The characteristic appearance of *B. thuringiensis*-killed larvae and the presence of vegetative rods formed the basis of the diagnostic method. Briefly, dead larvae collected in the field were immersed in a quaternary ammonium disinfectant (Hyamine) for one minute to surface-cleanse the cadaver, rinsed in distilled water, and then macerated on a microscope slide in a drop of 3% nigrosin. The dried smears were examined on the microscope and the presence of vegetative rods of *B. thuringiensis* was taken as presumptive evidence of infection.

(*c*) In laboratory tests, suspensions of Thuricide in oil-water emulsion were sprayed on to the surface of petri plates containing sterile Difco nutrient agar, and incubated at 30°C. In 18 hours, characteristic colonies of *B. thuringiensis* were visible indicating that the oil did not inhibit germination of the spores or multiplication of the vegetative rods. In the field test, disposable sterile plastic petri plates containing Difco nutrient agar were placed 12 inches above ground on small wooden platforms throughout the area to be sprayed and uncovered immediately prior to the application of the spray. Soon afterwards the lids were replaced, the plates removed to the laboratory for incubation and when the characteristic colonies were visible, they were counted. Areas of high and low spray density were thus indicated, and foliage samples were taken in the same areas and analysed by staff of the Bioferm Corporation.—T. A. Angus, A. M. Heimpel, R. A. Fisher.

Aerial Spraying of Thuricide Against the Spruce Budworm in New Brunswick.—Two formulations of Thuricide, 2 pounds in 1 U.S. gallon of furnace oil with an emulsifier, and 2 pounds in 1 U.S. gallon of water, were applied at 1 gallon per acre on two 30-acre plots of spruce budworm-infested balsam fir. Three types of check plots were employed: (*a*) sprayed with 12½ per cent DDT in oil at 1 gallon per acre; (*b*) sprayed with oil alone at the same dosage; and (*c*) untreated. A Stearman biplane fitted with boom and nozzles was used in the trials. The spray system was flushed with oil and with water and detergent before application of the different formulations.

Spray deposit and insect populations were measured at intervals along two lines at right angles to flight direction in each plot. Deposit was assessed as the number of bacterial colonies per 85 mm. diameter petri plate filled with nutrient agar. The plates were located about 12 inches above the ground in openings near the trees used for population sampling, and were exposed immediately before spraying. After spraying they were incubated for about 8 hours.

Insect populations were counted on 18-inch branch tips selected from the mid-crowns of dominant and co-dominant balsam fir trees at 100-foot intervals along the sampling lines.

At the time of spraying, the morning of May 30, 1960, the budworm population consisted of various instars as follows: III—1%; IV—49%; V—49%; VI—1%. Shoots were well extended and needle flare had taken place. A light shower had saturated the foliage immediately before spray application, but it was considered that this would aid dispersal and help to assure good contamination. Foliage was completely dry shortly after spraying. Application was followed by two days of weather favourable to rapid insect feeding, and absence of rain. A light drift of air from west to east occurred throughout the trials.

Counts of colonies on the petri plates revealed that deposit was almost uniformly higher than 500 colonies per plate throughout that portion of the plot tested directly with the oil formulation. Counts declined as distance from the swaths increased, but a few colonies still appeared about 2,000 feet downwind. Deposit was lighter on the plot treated with water formulation, probably due to the clogging of nozzles observed during application (a considerable residue of material was discovered in the spray system and all nozzles required cleaning). Counts of over 500 colonies per plate occurred over a smaller portion of the sample lines in the water-treated plot, and there was evidence that more easterly drift occurred.

Foliage samples from both plots were examined by the Bioferm Corporation for viable spores of *Bacillus thuringiensis*. Although spore counts are not necessarily indicative of insecticidal activity of *B.t.* sprays, the counts revealed that initial contamination above the level considered to be lethal occurred only on the day of treatment in those parts of the oil-treated plot on which plate counts were highest. In the remainder of the oil-treated plot, and in the plot treated with the water formulation, initial contamination was below the accepted lethal level. The counts revealed a rapid decrease of viable spores during the first five days, but spores were still present on the seventeenth day after treatment.

Larvae collected about 8 hours after spraying were reared in the laboratory at room temperature in sterile tubes. Mortality began June 3, and examination of nigrosin smears established the presence of vegetative rods of *B. thuringiensis* in the dead insects.

Population sampling at intervals of three days, however, failed to establish a discernible effect on population density produced by Thuricide sprays, or by spraying with oil alone, whereas the DDT treatment was followed by a clearly defined decline in density yielding about 95 per cent control.

However, the living and dead insects were counted in these collections, and diagnosis established that most of the dead larvae in the treated plots were contaminated with *B. thuringiensis*. The numbers of dead larvae expressed as percentages of the total number of larvae in each collection are shown in Table I.

TABLE I

Date	Percentage of dead larvae in collections				Differences in percentages		
	Untreated check	Thuricide in oil	Thuricide in water	DDT	Thuricide in oil-check	Thuricide in water-check	DDT-check
May 23	7	6	7	—	−1	0	—
29	6	16	15	15	10	9	9
30	13	9	6	17	−4	−7	4
Sprays applied May 30							
June 2	3	13	12	10	10	9	7
6	30	35	29	83	5	−1	53
9	7	20	18	73	13	11	66
13	3	25	21	56	22	18	53
16	4	35	26	33	31	22	29
20	4	7	11	—	3	7	—
23	11	13	11	33	2	0	22
27	6	9	8	0	3	2	−6
July 4	3	1	8	0	−2	5	−3

A higher percentage of dead insects occurred in the Thuricide-treated plots than in the check plots. The maximum differences between the check and treated plots were: oil treated, 31% on June 16; the water-treated, 22% on June 16; and DDT-treated, 66% on June 9. Thus, the Thuricide produced a reduced and later insecticidal effect in comparison with DDT.

Defoliation patterns mapped aerially and from the ground after budworm pupation revealed no discernible difference between the Thuricide-treated plots and the untreated plot.

Development of the Thuricide-treated populations appeared to be retarded, possibly because the larger, more rapidly feeding larvae were killed.

It is clear from these trials that Thuricide as formulated and applied from aircraft against the spruce budworm in 1960 produced some insecticidal effect. This was not sufficiently high to consider its use in place of DDT. An oil formulation yielded easier passage through the spray apparatus, a better pattern and degree of deposition of material, and slightly higher insecticidal effect than a water formulation.—D. G. Mott, T. A. Angus, A. M. Heimpel, R. A. Fisher.

Susceptibility of the Black-Headed Budworm to Thuricide.—Trials of Thuricide against the black-headed budworm were conducted on the Queen Charlotte Islands, British Columbia in 1960. Prior to aerial spraying a pre-test was carried out in a field laboratory. The purpose was to test the general susceptibility of the Queen Charlotte Islands strain of black-headed budworm to the Thuricide to be used in the aerial trials. On July 10, 24 field-grown western hemlock seedlings, approximately 2 feet tall, were placed individually in pots and divided into four lots of six seedlings each. All insects were removed from the seedlings. The mouths of the pots were covered with cloth and the pots were placed on the floor of a shed above strips of light brown wrapping paper. Two days later, 30 field-collected black-headed budworm larvae (mostly third instars) from unsprayed areas were placed on each seedling.

Seven days after their first introduction and just prior to application of the pre-test sprays, it was observed that only about one-third of the larvae originally introduced had established themselves on the potted seedlings. The amount of frass that had accumulated during larval establishment showed that considerable feeding by the survivors had taken place during the 7-day period. Fifty-six larvae were established on Lot 1, 69 on Lot 2, 60 on Lot 3 and 90 on Lot 4. The reason for such poor establishment is not known with certainty. The author suspects that handling of the delicate third-instar larvae during collecting, sorting, and introducing them to the seedlings contributed to this mortality.

The four lots of seedlings were sprayed as follows:

Lot 1	Thuricide SO-75	234 grams
	Wetting agent #3	40.1 grams
	Furnace oil #2	646.0 grams
	Tap water	59.6 grams
Lot 2	Two lb. Thuricide per U.S. gallon of tap water.	
Lot 3	Oil alone.	
Lot 4	Tap water alone.	

The spray, applied with a pressure-type hand sprayer, was directed above the seedlings and allowed to fall on them and on oil and water cards placed beside the oil treated (Lots 1 and 3) and water treated (Lots 2 and 4) seedlings, respectively. The droplet sizes on these cards indicated a very heavy spray dosage. There was no way of calibrating the hand sprayer used and, consequently, the spray deposits, although heavy throughout, were rather unevenly distributed.

The larvae which died during the tests were examined microscopically for vegetative cells of *Bacillus thuringiensis*. The cadavers of heavily infected larvae were light to dark brown, soft, and disintegrated under slight pressure. The cadavers were macerated on microscope slides and stained with nigrosin. Slides showing five or fewer vegetative cells were scored as negative.

Peak mortality for oil-Thuricide treated plants occurred in 24 hours and for water-Thuricide treated plants in 48 hours. The largest number of larvae positive for *B. thuringiensis* were found on the second and sixth days in the oil-Thuricide treatment, and on the fourth and sixth days in the water-Thuricide treatment. These "double peaks", it is presumed, merely indicate that some larvae ingested the microbial insecticide earlier than others.

In the oil-Thuricide treatment, all larvae died, but in the water-Thuricide treatment two remained alive at the end of the test. Sixty-six per cent of the dead larvae in the former group were positive for vegetative cells, compared with 51 per cent in the latter group. In the oil-alone and the water-alone treatments, four and two larvae, respectively, died. None of these were positive for *B. thuringiensis*.

From each of the four lots of sprayed seedlings, 25 small branches were removed immediately after treatment and each placed in a glass rearing tube. One larva, collected from an unsprayed field area and starved for 24 hours, was then placed on each branch. The tubes were placed in the field laboratory. Over a 7-day period mortality counts were taken and dead larvae examined for vegetative cells daily. Both the oil-Thuricide and water-Thuricide treatments produced peak mortalities on the third day following spray application and 23 out of 25 (92 per cent) were positive for vegetative cells.

In both oil and water controls, 7 out of 25 larvae died by the fifth day. In the oil controls, 5 out of the 7 dead were positive for *B. thuringiensis* and in the water controls, 3 out of 7 were positive. Presumably, there was bacterial contamination of these two treatments during the course of the experiment. This is not surprising because of the difficulty of ensuring aseptic conditions in the field laboratory.

The amounts of frass which accumulated in the feeding tubes were considerably larger for the untreated than for the treated groups. On the bases of frass accumulation, larvae on treated foliage appeared to have stopped feeding entirely within 24 hours. This suggests interference with normal digestive functions soon after ingestion of the microbial insecticide. Frass accumulations in the seedling test paralleled those in the rearing-tube test.

The mortality rate appeared to be slightly faster with the oil-Thuricide than with the water-Thuricide formulation. In the former, approximately 50 per cent of the infected larvae had died 48 hours after application of the spray in both seedling and rearing-tube tests. In the seedling and rearing-tube tests of water-Thuricide trials, 50 per cent mortality occurred approximately 96 hours and 72 hours, respectively, after spraying.

On the basis of these observations it is concluded that the black-headed budworm is susceptible to *Bacillus thuringiensis* and that the disease symptoms are similar to those of other susceptible Lepidoptera.—O. N. Morris.

Aerial Spray Trials Against the Black-Headed Budworm in British Columbia.—Advantage was taken of a DDT control project against a black-headed budworm outbreak on the Queen Charlotte Islands in 1960 to test Thuricide against this insect. Three 30-acre plots were selected in dense stands of small western hemlock trees, varying in height from 5 to 25 feet, which could be readily sampled from the ground. The budworm population in these young stands was light at the beginning of the season. When the Thuricide spray was applied on July 24, most of the insects had reached the fourth instar, but parasitism and cool, wet weather caused such heavy mortality that only a sparse population remained. Eighteen-inch branch samples taken the day before spraying yielded an average of only 3.3 living larvae per 10 square feet of foliage; over three-quarters of the samples were without living budworms.

In view of the low population, it was recognized before the experiment was begun that it would not be possible to obtain an adequate population reduction assessment, but that information could be gained on formulation, deposit, and viability of the material.

The Thuricide was suspended in oil similar to one of the formulations in the New Brunswick experiment, but additives were incorporated to reduce the tendency for clumping and settling. The formulation per 100 U.S. gallons was:

Thuricide SO-75	150 lb.	Sodium nitrate	8 lb.
wetting agent no. 3	4.5 gal.	Diesel fuel oil	73 gal.
Microcel A	35 lb.	Water	8.5 gal.

The Thuricide contained 60 billion viable *B. thuringiensis* spores per gram, and 3.6 per cent Petro A.G., a wetting agent. Wetting agent no. 3 was a 1:1 blend of Atlas emulsifiers Span 80 and Tween 80.

The equipment of the Grumman Avenger spray aircraft was modified to include manual agitating apparatus to prevent settling of solids in the small volumes of spray used. The aircraft was flown at 160 m.p.h. and the spray emission rate was 112 U.S. gallons per minute. With each pass over the $\frac{3}{8}$ mile centre lines of the plots about 16 gallons were released.

Two dosage levels were applied. On Plot 1, the aircraft sprayed two passes over the centre line, thus releasing 32 gallons containing 48 pounds of Thuricide. Three passes over the centre of Plot 3 gave a dosage of 48 gallons containing 72 pounds of Thuricide. If an effective swath width of 400 feet is assumed, the nominal dosages were 2.7 pounds of Thuricide in 1.8 gallons per acre on Plot 1, and 4 pounds in 2.7 gallons per acre on Plot 3.

Plot 2 was sprayed with 48 gallons of diesel oil containing all additives other than Thuricide.

In each plot, spray deposit assessment units were set on 4-foot stakes at 55 stations on five lines running at right angles to the line of flight. Oil-sensitive cards and 9 cm. petri plates containing nutrient agar were used to assess the deposits. Viable spores germinated quickly on the agar permitting colony counts within twelve hours after spraying, but deposits higher than 500 colonies per plate could not be estimated accurately.

The plots were almost twice as wide as the anticipated swath coverage to allow for lateral drift. As a consequence only about one-half of each plot was sprayed heavily. At stations where the colony counts exceeded 200 per plate, the average spray droplet density was 25.2 drops per square centimetre on Plot 1, and 29.0 on Plot 3. Both types of assessment units were in general agreement in delineating heavily sprayed portions of the plots. At most stations where more than 12 spray droplets per square centimetre were recorded, colony counts exceeded 500 per plate.

Where deposits were heavy, particles of solid material could be seen on rocks and foliage. On the oil-sensitive cards, clumps of spores and inert material could be seen in the centre of the oil stains with the unaided eye. The droplet counts indicate that not every oil droplet carried viable spores. The tendency for the material to agglomerate reduced the theoretical efficiency of the deposit.

Small samples of hemlock foliage were collected on July 24 and 27, and 12 and 80 hours after spraying, for residual spore analysis by the Bioferm Corporation. The first sample from Plot 1 yielded 1,390,000 viable spores per gram of fresh leaf, or about 6,040 spores per leaf. Corresponding values for samples from Plot 3 were 710,000 spores per gram (3,090 per leaf) on July 24, and 71,100 spores per gram (308 per leaf) on July 27. In comparison with Thuricide experiments on other insects, the initial deposits were high enough to cause budworm mortality, but by the third day after treatment the residues were at a presumed sub-lethal level. Apart from light overnight dews, the weather remained warm and dry for two weeks after the spray was applied.

On July 25, budworm larvae collected from an unsprayed area were placed on foliage from heavily sprayed trees in the spray plots. The insects were reared until either death or pupation at the Insect Pathology Research Institute at Sault Ste. Marie, Ontario. Diagnosis of the dead larvae revealed that 15 out of 20 larvae (75%) reared on Plot 1 foliage were infected with *B.t.* Of 77 larvae reared on Plot 3 foliage, 69 (90%) were infected with the bacterium.

Post-spray larval samples were taken on July 27, but only 41 living and 70 dead larvae were found on 412 samples from the two plots treated with Thuricide. Twenty-seven of 64 cadavers subsequently diagnosed at Sault Ste. Marie were