each examination, percent defoliation of each third of the crown was estimated, and condition of the top, whether dead or alive, was noted. Individual tree records were used to assess degree of recovery from initial damage.

The relationship between number of larvae surviving the spraying and percent defoliation recorded in 1963 was tested by statistical correlation, using individual plot trees. Numbers of larvae 16 and 19 days after spraying were averaged and expressed on the basis of ten 18-inch branch tips. Separate correlations were attempted for each of the crown thirds and for an average of low and middle crown thirds.

Results

Population Trends

Larval populations were considerably lower in 1964. Greatest reductions were at plots having high populations and showing heavy defoliation in 1963. In contrast with 1963, no change in larval populations was found up to the fourth instar. It is significant that in 1963 the peak of the fourth instar was 19 days after the Thuricide spraying. Population estimates for similar developmental periods for the 2 years were as follows:

Average number of larvae per 15 branch tips

	Instar II	Instar IV	Instar V
1963	60	40	28
1964	22	22	17

Larval populations were further decreased in 1965. Weather during the fall of 1964 was generally unfavorable for flight and mating, with daily maximum temperatures of 55° F. for much of this period. Intensive sampling in 1965 at the peak of the fifth instar yielded only 70 larvae, collected in 15 man-hours at 15 plots.

Trends determined from sampling pupae were similar to those found for larvae and more definitive. As shown below, the population reached a very low level by fall 1965.

	Pupae found	Man-hours in collecting	Pupae per man-hour
	(Number)	(Number)	(Number)
1963	161	9	18
1964	234	19	12
1965	20	9	2

Tree Damage

Examination of plot trees in fall of 1963 showed that 5 of the 60 trees had suffered serious damage, with 90 percent or more of their foliage removed. One of these trees died in fall of 1963; three other trees had extensive top damage. In June 1964, foliage remaining on heavily defoliated trees was almost entirely 1963 needle growth. These trees showed progressive refoliation and no further mortality occurred. Two of three trees showing serious top damage recovered in 2 years; the other survived even though the top was killed. One of the plot trees showing light defoliation succumbed to bear damage in spring of 1964.

Tree recovery was abetted by the reduced looper population level in 1964; feeding was scarcely visible. For all practical purposes, no additional damage occurred in 1964 or 1965.

Tree damage was correlated with number of larvae surviving the Thuricide application. The best relationship was obtained when damage was expressed by an average of percent defoliations recorded for low and middle thirds of the crown. A probit transfer (probit = 0.44 + 2.5 log N) produced the curve shown in figure 12.

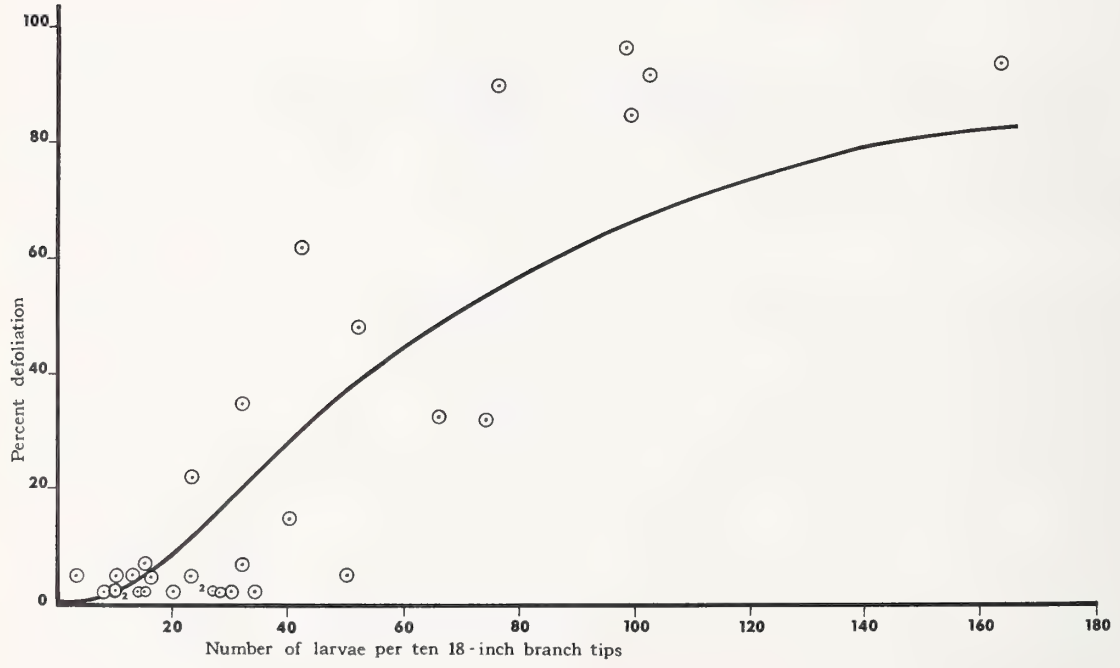

Figure 12.--Relationship between number of larvae in fourth instar and percent defoliation of codominant western hemlock trees.

DISCUSSION AND CONCLUSIONS

The field test, conducted under rigorous operational conditions, resulted in insufficient kill of the hemlock looper to achieve economic control. The failure was related to limited persistence of lethal doses of the *Bacillus*, inadequate spray distribution within the tree crowns, and the slow feeding rate of the looper. Also pertinent is the fact that larvae in the last three instars feed on all ages of foliage in an erratic pattern of feeding and wandering.

Lethal doses of the *Bacillus* persisted about 9 days, a short period for a material acting only as a stomach poison. Looper larvae are widely distributed throughout the tree crown and, since spray particles were sparsely distributed within the large lower crown areas, substantial mortality could result only from rapid and systematic consumption of foliage. However, larvae fed slowly under the cool temperatures prevailing during the 20-day period after spraying and thus had limited opportunity to encounter lethal doses of the *Bacillus*.. An increased rate of feeding in the next 20 days, associated with attainment of the fifth instar, took place after the *Bacillus* effect was ended, and several trees were heavily defoliated without any significant larval mortality occurring.

The influence of temperature on the rate of feeding was shown by the relatively high mortalities resulting from laboratory tests using foliage from the treated area. No evidence of rejection of foliage was found in these tests. It is conceivable that a repellent effect may exist but is overcome by the feeding stimulus of higher temperatures.

Increased persistence of the *Bacillus* deposit is needed to increase control effectiveness, and it is possible that new formulations of Thuricide, now appearing on the market, will answer this need. Thorough spray distribution, particularly penetration into the lower crowns of hemlock trees, is also required for improved control. Unless both persistence of deposits and spray distribution are improved, increasing the rate of application would have only minor value in increasing control effect. We know that larvae must ingest the *Bacillus* to be killed; therefore, the characteristics of the material and its distribution must be superior to that of a contact insecticide in order to be equally effective.

Although the concentration of viable spores in the material used in the 1963 tests was lower than the manufacturer's tests indicated, this factor probably had little effect on the results of the tests. A certain minimum concentration is, of course, needed. Field experiments to control the spiny bollworm on cotton showed that higher dosages of the *Bacillus* did not produce higher protection of the plant (Al-Azawi 1964). However, high spore concentrations of a preparation permit greater dilution with water and an opportunity to reduce costs of operations.

Further laboratory tests are needed to evaluate the susceptibility of the western hemlock looper to *B. thuringiensis* in terms of minimum effective dosage. In addition, as pointed out by Klein and Lewis (1966), certain factors connected with the micro-organism itself need to be explored in the laboratory. These include toxic components and their effects alone and combined, development and evaluation of selected strains, determination of LD50 and LD95, and effects of commercial additives on the biological effectiveness of various toxic components. Morris (1963) had previously suggested that the concentration of toxic material in commercially prepared *B. thuringiensis* might vary with methods of propagation, recovery, and formulation, even with identical bacterial strains.

A combination of sophisticated laboratory and field tests with an improved formulation of the *Bacillus* is needed to evaluate its usefulness in control for a variety of forest defoliators. Successful use may be limited by climate or weather conditions, crown characteristics of infested trees, and habits of the insect to be controlled. Results from testing *B. thuringiensis* in the Northeast for control of gypsy moth (Doane and Hitchcock 1964, Lewis and Connola 1966) and spruce budworm (Klein and Lewis 1966) show promise for effective control under those conditions. However, under other conditions, control may be difficult to achieve by use of the *Bacillus* alone.

As with all control applications, improved sampling techniques are needed to evaluate control results accurately. Hemlock looper populations are particularly difficult to sample because of the habits of the insect and the typical occurrence of outbreaks in stands 80 years of age or older. To lessen variation in larval population estimates based on foliage sampling, more study of sampling units is needed. Use of frass production as a guide to larval mortality appears to have limited possibilities, because of temporary depression of the feeding rate immediately after the Thuricide application due in part to sublethal effects. Measurement of pupal populations as a measure of control effectiveness is difficult because larvae leave the foliage and pupate in a great variety of sites, both in the overstory and understory. Measurement of egg populations would be unrealistic, because unfavorable weather conditions at the time of moth flight often cause large population reductions.

Improvement in direct methods of larval sampling therefore offers the greatest promise of accurately recording mortality resulting from Thuricide applications.

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Helicopter spraying with 1 gallon of Thuricide 90-T plus 1 gallon of water per acre resulted in some significant kill of the looper, but less than needed for economic control. Lethal doses of the *Bacillus* stayed on the foliage about 9 days, too short a period to obtain substantial looper mortality. Further tests are needed with an improved formulation and increased application rate, using improved foliage-sampling techniques.

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SUMMARY

Field-testing of *Bacillus thuringiensis* Berliner against the western hemlock looper was conducted under operational conditions in a 60- to 100-year-old hemlock stand in southwest Washington. Application by helicopter at a rate of 1 gallon of a commercial preparation (Thuricide 90-T) plus 1 gallon of water per acre resulted in some significant kill of the looper, but less than is needed for economic control. Under typical coastal weather conditions, lethal doses of the *Bacillus* remained on foliage for about 9 days, too short a period to obtain substantial looper mortality. Spray distribution, as recorded on spray cards, appeared good, but coverage of branches in lower crowns was poor. With the generally low control obtained, foliage sampling methods were inadequate to measure most population reductions with accuracy. Amounts of frass collected under sample trees indicated a temporary reduction in feeding activity during the first 4 days after spraying, but they were of limited value in demonstrating mortality due to spraying. Improvement of foliage sampling methods offers the greatest promise for improving the assessment of larval mortality. The spray application had no obvious effects on biotic control agents attacking the looper, but more intensive studies are needed. Number of larvae surviving the spraying was correlated with subsequent tree defoliation. Further tests are needed with an improved formulation and increased application rate to determine whether economic control of the hemlock looper is feasible with this microbial insecticide.


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