Forest Research Note

NE

FOREST SERVICE, U.S. DEPT. OF AGRICULTURE, 102 MOTORS AVENUE, UPPER DARBY, PA.

No. 130
1962

HOW TO COLLECT AND PROCESS LARGE POLYHEDRAL VIRUSES OF INSECTS

Polyhedral viruses have proved highly effective and very practical for control of certain pine sawflies; and a method of collecting and processing the small polyhedra (5 microns or less) characteristic of sawflies has been described.[1] There is experimental evidence that the virus diseases of many Lepidopterous insects can be used similarly for direct control. The polyhedra of most caterpillars, such as the gypsy moth and forest tent caterpillar, are larger (6 to 15 microns) and the method of processing them for control use is somewhat simpler. The preparation of polyhedral material from the gypsy moth, Porthetria dispar (L), will be used as an example.

HOW TO OBTAIN THE POLYHEDRA

The polyhedra containing the infectious agent, the virus rod, are found in tremendous quantities in the caterpillar immediately after death. Care is necessary when collecting diseased caterpillars, since the integument of the dead larva is very fragile and easily punctured. Much of the polyhedral material will be lost if the insect is ruptured. Two general methods may be used to obtain the polyhedra.

[1] Lewis, F. B. How to collect and process small polyhedral viruses of insects. Northeast. Forest Expt. Sta. Forest Res. Note 109. 8 pp. 1960.

Field collection.--In noticeably infested areas, the polyhedral disease may be present in an ample number of caterpillars so that field collection is easy and rapid.

Gypsy moth, tent caterpillar, and other Lepidopterous larvae die of polyhedrosis in a characteristic posture, sometimes described as "wilting". The dead insect hangs from the twigs, leaves, or other parts of a host tree by a proleg in an inverted V position.

The diseased larvae are collected in a dry pint or quart glass jar. Each larva is loosened and removed from the hanging position with tweezers. The collecting jar is held immediately beneath the insect to catch any liquid material (containing huge quantities of polyhedra) in case the larva inadvertantly is ruptured. It is possible for two people to collect 16 to 20 pints of diseased material per day in this way. A pint of virus-killed gypsy moth caterpillars in the V-VI instar will yield up to 10 grams of purified polyhedra powder, depending upon the condition of collected material.

Laboratory rearing.--When the host insect density and disease incidence are not sufficient for making field collection of diseased specimens efficiently, it is possible to rear artificially infected larvae in the laboratory. With the gypsy moth, 50 larvae are placed in a cage and fed suitable foliage thoroughly sprayed with the disease agent. A single feeding of contaminated foliage usually is sufficient to initiate the disease. Unsprayed foliage is used to feed the larvae until the disease kills them. Approximately $\frac{1}{2}$ gram of purified polyhedral powder may be obtained per cage lot.

Young larvae, usually in instars II-III, are fed the polyhedra because death does not occur till 12 to 20 days after infection, depending on rearing conditions (80-90° F. and 90 percent relative humidity are optimum for polyhedrosis). By the time mortality occurs, the larvae will be large enough to produce a good yield of polyhedra, but not old enough to pupate and emerge as adults. The dead larvae are collected as soon as they die, in the same manner as described for field collecting.

STORAGE OF DISEASED CADAVERS

The polyhedra-filled cadavers are usually kept in closed glass jars under refrigeration (40° C.) until the

final clean, dry powder is prepared. The larvae can be kept in this manner for several months without loss of viral virulence. It is advisable, however, to prepare the purified polyhedra powder the same year the diseased material is collected.

HOW TO PREPARE THE PURIFIED POLYHEDRA POWDER

Preparation of the purified polyhedra powder from the diseased caterpillars requires the following equipment: (1) a good compound microscope, (2) an electric centrifuge capable of speeds to 5,000 rpm, (3) a Waring-type blender, (4) a hemacytometer, and (5) pipettes, large beakers, large watch glasses, and miscellaneous glass containers. It is necessary that the operating characteristics--particularly the range of speeds--of the centrifuge be known. Any one of several types of hemacytometers or particle-counting instruments can be used.

The process of purifying the polyhedra is essentially one of freeing the polyhedra from the tissues of the cadaver, removing the insect debris by filtering, concentrating the polyhedra by centrifugation and gravity, and finally decanting and evaporating the supernatant (liquid) from the polyhedra.

To start, place the stored diseased material in a Waring-type blender. Do not fill the blender more than half full. Rinse the storage jar carefully with a small amount of tap water and add to the blender. The rinsing must be done because many of the polyhedra will have settled to the bottom of the jar. Blend this material for 2 to 3 minutes and then follow the process outlined on the flow sheet (p.4).

The critical steps in the process are the decanting and evaporation. Careful decanting of the water from the settled polyhedra will remove a large amount of contaminating small debris and bacterial material. During evaporation, additional brown contaminating material can be removed by very careful aspiration with a fine-tipped pipette. Since the large polyhedral bodies settle quickly to the bottom of the beaker or watch glass as a white layer, the brown, smaller contaminants can be seen on the surface of the polyhedral layer and removed.

FLOW SHEET FOR PREPARATION OF PURIFIED POLYHEDRA POWDER

Blend diseased larvae

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Filter twice through 40-gage plastic screen. Squeeze material on the screen dry and discard.

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Centrifuge at 5,000 rpm for 5 minutes.

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Sediment — Supernatant (discard) ⇨

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Thoroughly resuspend in small amount of water.

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Filter through a double layer of 40-gage plastic screen several times, alternating between two beakers. Squeeze screen dry and discard material on screen.

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Place beaker(s) in cold (4° C.) and let polyhedra settle for 2 weeks.

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Carefully siphon off supernatant and discard. (CAUTION --sediment very easily resuspends.)

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Resuspend polyhedra layer on bottom with a very small amount of water.

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Place suspension in large water glass and evaporate water off with the aid of a light bulb placed directly over the water glass.

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Scrape dry film of polyhedra off water glass. Grind in mortar and place in stoppered glass containers.

STORAGE OF THE PURIFIED POWDER

The purified powder is weighed and placed in tightly capped glass containers. The powder can be kept at room temperature, but by preference it is kept under refrigeration (40° F.). The powder will keep its potency for several years. One gram of purified gypsy moth polyhedra powder will contain approximately 6×10^{12} polyhedra.

USING THE PURIFIED POLYHEDRAL POWDER

Determine the number of polyhedra per gram of the purified powder by taking several 0.1-gram samples and place each sample in 100 ml. of water. Blend each thoroughly and count the number of polyhedra per ml. by using a hemacytometer or similar counting instrument.[2] Multiply the number of polyhedra per ml. (as counted in the hemacytometer) by 100 to get the total number of polyhedra in each 0.1-gram sample. Average these sample totals and multiply by 10 for the estimate in terms of grams.

Weigh out the grams of powder required to treat the infested acreage at the specified dosage. For example, gypsy moth polyhedra powder with a low count of polyhedra, say 25×10^9 polyhedra per gram, is to be applied to 50 acres at a concentration of 50×10^9 polyhedra per acre in 1 gallon of water. Two grams of this powder will be required per gallon, and a total of 100 grams of purified powder will be needed to spray the entire plot to give the required dosage.

For mixing, 100 grams of the powder would be weighed out, placed in 400-500 ml. of water, blended thoroughly to make a homogenous suspension, and diluted with water and sticker to make 50 gallons of the finished spray. The blended suspension of powder can be prepared several days in advance or can be left in the finished spray should application be delayed. No loss of potency will occur in this short space of time.

The insect pathology section of the Northeastern Station's Forest Insect Laboratory at New Haven has on hand a small supply of virus polyhedra powders of the gypsy moth,

[2] Instructions for use generally accompany the instrument.

forest tent caterpillar, and eastern tent caterpillar, and will send a sample to anyone interested in examining the material or building up stock.

Further information on collecting, processing, determining concentrations, or other matters relating to the larger polyhedral virus diseases will be gladly sent upon request.

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