A WHOLE-BARK METHOD OF REARING DRYOCOETES CONFUSUS SW.

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Introduction

During studies on the relation of a fungus, *Ceratocystis* sp., to the western balsam bark beetle, *Dryocoetes confusus* Sw., it was found necessary to rear the insect in the laboratory. Lacking proven methods, a search was made among those used for other cambium-feeding insects, to find one which would be suitable for *Dryocoetes* or which would at least serve as a starting point for developing a satisfactory method. With a few modifications, a whole-bark rearing method used by Finnegan (1) to rear the pine weevil, *Pissodes approximatus* Hopk., was found very satisfactory. All stages of *Dryocoetes confusus,* from egg to adult developed well in alpine fir, *Abies lasiocarpa* (Hook.) Nutt., whole bark and were readily observed during their development.

Method

Finnegan placed newly hatched larvae in small grooves in the cambial surface of freshly cut Scots pine, *Pinus sylvestris* L. bark discs. The discs were then pressed tightly, cambial surface down, in the bottom of petri dishes by filling them with moist sand and applying pressure on the cover with rubber bands. He indicated the importance of using sterilized glassware and sand and keeping to a minimum the exposure of the cambial surface of the inner bark to the air during preparation.

Finnegan's method as used for *Pissodes approximatus* was moderately satisfactory for *Dryocoetes confusus* but a number of changes effected a marked improvement, particularly where the insects were reared throughout their life stages in a single rearing chamber. The changes made were largely in connection with improving the maintenance of suitable moisture conditions and with getting rid of the objectionable feature of sand sifting out of the chambers during handling. The sand was replaced by vermiculite, for vermiculite dried less quickly and more evenly. It also had a slight tendency to sift out but this was overcome by placing two slightly oversized pieces of filter paper in the chamber, one next to the bark and one next to the lid, to enclose the vermiculite. The filter paper provided the additional advantage of assuring an even distribution of water, which was added after about five weeks in long term observation chambers.

The procedures used for the preparation of materials and setting up rearing chambers for *D. confusus* were as follows:

1. Preparation of bark discs

Discs were generally cut from fresh alpine fir logs with non-corky bark, but equally satisfactory results were obtained using logs several weeks old when the ends were sealed with wax at the time of cutting and the logs were kept in a cool place. Immediately before cutting the discs the logs were scrubbed with 70 per cent methyl alcohol and the thin outer bark was sliced off with a clasp knife or draw knife. Discs of the right size to fit the 100 mm. dishes used were readily obtained by cutting around a template with a sharp-pointed, sturdy knife and slicing through between the inner bark and cambium. To maintain the bark as aseptic as possible until being used in the rearing chambers, the discs were stacked in sterilized, metal petri dish holders alternately

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with fresh filter paper. It was found desirable to use the bark within a few hours after cutting, although overnight storage in a refrigerator did not appear to be detrimental. Complete asepsis is not possible, of course, for even if the bark were rendered aseptic, beetles or larve gathered in the field would introduce their naturally associated flora and fauna. These were in fact found to develop in the rearing chambers but very rarely inhibited the development of the insects.

2. The moistening medium

The moistening medium was prepared in advance by dry-sterilizing fine-grained vermiculite at 95° C for 48 hours. To this was added sterile water at the rate of one volume of water to four volumes of vermiculite. Two gms. of sodium benzoate were added to 1,000 mls. of the sterile water to inhibit bacterial activity.

3. Setting up rearing chambers

The procedure for setting up the rearing chambers was essentially that used by Finnegan except that some of the materials were different as indicated. A number of features were particularly critical to the success of the rearings and should be emphasized:

a) The nature and size of the bark niches in which the insects were started was important for eggs and larvae. A size just large enough to hold the larvae, preferable triangular in shape, appeared to be the best. A small triangular niche cut with a scalpel provided the necessary purchase for the larva to start feeding. Adults were less critical in this regard and a round hole to simulate a nuptial chamber, cut with a cork borer, served well.

b) After placing the insects in the niches care was necessary to make sure the inner bark was tightly pressed against the bottom of the dish by inverting the dish over it and holding it firmly until the first filter paper, vermiculite, second filter paper, lid, and rubber band were added. Space between the bottom of the dish and bark permitted insects to escape into these spaces where they were often unable to start feeding again.

c) It was necessary to avoid adding water to the extent that free moisture formed in the bottom of the dish, which tended to hold the insect immobile and often drowned it.

d) The rearing chambers were kept in a dark cupboard at room temperature.

Efficacy of Method

The primary purpose of the rearings of *Dryocoetes confusus* was to produce adults free from contamination by the apparently pathogenic fungus under study. Since the egg stage was the only one amenable to sterilization, it was necessary to rear the insect throughout its life stages. The results of rearings, however, suggested the suitability of the method for rearing studies beyond the scope of the present investigation. It appears suitable to life history studies and other laboratory investigations involving detailed observations on active living insects of this and probably other bark and cambial feeding species. Some of the results of rearings, especially survival figures, are summarized below to permit the reader to evaluate the method in terms of his own requirements.

Survival

Field collections of *Dryocoetes* for rearing purposes were confined to larvae and adults but transfers to fresh rearing chambers were carried out at the egg and pupal stages as well. The survival figures for rearings and transfers are summarized in Table 1. The relatively poor survival obtained with field-collected larvae

TABLE 1—Survivors from Rearing Studies of **Dryocoetes confusus** Sw. in Whole Bark-Petrie-Dish Rearing Chambers.

Time from start (weeks)	A Field larva (Oct. 1958 collection)	B Transferred larvae off-spring of "A"	C Transferred pupae off-spring of "A"	D Field adults (May 1959 collection)
		Number living		
0	289	71	21	55
2	129	57	—	44
4	119	55	16	44
8	102	—	—	—
16	73	—	—	—
Final disposition	Transferred for rearing studies	All survivors adults; discarded	All survivors adults; discarded	Rearing study; eggs transferred as needed, rest discarded

was not considered a reflection on the rearing method, for the larvae were sent by mail from Vernon to Victoria and nearly a week elapsed before they were placed in rearing chambers. In addition, they were first started with sand as the moistening medium, then changed to vermiculite when it was found that the sand would prove troublesome. It can be seen that 55 per cent of the mortality occurred in the first two weeks of rearings. In the other columns of this table it can be seen that relatively little difficulty was experienced in transferring laboratory-reared larvae and pupae and field-collected adults.

All eggs used in the egg-transfer studies came from breeding experiments using the wholebark rearing chambers described. Transfers to fresh rearing chambers were carried out using two methods of egg sterilization.

In the first method of egg sterilization, eggs were freed from boring dust and washed for 30 seconds with a 1:1000 solution of Hg Cl, followed by a sterile water wash for 60 seconds. The eggs were then placed in the described niches in fresh bark. In the second method eggs were washed with 95 per cent ethonal with a brush on sterile agar plates and moved to a clean part of the agar. A day later the eggs were transferred to bark niches with a sterilized needle.

There did not appear to be a significant difference in survival between the various methods and control used except for the poor results obtained in the second Hg Cl treatment (Table 2), but the writers favoured the

TABLE 2—Survivors of **Dryocoetes confusus** Egg Transfers

Time from transfer (weeks)	Eggs from adults reared from larvae (Table 1"A")		Eggs from field adults mated in whole bark chambers		Eggs from field adults mated in whole bark chambers	
	sterilized Hg Cl	not sterilized	sterilized Hg Cl	not sterilized	sterilized ethanol	sterilized ethanol, inoculated with **Ceratocystis**
			Number living			
0	6*	15*	15*	15*	15*	25*
1	4	13	3	11	10	17
2	3	13	1	10	10	17
3	3	13	1	10	10	17
4	—	—	—	—	10	17
5	—	—	—	—	9	15
Final disposition	Broken up for isolation experiments. Larva only.		Broken up for isolation experiments. Larva only.		Broken up for isolation experiments; 3 adults remainder larvae	

* Healthy appearing eggs but some may not have been viable.

ethonal method because it involved less handling of the eggs and afforded less opportunity for over-exposure of the eggs to the sterilizing agent. It should be pointed out that the chambers with sterilized eggs did not remain entirely free from micro-organisms but they were free from the pathogenic *Ceratocystis* under study. To obtain aseptic chambers a suitable method of sterilizing bark will have to be developed which does not change its essential properties. Steam sterilization, a method recommended by Holst (2), rendered alpine fir bark somewhat plastic and unacceptable to both larvae and adults of *Dryocoetes*.

Development of *Dryocoetes confusus* in rearing chambers

Detailed observations and measurements on the insect's development were not included in the scope of these experiments and, since relatively little is known of its development in nature, little can be said concerning the effects of artificial rearing on development. A number of observations, however may be worth recording here.

1) The life cycle of *D. confusus* was carried to completion and viable eggs were produced by females reared in the chambers.
2) It was evident that the life cycle can be greatly accelerated through artificial rearings by reducing the normal periods of inactivity induced by unfavourable weather conditions. The progeny of the first set of larvae collected in the fall of 1958 attained maturity by the following May, a full year before this would have occurred under field conditions.
3) Survivors of larvae collected in the field in October attained adulthood at nearly a 1:1 male-female ratio, while 250 adults collected the following spring in the same area at Bolean Lake were in the ratio of 1:12 males to females.
4) Adults reared from larvae in the chambers started oviposition 6-7 days after mating on fresh bark, while field-collected adults started oviposition within 3 days of being placed on fresh bark in the chambers. The field collections were made as much as 2 months before normal oviposition when the insects were still frozen in the host trees. Thus, a much wider time scope is available for laboratory work with eggs than would be possible with those collected in the field; in addition field-collected eggs pose difficult handling problems and require time-consuming observations to collect the right stage.
5) Apparently mating in the spring is not required for females collected in the field. Females collected from frozen trees in the spring and placed unmated in rearing chambers produced viable eggs in abundance.

Conclusion

Finnegan's method for rearing weevils in whole-bark rearing chambers, with minor modifications, met the requirements for the laboratory rearing of *Dryocoetes confusus*. The results of experiments indicate the suitability of the method for detailed observations on all phases of the insect's development from egg to adult. The adaptability of the method to other bark and cambial feeding insects is suggested by its success with two widely separated species.

The advantages of the method are the simplicity of the set-up and procedures, readily available materials, the lack of a troublesome moisture problem, and the ease of observing development at any time (Figs. 1, 2, and 3). While 100 mm. petri dishes were used in these studies, larger sizes are available if desired.

The possibilities of adapting this rearing method to standard insectary procedure for rearing larvae of unknown bark and cambial feeding insects sent in through the Forest Biology Survey are worth investigating. Materials and procedures could be readily set up. The main problem would stem from the unavailability of bark of the right species; but this difficulty could be overcome by one or both of two ways. Logs with waxed ends could be kept in cool storage for considerable periods without the bark losing its desirable qualities, or the collector could air-mail a section of wood and bark with his collection of unknown larvae.

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References

Finnegan, R. J. 1958. The pine weevil, **Pissodes approximatus** Hopk., in southern Ontario. Can. Ent. 90: 348-354.

Holst, E. C. 1937. Aseptic rearing of bark beetles. J. Econ. Ent. 30: 676-677.

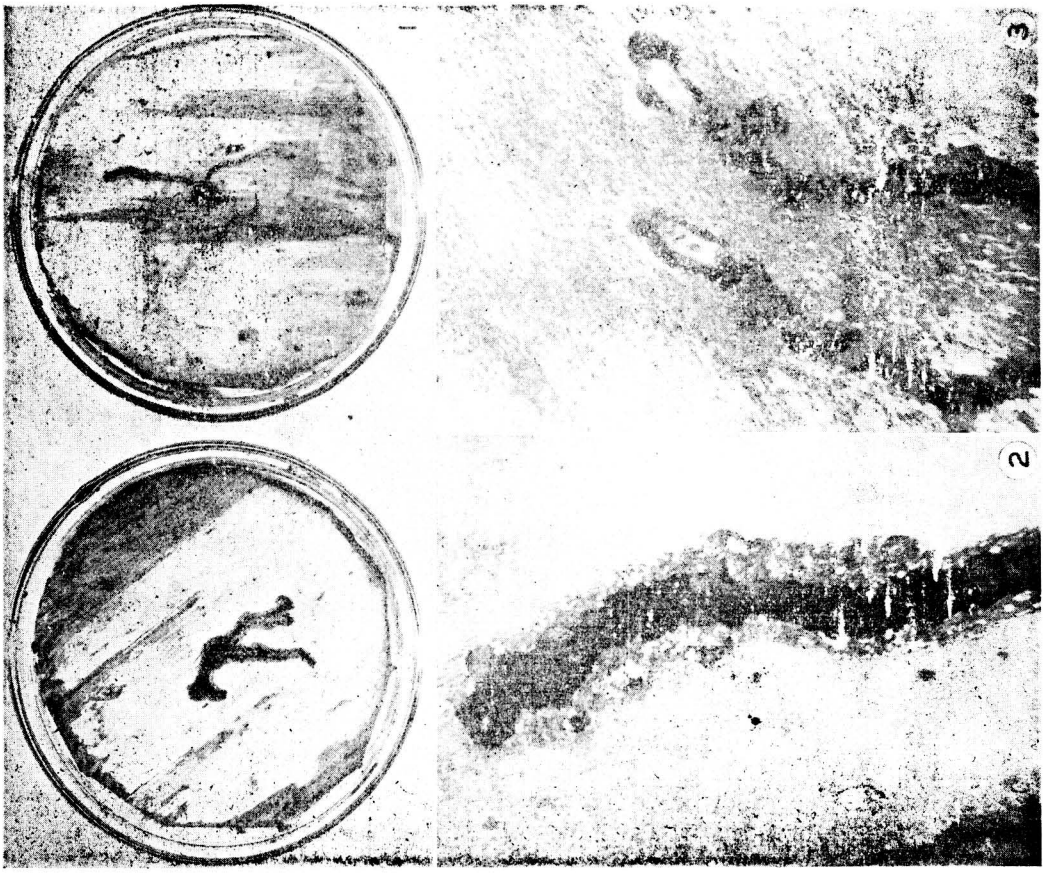

Fig. 1.—Underside of whole-bark rearing chambers showing **Dryocoetes confusus** feeding galleries on the left and egg galleries on the right. 0.6 X.

Fig. 2.—Egg gallery in whole-bark rearing chamber showing deposited eggs and female **Dryocoetes** in process of extending gallery. Approximately 8 X.

Fig. 3.—Newly hatched larvae of **D. confusus** in whole-bark rearing chamber. Approximately 8 X.