

Missoula, Montana
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WORK PLAN FOR AN AERIAL APPLICATION OF NUCLEAR
POLYHEDROSIS VIRUS TO CONTROL DOUGLAS-FIR
TUSSOCK MOTH LARVAE IN LATAH COUNTY, IDAHO
1965

Division of State and Private Forestry

PROBLEM

Periodic epidemics of the Douglas-fir tussock moth, Hemerocampa pseudotsugata McD., severely damage stands of Douglas-fir and true fir trees in the western American continent. Small infestations became apparent around private homes and farmyards near several towns in northern Idaho during 1961. By 1964, defoliation visible from the air covered about 70,000 acres in Benewah and Latah Counties, Idaho. Ground surveys in the fall indicated that various numbers of tussock moth egg masses could be found within the boundaries of a 300,000-acre area that extended into both counties. The potential hazard to these fir stands during 1965 from tussock moth feeding is very high.

DDF is the only tested insecticide, at present, highly toxic to tussock moths. Due to its unpopular side effects on other organisms, its replacement by another insecticide or biological control agent is being sought. A nuclear polyhedrosis virus has already been tested^{1/} and sprayed from the air to kill tussock moth larvae.^{2/} This material shows great promise as a tussock moth killer and needs further testing when applied from the air.

Plans have been made to spray about 120,000 acres with DDF and about 6,500 acres with virus during 1965 to control tussock moth larvae in the Idaho epidemic. The virus will be applied over lands classed as city watersheds.

OBJECTIVES

The main objectives of this operational-type pilot test are to:

1. Perfect a method for controlling tussock moth larvae other than with a chemical spray.

^{1/} Morris, O. N., The natural and artificial control of the Douglas-fir tussock moth, Orgyia pseudotsugata, McD., by a nuclear polyhedrosis virus. Jour. Insect Path. 5(4) : 401-413, 1963.

^{2/} Washburn, R. I. and W. E. Cole, The use of virus to control tussock moth. Unpublished report, Div. For. Insect Res., Inter. For. and Range Exp. Sta., USFS, Ogden, Utah, 11 pages, 1960.

2. Develop methods in the laboratory for mass rearing of tussock moth larvae and inoculating them with virus, then processing polyhedral bodies from their cadavers.

3. Determine the effectiveness of applying 1 billion polyhedra per acre.

4. Develop a sampling method for determining the amount of mortality caused by introducing virus from the air.

MATERIALS AND METHODS

Rearing tussock moth larvae.--Douglas-fir tussock moth larvae will be reared from eggs collected in Idaho from areas that contain a low incidence of virus. Egg hatch will occur in a humid environment created within closed Petri dishes. Hatched larvae will be reared in plastic boxes under high humidity and fed larch foliage. Larch twigs from the field, placed in water, should provide foliage within 2 weeks.

Inoculating larvae with virus.--When the larvae in the plastic boxes are in the last part of the third instar, they will be placed in larger cardboard boxes with plastic windows and tops, and fed Douglas-fir foliage or larch dipped in water containing 1 billion polyhedra per gallon. The object is to have them start dying when they reach the last instar. The larger the larvae, the greater amount of virus produced.

Processing polyhedrosis virus.--As the larvae begin to die of polyhedrosis, their bodies will be collected, placed in dry, glass bottles and stored at 4° C. Hatches of these cadavers will be pulped in a Waring blender with distilled water. This blended material will be filtered through a 40-gage plastic screen, through cheesecloth, and finally filtered through fine nylon cloth. Deposits left on the filters will be washed off with distilled water, blended again, and filtered as above. This step will obtain many polyhedra that remained in the deposit. This filtrate will be stored in darkness at 4° C. The numbers of polyhedra per milliliter of the filtrate will be determined with a hemacytometer slide. If this material is going to be stored for more than 6 months, polyhedra should be reduced to a powdered form.^{3/} Solutions having a pH of 4.0 to 5.0 and 8.0 to 8.5 are critical and can dissolve the virus.^{4/}

Mixing spray solution.--The desired numbers of polyhedra per milliliter are taken from the filtrate solution and added to each gallon of water.

^{3/} Rollinson, W. D. and F. B. Lewis, How to collect and process large polyhedral viruses of insects. For. Res. Note No. 130, NE For. Exp. Sta., For. Ser., USDA, Upper Darby, Pa., 1962.

^{4/} Smirnoff, W. A., Preparation and application of viral material in biological control of the jack pine sawfly. Forestry Chron. 40 : 187-194, 1964.

For example, to formulate 1 gallon of spray, use:

- 0.9 gallon of nonchlorinated water
- 0.1 gallon of light corn sirup (evaporation inhibitor)
- 0.01 gallon of Leucophor C 6208 (U) (fluorescent tracer)
- 2.00 milliliters of filtrate containing 500 million polyhedra per milliliter. (This will prepare a dosage rate of 1 billion polyhedra per gallon.)

The virus filtrate should not be added to the formulation until the spray mixture is to be used. Polyhedra will settle to the bottom of the mixing tank; therefore, the spray mixture should be lightly agitated after the virus is poured in. Fluorescent tracer has to be thoroughly mixed in also. Corn sirup will mix with water faster if both are heated to 100° F. There may be times, because of wind or rain, that a mixed batch of spray cannot be utilized immediately. Corn sirup might ferment in a few days; or when cool, it could adhere to the sides of the mixing tank. Therefore, the best policy will be to mix only enough solution for each plane load and add the virus at the last moment. The loss of any virus material could reduce the number of acres treated.

One 2,000-gallon mixing unit and one 150-gallon unit will be borrowed from the Coeur d'Alene National Forest. The larger unit is to be placed at the Moscow-Pullman airport, and the small one set up at a heliport. Water can be metered into them and circulated to blend the formulation. These tanks will have to be thoroughly cleaned with the flushing solvent "Nutra-Sol" and rinsed with nonchlorinated water to remove all oil and insecticide residues.

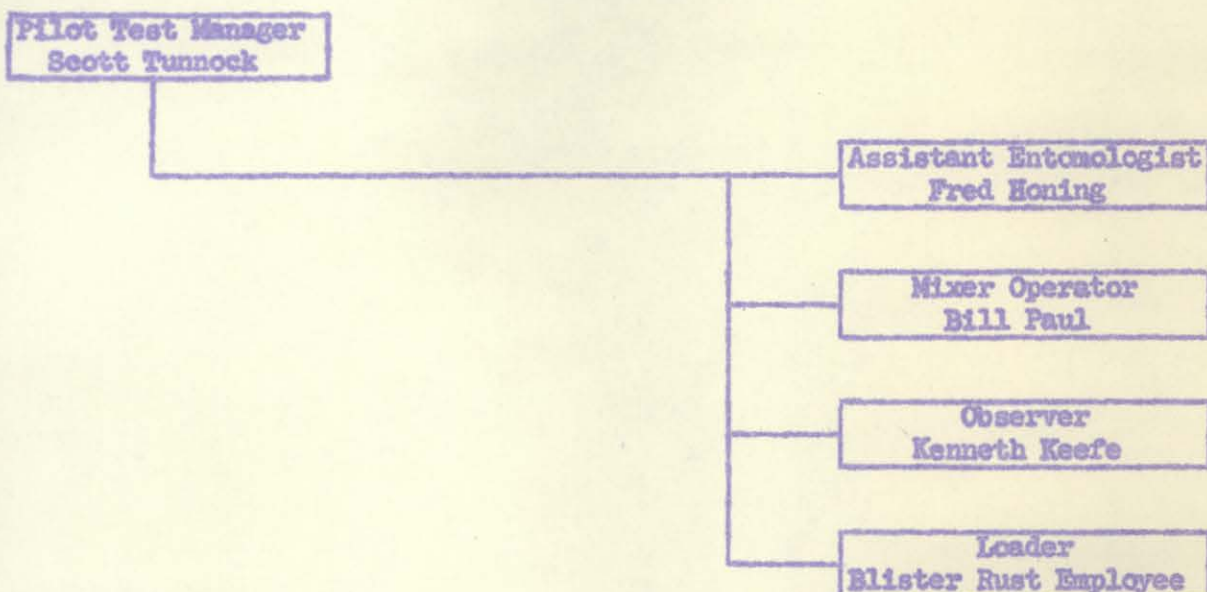
Treatment.--Treatment will start when about half the larvae on the sample trees reach the second instar. Approximately 1,500 acres are to be sprayed by a helicopter and 5,000 acres by a Ford Trimotor airplane. The helicopter can carry 70 gallons of solution per trip. If it averages four trips per day, approximately 5 days will be needed to treat the 1,500 acres. The capacity of the Trimotor is 350 gallons. On the basis of four trips per day, it should take about 4 days to treat the 5,000 acres.

An aerial observer will follow the Ford Trimotor to check the coverage. Spraying will be discontinued when temperature inversion occurs or wind velocity reaches 3 m.p.h.

Spray deposit will be sampled by placing Kromekote cards on branch tips of sample trees. To check for spray drift, cards are needed on check trees. Leucophor C in the spray can be detected on the cards by placing them under a "black light."

The dosage rate will be 1 billion polyhedra in 1 gallon of water per acre. Morris¹ fed larvae Douglas-fir foliage that was dipped in a suspension containing 1 billion polyhedra per milliliter, and 100 percent of them died of the virus.

ORGANIZATION CHART



Sampling methods.--Fifty sample trees, 10-15 feet in height, will be located along string lines within the 1,500-acre block. The sample trees will be open-grown, well foliated, and harbor a moderate amount of egg masses. Additional egg masses from neighboring trees will be added where it appears necessary.

Twenty-five check trees will be uprooted prior to spraying and removed from the 1,500-acre block. These trees will be transplanted about 2 air miles south of the spray block. The trees will be planted in rows to form a fairly close grouping.

Just prior to spraying, separate collections will be made of about 100 to 200 tussock moth larvae from near the original location of each check tree. These larvae will be placed on the respective check trees for development.

Under each sample and check tree, two 3- by 6-foot sections of cheese-cloth will be suspended between stakes about 2 feet off the ground. The middle will be tied down to keep the cloth from flapping. These cloths will be checked every other day after spray application, and all dead and dying larvae will be collected and placed in a container. This will afford a record of larvae killed by polyhedrosis that fell off the branches, which otherwise might be missed if sampling was confined to collecting larvae from the foliage.

A prespray collection of five larvae will be made from each sample and check tree. Each larva will be placed in a 4-ounce, clear plastic container and reared on artificial media^{5/} until it pupates. This procedure will be

^{5/}McMorran, A., A synthetic diet for the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae), Can. Ent. 97: 58-62, 1965.

repeated after treatment at the end of 7, 14, 18, 21, and 28 days. Death due to polyhedrosis, other diseases, parasites, and unknown causes will be determined. Abbott's formula can be used to correct for natural mortality in the checks, and an analysis of the variance will be used on the corrected data to determine between-plot variation.

Observations in the 5,000-acre block will consist of estimating the number of egg masses present in four areas before spray application. The egg mass population will be rated as follows: very abundant, numerous, moderate, few, or scarce. These four areas will be examined in October, and the number of egg masses rated again. Degree of success can be judged by the differences in ratings. If a rating dropped from numerous to scarce, moderate control would be indicated.

As soon as larval mortality from virus infection is detected in the sprayed area, a collection of dead and dying caterpillars will be made to recover a supply of virus for future use. Twenty gallons will be collected, if possible.

During the fall of 1965, egg masses will be collected from sample plots located near the sample trees and held at outside winter temperature until about February 1966. These egg masses will be sent to the Forestry Sciences Laboratory in Corvallis, Oregon, for hatching to determine virus carryover.

MATERIALS, LABOR, EQUIPMENT, TRAVEL, AND COSTS

	<u>Total Cost</u>
Miscellaneous supplies and materials	\$2,920.00
Labor	3,099.00
1. 3 man-days to dig Douglas-fir trees for greenhouse	\$75.00
2. 20 man-days to transplant 25 fir trees (GS-4 at \$2.15 per hour)	344.00
3. 60 man-days for various jobs on plots and during spray operations	2,100.00
4. Per diem for labor	580.00
Equipment	7,172.00
1. Observation plane (16 hours @ \$35)	560.00
2. Ford Trimotor (16 hours @ \$135)	2,160.00
3. Ford Trimotor, ferry time (4 hours @ \$135)	540.00
4. Airport fee for Ford (6 days @ \$25)	150.00
5. Handling charge for the above costs	500.00
6. Helicopter (15 hours @ \$128)	1,920.00
7. Ferrying time for helicopter	100.00

	<u>Total cost</u>
Equipment (con.)	
8. Truck, 1½-ton (500 miles @ 22 cents)	\$110.00
9. Truck, 1½-ton, to be used as tanker (300 miles @ 22 cents)	66.00
10. Truck, 1½-ton, with 150-gallon mixing unit, (300 miles @ 22 cents)	66.00
11. Truck-tractor, to haul 2,000-gallon mixing unit (200 miles @ \$1)	200.00
12. Back-hoe and driver for transplanting trees	300.00
13. Miscellaneous	500.00
Travel	\$2,570.00
1. Per diem for Tumock and Keefe (April-July)	1,600.00
2. Mileage	
Sedan delivery (April-July), 6,500 miles @ 8.5 cents	552.00
Pickup, ½-ton (May-July), 4,400 miles @ 9.5 cents	418.00
Detail of Fred Honing from Region 4	600.00
F.Y. 1966	
Labor - 80 man-days @ \$25 per day for harvesting 20 gallons of diseased larvae	<u>2,000.00</u>
Grand total	\$18,361.00