

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 $\frac{1}{2}$ 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 Bioform 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

infected. Nine of the infected larvae were from portions of the plots that received only light or trace deposits. The living larvae from this collection were reared on clean foliage until either pupation or death. Of 32 that died as larvae, 14 were infected.

A number of branch tips in Plot 1 were colonized with healthy larvae prior to spraying. Twenty-five per cent of the dead larvae found on these branches on July 29 and August 1 were positively diagnosed, although almost one-half were from lightly sprayed trees.

No general statement on population reduction can be drawn from the sparse population that was present. Nevertheless, the field trial indicated that the black-headed budworm is susceptible to commercially produced *Bacillus thuringiensis*, that the toxicity of the preparation is not destroyed by the oil carrier, and that it is possible to apply a lethal deposit from aircraft. If the residual life of the deposits can be extended, and if the clumping tendency can be overcome, one may expect an increase in the efficiency of aerial applications of *Bacillus thuringiensis* suspensions.—J. M. Kinghorn, R. A. Fisher, T. A. Angus, A. M. Heimpel.

CURRENT ACTIVITIES ROCKY MOUNTAIN REGION

Miscellaneous New Records of Fungi Occurring on Pine in Alberta.—In the course of cull surveys made in Alberta, two new fungi were recorded. These fungi were isolated in culture from living, 110-year-old pine trees, believed to be natural hybrids between *Pinus banksiana* Lamb. and *Pinus contorta* Dougl. var. *latifolia* Engelm., near Whitecourt, Alberta (Ref. Moss, E. H., Natural Pine Hybrids in Alberta. C. Journ. Res., C, 27:218-229. Oct. 1949). Only single isolates were obtained in each case.

Poria asiatica (Pilát) Overh. was isolated from brown cubical rot at stump height occurring in lens-shaped pockets which often contained white mycelium. The fungus apparently entered through a fire scar and the volume of associated decay was about 0.2 cu. ft. *Poria asiatica* has been reported as the cause of a butt and trunk rot of jack pine in Ontario (Forest Resources Inventory, Div. of Timber, Ontario Dept. of Lands and Forests, 1958) but there seems to be no record of its occurrence on lodgepole pine from any part of the range for that tree species. It has not been previously reported on any tree species in Alberta.

Peniophora phlebioides Jackson and Dearden was isolated from a very pale brown, soft rot at stump height in the vicinity of a fire scar. The volume of associated decay was about 0.2 cu. ft. This fungus has been found quite commonly on lodgepole pine slash in Alberta but this is the first report of its occurrence on living pine.—Robena C. Robinson and Glen D. Paul.

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ROGER DUHAMEL, F.R.S.C., Queen's Printer and Controller of Stationery, Ottawa, 1961

O. H. M. S.

Robena C. Robinson

F.L.
D. C. EIDT,
FOREST BIOLOGY LABORATORY,
COLLEGE HILL,
FREDERICTON, N.B.

DEPARTMENT OF FORESTRY
OTTAWA