United States
Department of
Agriculture

Combined Forest Pest
Research and
Development Program

Agriculture Handbook
No. 520

Douglas-Fir Tussock Moth Handbook

Rearing the Douglas-fir Tussock Moth

Rearing the Douglas-Fir Tussock Moth

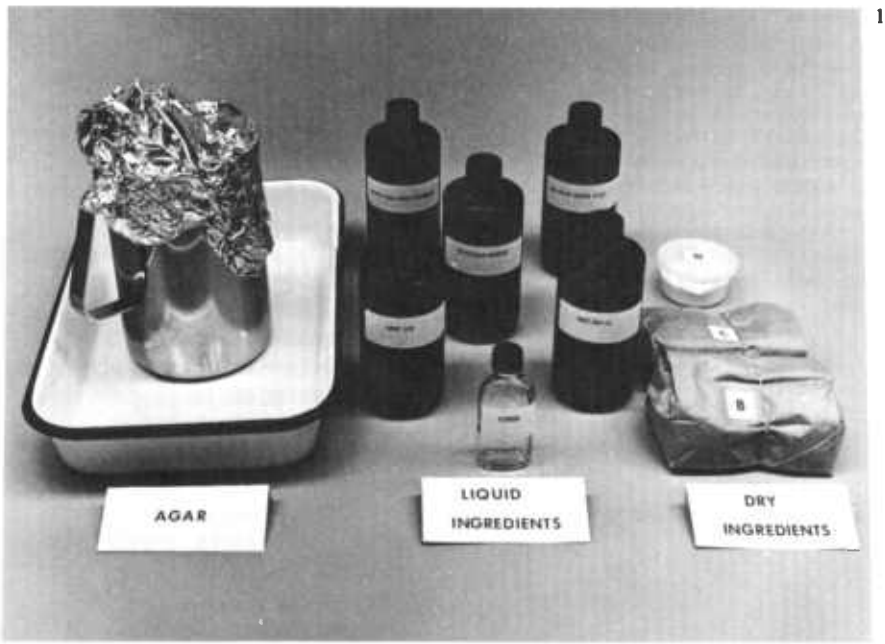
by Clarence G. Thompson and Linda J. Peterson[1]

Introduction

In 1974 the U.S. Department of Agriculture initiated the Combined Forest Pest Research and Development Program, an interagency effort that concentrated on the Douglas-fir tussock moth in the West, on the southern pine beetle in the South, and on the gypsy moth in the Northeast. The work reported in this publication was funded in whole or in part by the program. This manual is one in a series on the Douglas-fir tussock moth.

This leaflet provides laboratory rearing procedures for the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough). These procedures have been developed over the past 14 years of continuous rearing of the tussock moth at the Forest Service's Forestry Sciences Laboratory in Corvallis, Oregon. The artificial diet was developed over a period of several years with advice and suggestions from many sources. Similar diets have been described elsewhere (Lyon and Flake 1966); however, the recipe given here has been used in the Corvallis laboratory and by our cooperators with success for a number of years. The techniques can be used for continuous rearings or for one-generation rearings of field-collected eggs or larvae in order to assess levels of disease, parasitism, and other aspects of population quality.

[1] Research entomologist and formerly laboratory technician, respectively. Forest Service, Pacific Northwest Forest and Range Experiment Station, Forestry Sciences Laboratory, Corvallis, Oreg.



Following are instructions for preparing Douglas-fir tussock moth diet 65-W-PEN (the 65th modification in development of the diet). We find it convenient to package ingredients for several batches of diet at one time (fig. 1). The recipe makes one batch—four liters (5 pans)—of diet.

Figure 1.—Components of the tussock moth artificial diet prepackaged for convenient use.

Equipment

5 flat-bottom enamel or stainless steel pans, (25 × 40 cm)
1 stainless steel beaker (4-liter capacity)
1 graduated cylinder (100 ml)
1 graduated cylinder (1000 ml)
1 glass stirring rod
Disposable 10-ml pipettes
1 high-speed commercial blender (4-liter capacity)

Ingredients (Diet No. 65-W-PEN):

Package A	Agar, granulated	64.0 g
Package B	Casein, technical	210.0 g
	Sugar, granulated	210.0 g
	Wesson's salts "w"	60.0 g
	Alphacel	30.0 g
Package C	Wheat germ	180.0 g
Package D	Ascorbic acid	24.0 g
	Chlorotetracycline	280.0 mg
Package E	Penicillin G, K salt	800,000 units
Liquids:	Sorbic Acid (10% in 95% ETOH. Mix 10 gm/100 ml or multiples of this ratio)	32.0 ml
	Methyl parahydroxybenzoate (15% in 95% ETOH. Mix 15 gm in 100 ml or multiples of this ratio)	40.0 ml
	Vitamin solution[3]	10.0 ml
	Choline chloride (10% in H_2O)	60.0 ml
	Potassium hydroxide (4M)	30.0 ml
	Wheat germ oil, cold pressed	20.0 ml
	Distilled water	3000.0 ml

[3] Prepare vitamin stock solution in 100-ml quantities. The solution should be refrigerated.
Ingredients include:

Niacin	600 mg
Calcium pantothenate	600 mg
Riboflavin	300 mg
Thiamin hydrochloride	150 mg
Pyridoxine hydrochloride	150 mg
Folic acid	150 mg
Biotin	12 mg
Vitamin B_{12}	1.2 mg
Sterile distilled water	100 ml

Procedure

1. Place 1400 ml distilled water in a 4-liter stainless steel beaker. Stir in the agar (package A), and cover with aluminum foil. Autoclave along with the 4-liter blender and 5 pans for 20 minutes at 15 pounds pressure and slow exhaust.
2. Place 1600 ml sterile distilled water in the blender. Add the preweighed packaged ingredients (packages B, C, D, E). Add the liquid ingredients in their proper amounts. Blend on low for 4 minutes. The temperature of this mixture after blending should be about 38° C.
3. Allow the autoclaved hot agar to cool to 55° C with intermittent stirring. It takes 30–40 minutes for the agar solution to cool to 55° C.
4. Add the 55° C agar solution to the mixture in the blender and blend for 4 minutes on low. The temperature of this mixture should be about 52° C.
5. Immediately pour equal amounts of diet into each pan to a depth of about 1 cm.
6. Allow the pans to remain uncovered for about 10 minutes; then cover loosely with aluminum foil. After 10–20 minutes, cover tightly, label, and refrigerate (4° C).

Egg stage

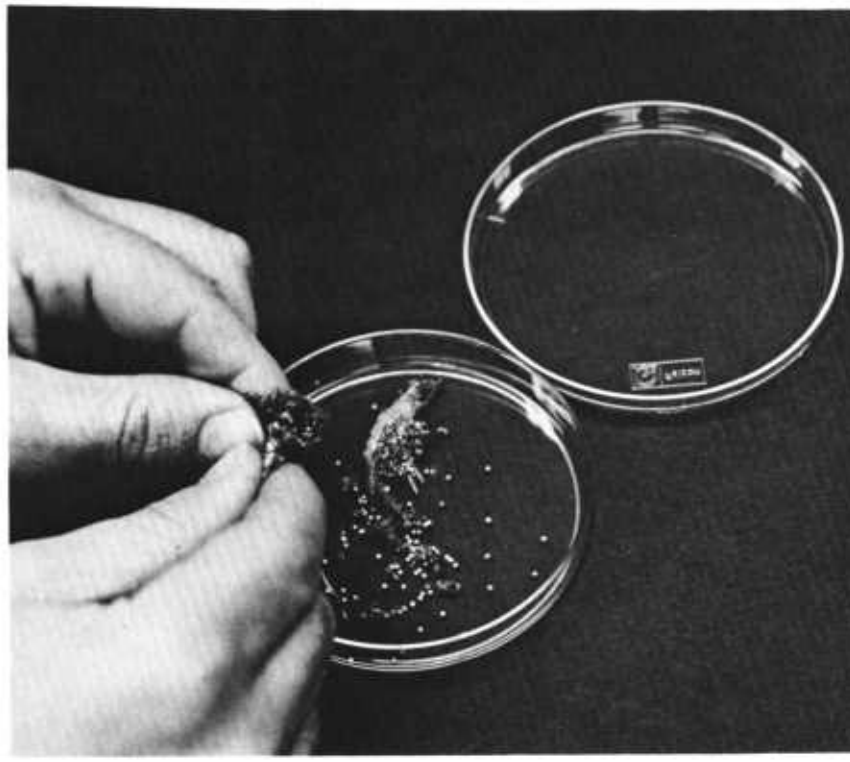
In nature, a diapause takes place in the overwintering eggs. At least three and preferably four months exposure to cool temperatures is necessary to break the diapause. Usually eggs collected in the field in January or later have completed their cold exposure requirement and will hatch after about 14 days incubation at a constant temperature of 21–23° C. Eggs collected in the field in the fall or produced in laboratory rearings should be stored for 3–4 months at 2–5° C before attempting to hatch them.

Following cold treatment, the eggs are surface sterilized to remove microbial contaminants such as nucleopolyhedrosis virus (NPV), *unless* the purpose of the rearing is to measure field incidence of NPV. The technique for surface sterilization is as follows:

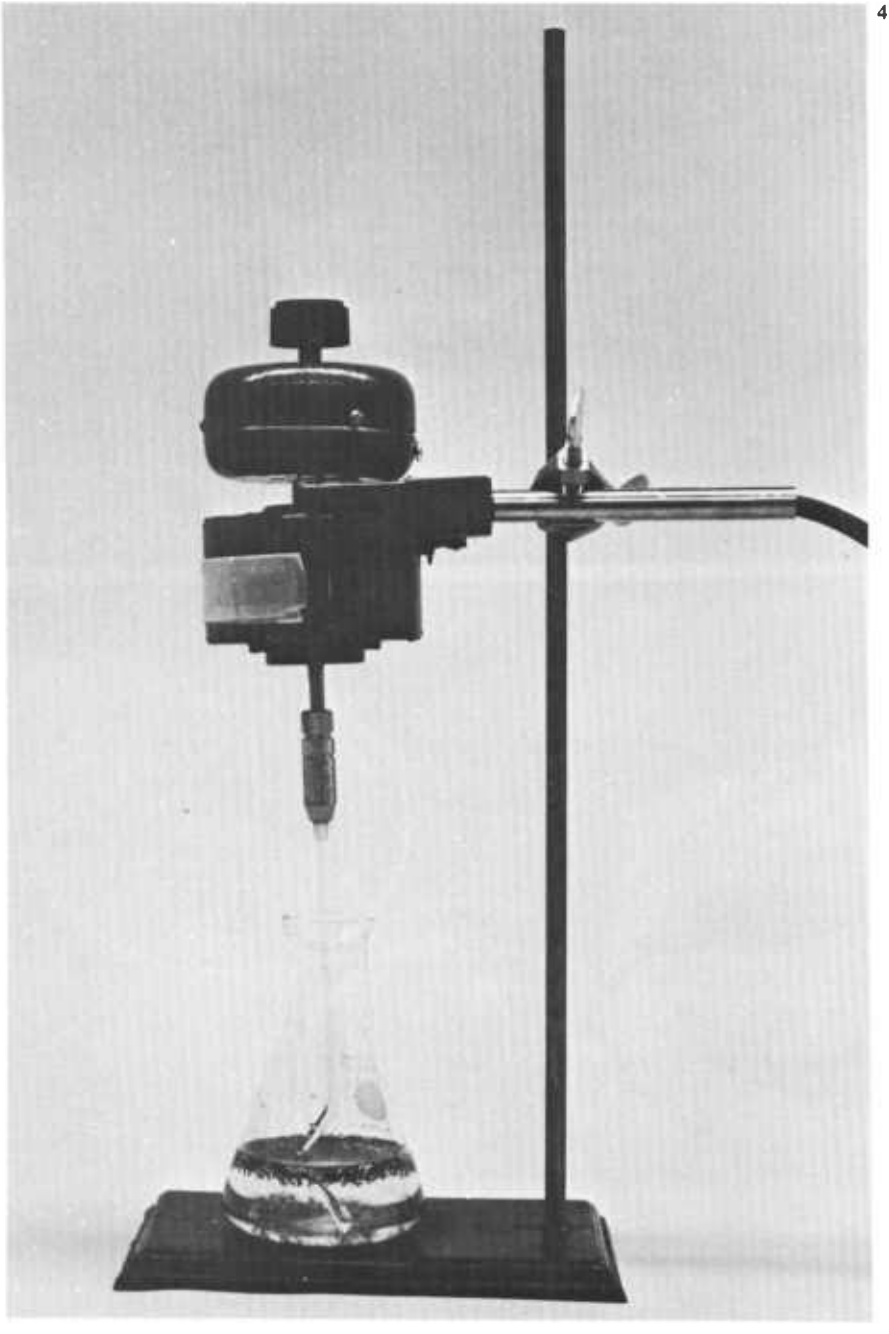
1. Remove the eggs from the cocoon (figs. 2 and 3).
2. Gently break up the egg mass without damaging the eggs (fig. 3).

Figure 2.—Field-collected egg masses of the tussock moth.
Figure 3.—The egg masses are stripped from the maternal cocoon and gently broken up.

2



3



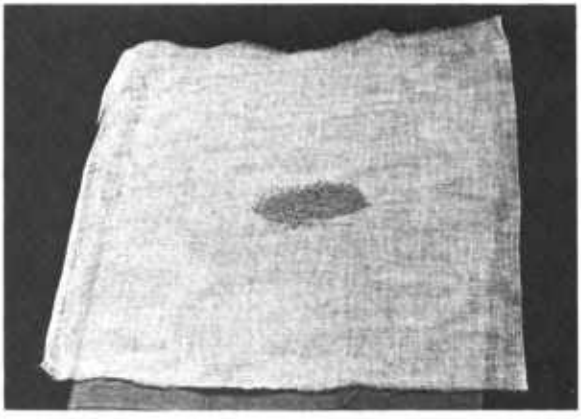
4

3. Place the eggs in a flask or beaker containing 0.1 percent sodium hypochlorite (this is roughly equivalent to a 2 percent solution in water of common household bleaches such as Clorox^{R} or Purex^{R}.)
4. Briskly agitate the sodium hypochlorite solution containing the egg-mass fragments, preferably with a laboratory shaker or stirrer. If it is not necessary to sterilize each egg mass separately, up to 10 egg masses can be sterilized in one 600-ml beaker or flask with a lab stirrer (fig. 4). If it is necessary to keep each egg mass separate, a laboratory shaker capable of handling a number of flasks is preferred (fig. 5).

Figure 4.—Eggs from up to 10 masses can be surface sterilized in 0.1 percent sodium hypochlorite using a simple lab stirrer.

Figure 5.—Where egg masses must be treated individually or where very large numbers of eggs must be surfaced sterilized, a laboratory shaker with multiple receptacles is used.

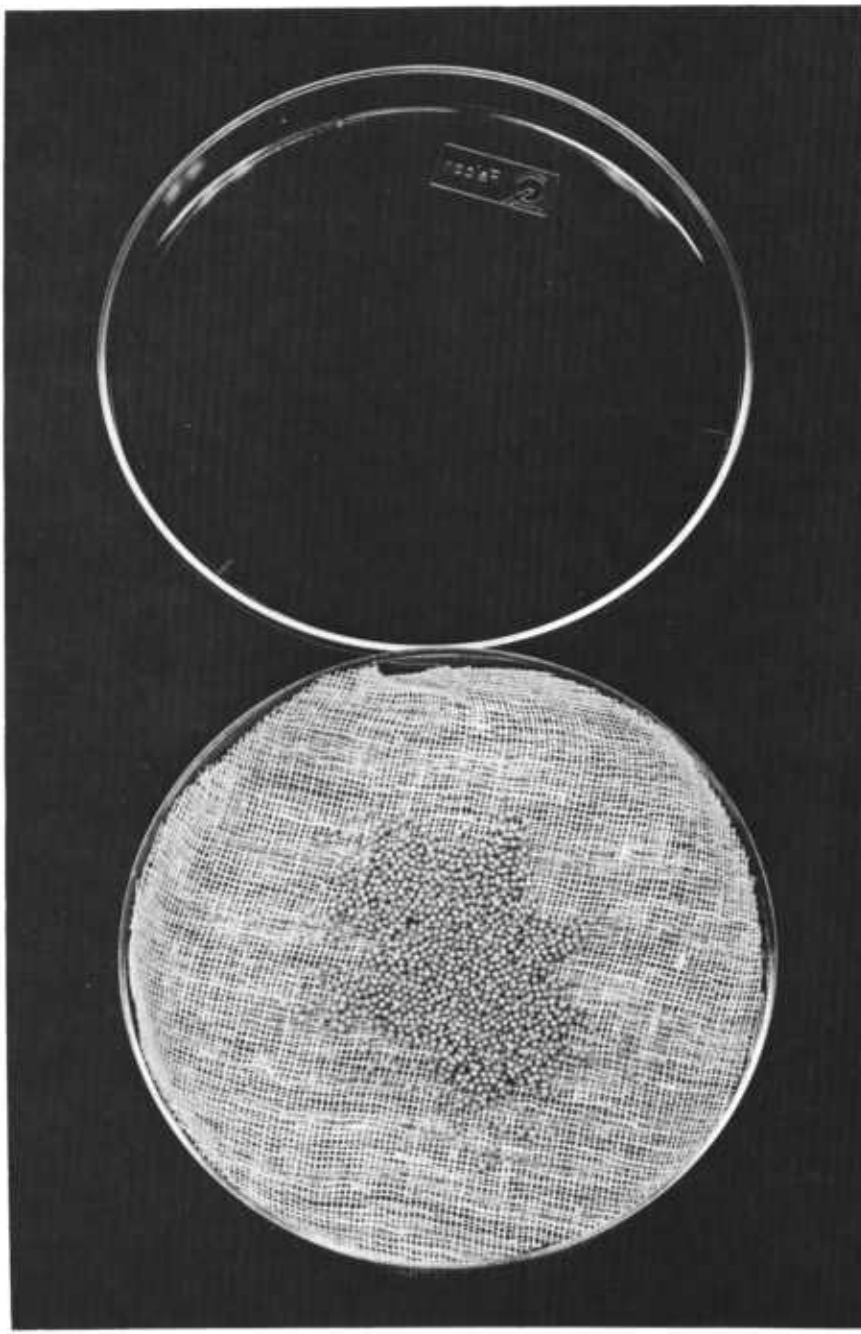
6



7

5. After 15 minutes agitation, replace the spent sodium hypochlorite solution with a fresh solution and agitate for 15 minutes more.
6. Remove the tussock moth eggs by pouring the sodium hypochlorite solution through cheesecloth (fig. 6) where the eggs are retained. Thoroughly rinse the eggs with water, and place them in a flask or beaker with fresh water. Agitate for 5 minutes to remove the last traces of the disinfectant.
7. Again remove the eggs from the rinse water by pouring through cheesecloth and gently blot to remove excess water (fig. 7).
8. Hold the eggs (fig. 8) at 25° C, preferably at 45 percent RH and with alternating 12-hour periods of light and dark. Hatch usually occurs in 12–14 days.

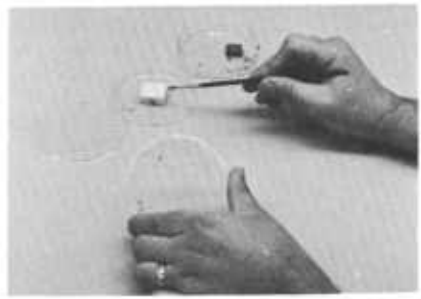
Figure 6.—Sterilized eggs are removed from the sodium hypochlorite solution by straining with cheesecloth.
Figure 7.—After washing the eggs, excess moisture is removed by blotting with paper towels.
Figure 8.—Following surface sterilization, the eggs are set up in desired numbers for hatching.



8

Larval stage

1. Gently transfer newly hatched larvae with sterile toothpicks or camel's hair brushes to rearing containers (disposable plastic 15 × 100 mm petri dishes are recommended) containing a block of artificial diet (fig. 9). For stock cultures, put 10 larvae in each dish. For virus incidence determination (from unsterilized eggs) put 25 larvae in each dish and maintain these dishes for 14 days only (fig. 10).
2. Feed the insects every 7 days. Remove the unused old diet at each feeding. Where virus incidence rearings are made, use a sterile toothpick to add new diet and to remove the old from each dish (figs. 11 and 12).



9

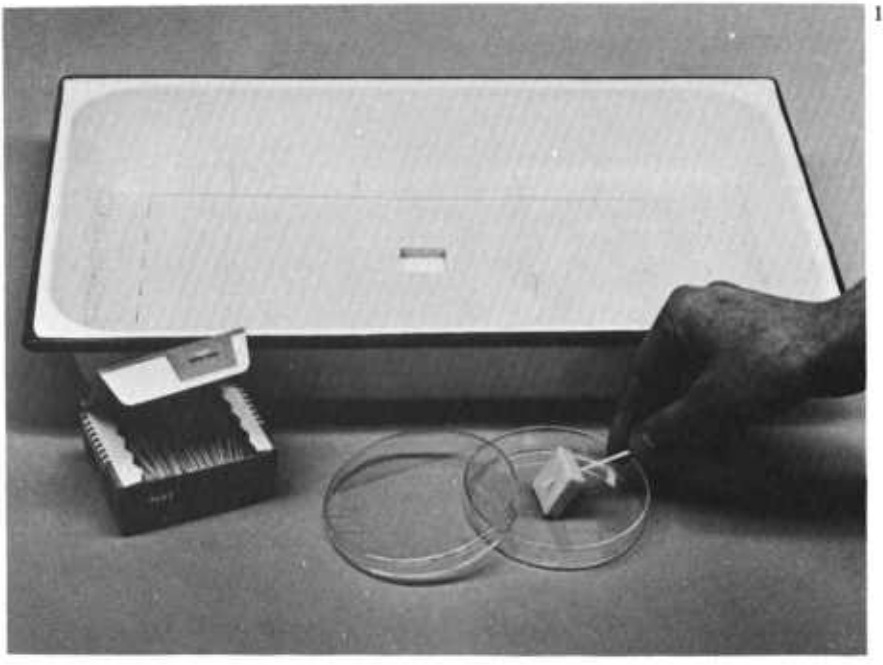
10

Figure 9.—Following egg hatch, the larvae are transferred in groups of 1 to 25 larvae, depending on intended uses, to petri dishes containing blocks of artificial diet. The diet is changed at weekly intervals.

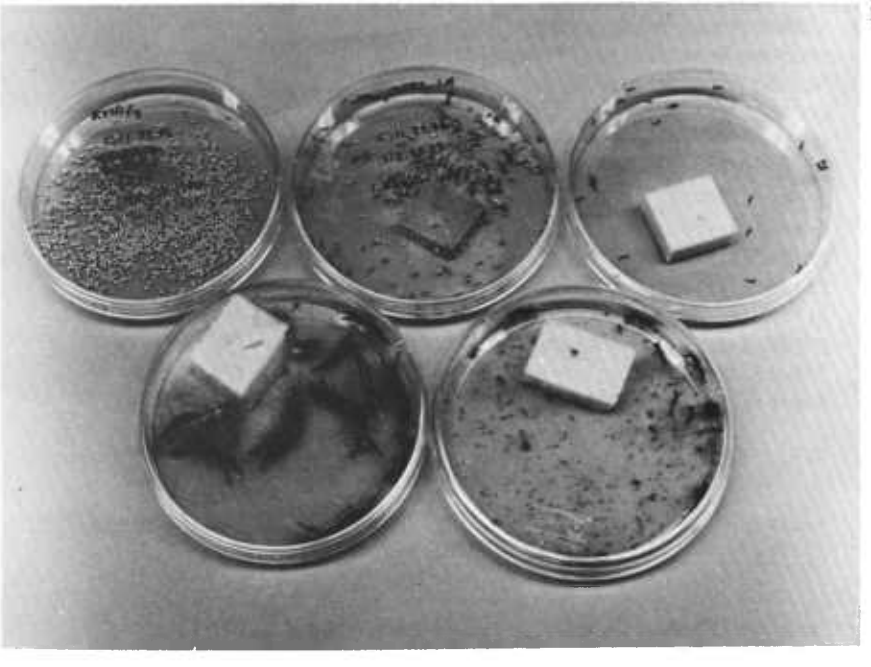
Figure 10.—A convenient system of rearing tussock moth larvae. Each tray is 60 cm × 60 cm and will hold up to 36 standard petri dishes.

Figure 11.—The artificial diet is cut in squares (1 or 2 cm) with a spatula (knives may scratch the enamel pan) and removed with sterile toothpicks. When feeding, it is best to use a fresh toothpick for each petri dish to avoid transferring virus or any other contaminating agent.

Figure 12.—Egg and various larval stages in laboratory rearing.



11



12

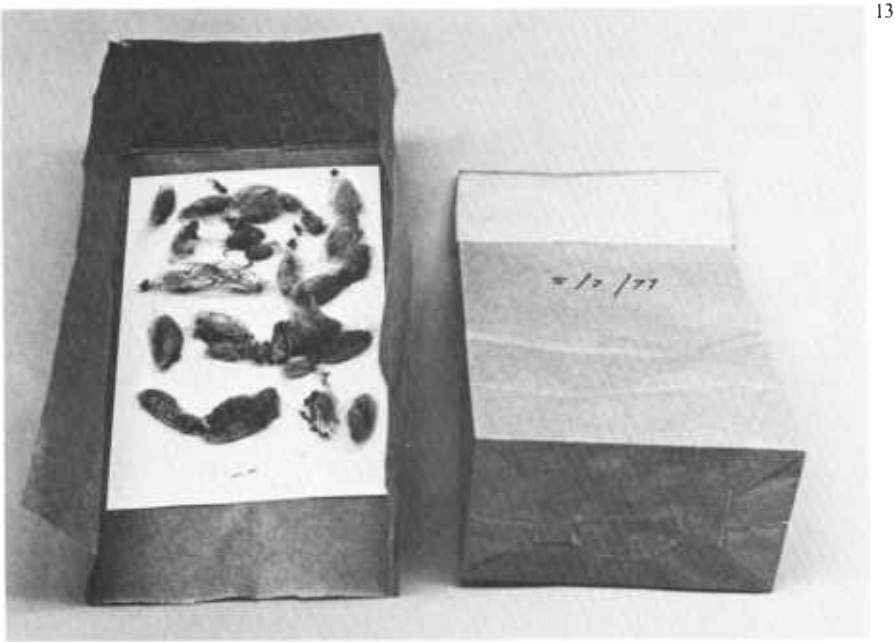


Figure 13.—Female pupae within their cocoons glued to cards in preparation for emergence, mating, and oviposition. Twenty-five male pupae are added to the sack before it is closed.

Pupal stage

1. Collect pupae, within their cocoons, as soon as they form.
2. Sex the pupae by placing the cocoons on a photographic light-table where the much larger female pupae can be identified inside their cocoons.
3. Glue cocoons containing female pupae 20 to a card (approximately 15 cm × 25 cm) (fig. 13) and place in brown paper bags. Place 25 male pupae, 3 days older than the females, loosely in the bag and staple the bag closed.

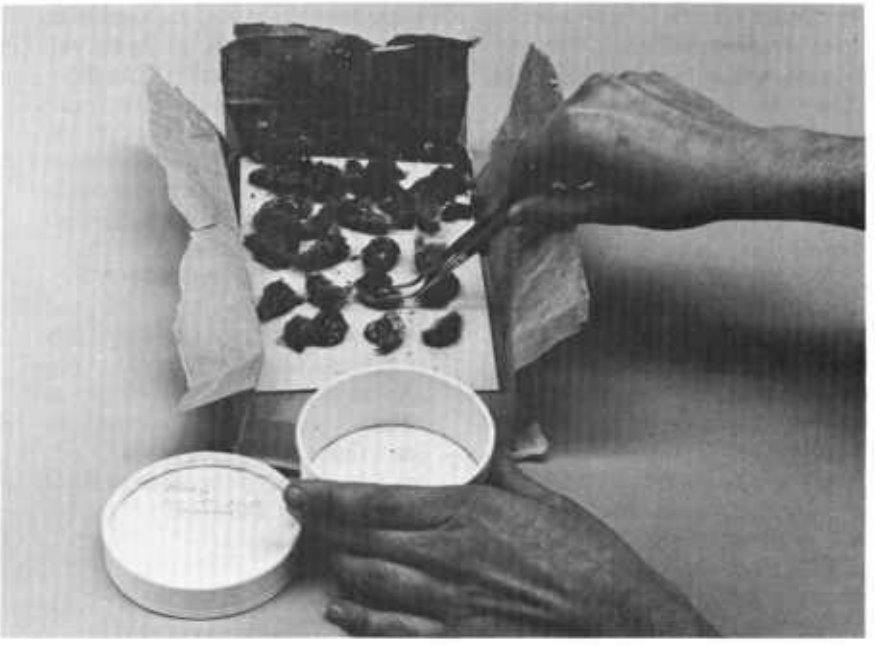


Figure 14.—Egg masses are removed from the oviposition cages and stored at 2°–5° C for a minimum of 4 months to break diapause.

Adult stage

The adults emerge, mate, and oviposit in the paper bags.

1. Thirty days later, remove the cards with cocoons—now bearing egg masses—from the bags (fig. 14) and remove the egg masses from the cards.
2. Store the eggs at 2°–5° C for 4 months to complete the cycle.

General Laboratory Procedure

The tussock moth is subject to several extremely virulent diseases. Very minute amounts of NPV can infect and kill tussock moth larvae, and once established, the virus can spread rapidly and wipe out entire cultures unless a strict regime of sanitation is maintained. Bacterial, fungal, and protozoan diseases can be nearly as serious.

All work surfaces should be kept clean and sponged daily with a good disinfectant (a 2 percent solution of common household bleaches containing 5–6 percent sodium hypochlorite will do very well). Wherever practical, disposable rearing containers should be used and discarded after one use. Healthy stock cultures should be isolated from any work involving, or potentially involving, diseased tussock moth larvae. If at all possible, the individual preparing the diet and maintaining healthy stock cultures should be prohibited from any contact with areas where disease experiments are being conducted. Post this sanitation information in the work area and make sure it is rigidly followed. One slip can wipe out months of work!

Almost everyone who has worked extensively with tussock moths has experienced some irritation from the barbed hairs of the larvae. Some individuals are so allergic that they may require medical treatment after a brief exposure. Certain precautions can be used to reduce the irritation. The dry cast skins and the cocoons are the primary sources of irritation. If the cocoons and egg masses are handled in an exhaust hood, the problem will be greatly alleviated. Rubber gloves offer additional protection. It is wise to take all possible precautions because it appears that some individuals who are not initially allergic become sensitized with continued exposure.

References

Lyon, R. L., and H. W. Flake, Jr. 1966. Rearing Douglas-fir tussock moth larvae on synthetic media. J. Econ. Entomol. 59:696–698.

Photos by Alex Yusha