METHODS OF COLLECTING, REARING AND HANDLING THE LARCH SAWFLY FOR EXPERIMENTAL STUDIES

by

R. J. Heron and J. A. Drouin

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ILLUSTRATIONS

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Fig. 1 - D. N. Smith; Fig. 2 & 9 - B. B. McLeod; Fig. 3 - C. Burdall; Fig. 4 & 10 - R. J. Cheale; Fig. 7 - J. A. Drouin; Fig. 5, 6, 11 and appendices photos - E. R. Rayner. Drawings in Appendix II - J. A. Drouin

INTRODUCTION

The larch sawfly, *Pristiphora erichsonii* (Hartig), an important pest of tamarack, *Larix laricina* (DuRoi) K. Koch, and western larch, *L. occidentalis* Nutt., in North America, has been studied intensively, for several years, by entomologists of the Forest Research Laboratory in Winnipeg. During this time, various techniques and procedures for rearing and handling this insect have been developed or adapted and tested by repeated use. Some procedures have been described briefly in research papers by personnel of this laboratory, but others have not. The only published material dealing specifically with rearing methods for the larch sawfly are the brief accounts by Graham (1937) and Heimpel (1957). The detailed treatment of the subject presented in this report was undertaken in response to enquiries received for this information. It is presented with the hope that it will be of interest to persons undertaking to rear this insect and that it will help them avoid some of the difficulties that can be encountered. Some of the techniques may also be useful in the rearing of other phytophagous insects.

The following account is divided into three sections. The first section deals with methods of collecting larvae and rearing mass collections to obtain stocks of cocoons for experimental use. The second section concerns methods of handling and rearing the larch sawfly during the period of its normal seasonal development, from the time the experimental cocoon stock is placed in cold storage until the new cocoons are spun by the ensuing generation. The final section describes procedures for rearing the insect during the winter months when its normal food is not available in the field.

A brief, illustrated life history of the insect is given in Appendix I. Detailed descriptions and illustrations of the various types of rearing cages used are presented in Appendix II.

Throughout this report, unless otherwise stated, references to seasonal development relate to conditions as they normally occur in south and central Manitoba and Saskatchewan.

I MASS REARINGS

Larval Collections

To conduct experiments on the larch sawfly it is often convenient to have a supply of overwintering cocoons to provide a stock from which larval rearings can be established. The cocoons can be most easily obtained by collecting late-instar larvae from heavily infested tamarack stands. Suitable collection areas can be readily spotted because the upper crowns of infested trees soon become stripped of foliage and are quite conspicuous (Fig. 1).

Fig. 1. Mixed stand of tamarack and black spruce showing heavy defoliation of tamarack by the larch sawfly.

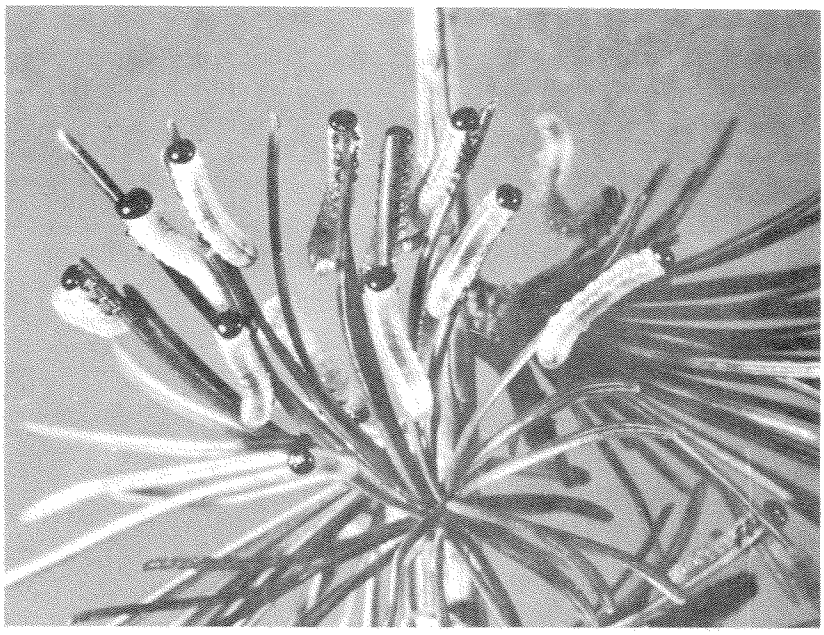

Fig. 2. Colony of larch sawfly larvae.

Due to the prolonged period of adult emergence and oviposition, feeding larvae are present from mid-June to the first week of September. About 20 days are required to complete feeding and larvae begin dropping to form cocoons about the end of the first week of July. The peak of larval drop usually occurs about the end of July and all but a small portion will have dropped by mid-August. There is considerable variation in the timing from year to year depending on weather conditions. Mass collections of larvae should be made at the peak of the feeding period when the majority of the larvae are in the fourth or fifth stadia. This is usually about the third week of July.

The larvae are gregarious feeders and normally remain in a colony (Fig. 2) until after the fourth stadium. During the fifth stadium, larvae may wander considerably in search of food. Their behavioral responses result in a tendency to aggregate at the periphery of the branches and in the upper portion of the crown. Due to their green coloration the feeding colonies blend with the foliage. Their presence can be detected most readily by searching for the defoliated branch tips.

In making collections of larvae to obtain a stock of cocoons only fourth- and fifth-instar larvae should be included. Too much labor in rearing is required when early-instar larvae are collected and there is high mortality during rearing and handling. For efficient handling the collector should be equipped with a sleeve cage (household screen approx. 8 inch diameter by 12 inches long with cloth ends), closed at one end, with a lanyard arrangement around the neck leaving both hands free. Avoid touching the larvae if mosquito repellent has been used on the hands. In the fourth and early fifth stadia the larvae are generally in colonies and will not drop to the ground when disturbed. The collection of larval colonies is facilitated by using hand pruners to remove the part of the branch on which the larvae are feeding. Since late fifth-instar larvae tend to drop immediately when the branch is disturbed, a cotton beating sheet of adequate size should be used to catch larvae dislodged by shaking or beating the tree or branches.

In mature stands, where populations may be confined to the top 2/3's, sectional pole pruners can be used. The pole operator can locate, cut and lower the infested branches gently to an assistant. Generally, collecting is fastest along the edges of a stand, road allowances or natural openings, where the trees are heavily branched.

Once the collection is completed the sleeve cages should be kept in a cool, ventilated location during transport to the laboratory or better still the larvae can be transferred to rearing cages containing a good supply of fresh foliage for transport.

Rearing and Handling of Larvae

When the field collected larvae arrive at the laboratory or insectary they should be transferred as soon as possible to rearing cages prepared as described below.

Before transferring larvae to the rearing cages however they may be examined to segregate those parasitized by Bessa harveyi (Tnsd.). Larvae parasitized by this tachinid can be recognized by the presence of eggs or egg scars on the integument (Fig. 3). The egg chorion is white and readily detected by the naked eye. If the egg has been sloughed off, after hatching, the point of entry of the maggot will be indicated by a dark area. Initial segregation saves time during later stages of incubation and rearing. Some of the parasites will emerge in the fall prior to cocoon storage (Turnock and Melvin 1963). Not all the larvae that had eggs or egg scars will produce parasites. Because of unsuccessful hatching or establishment of the parasite, some sawfly adults will also emerge. The two groups of larvae should be kept separate but handled in a similar manner.

Large screened cages are used for the mass rearing of larvae. The cages are illustrated and described in detail in Appendix I.

Prepare the cages for rearing as follows:

(a) Place four 1/2-pint, wide-mouth jars filled with water in each cage. Cover the mouths of the jars with a sheet of acetate plastic 1/4-inch thick foam, held in place with elastic bands. Cover the floor of the cage around the jars with clean, slightly moist sphagnum moss to a depth of 3 or 4 inches.

(b) Insert 4 or 5 freshly cut tamarack branches in each jar. The plastic foam should fit tightly around the butts of the branches to prevent the larvae from crawling into the jars and drowning. Some branches should be arranged so that foliage contacts the moss thereby enabling wandering larvae to find food.

(c) Once the cage contains sufficient fresh foliage, scatter the larvae gently over the foliage.

(d) Place the cages in a bright, airy but sheltered area and avoid temperature extremes and direct sunlight. The cages should be checked daily for food reserve and water. The larvae may tend to congregate on the screen particularly on the side toward the light source. A gentle tapping will dislodge the larvae onto the foliage or moss and turning the cage will reactivate them.

Foliage consumption of fourth-instar larvae will not be great and the addition of fresh foliage will not be necessary until the third or fourth day. At this time, all the contents of the cage should be removed taking care not to disturb the moss. Refill water jars, top with new plastic foam and insert fresh foliage as before. Pick out dead or diseased larvae and discard. Remove the larvae from the old foliage into pans and redistribute

them throughout the fresh foliage. Check the moss for moisture and dampen if dry. Since fifth-instar larvae consume foliage more rapidly than fourth-instars its depletion is accelerated and cages should be checked daily if the numbers of larvae warrant it. At completion of larval feeding, leave the moss containing the cocoons undisturbed for a week to assure that cocoon spinning is completed.

Rearings of larvae parasitized by the introduced ichneumonid, Olesicampe benefactor Hinz, should be handled with special care to prevent loss of larvae or contamination of other study material. Because these larvae are dwarfed and able to crawl through household screen, special cages, tightly constructed, with fine mesh screen (smaller than 18 x 14 mesh) should be used.

Handling and Storage of Cocoons

Seven to 10 days after feeding has finished, examine the moss, remove the cocoons and handle them as follows:

(a) Examine all the cocoons carefully and remove all those that obviously contain dead larvae. The dead material will consist of poorly spun cocoons with dead larvae visible, flattened cocoons and cocoons with holes made by fall emerging parasites.

(b) In areas where the introduced parasite O. benefactor is present it may be desirable to separate the cocoons containing larvae parasitized by this species from the rest of the cocoons. The growth rate of larvae parasitized by O. benefactor is considerably reduced. Among cocoons collected at two release sites in Manitoba it was found that almost all the cocoons of parasitized larvae measured less than 9.25 mm in length (Muldrew 1967). With a little experience it is possible to recognize the small cocoons without recourse to actual measurement (Fig. 4). Most of the male sawflies and some partially-starved female specimens will also fall into the small cocoon category. The "small" and the "large" cocoons can be treated in the same manner but keeping them separate will simplify handling when the adults emerge.

(c) Package the apparently sound cocoons in lots of 100, in moist sphagnum moss wrapped in dacron marquisette, taking care to avoid squashing the cocoons (Fig. 5). A label identifying the area or date may be attached if necessary. Numbered aluminum poultry bands are useful for labelling the packets by code number. ("Moist" moss feels damp but no water can be squeezed from it by hand.)

(d) The marquisette packages should then be layered between moist sphagnum moss in plastic refrigerator trays (Fig. 6) for storage in cold rooms or layered in the same manner in screened containers (Fig. 7) that are buried under 12 or more inches of moss for storage outdoors in a semi-natural environment.

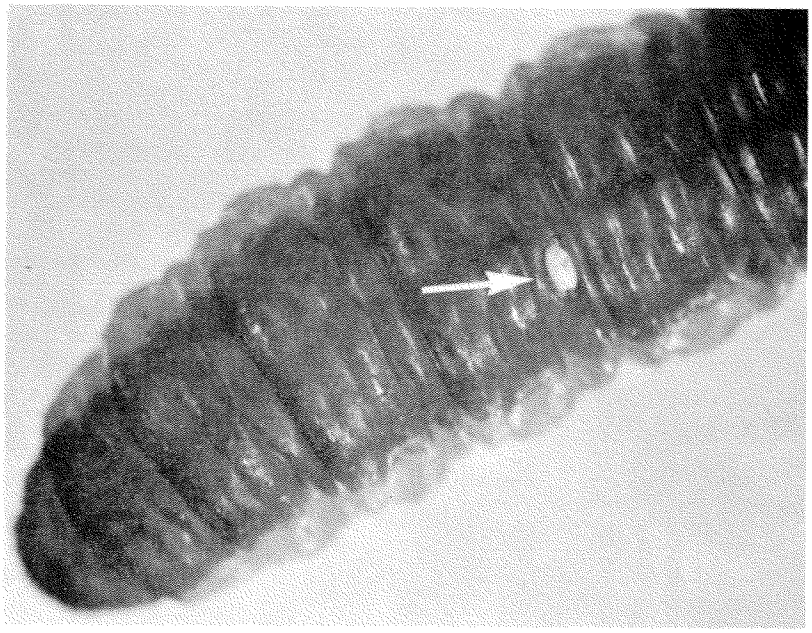

Fig. 3. Close-up of last-instar larva with hatched egg of Bessa harveyi on dorsum of abdominal segment (indicated by arrow).

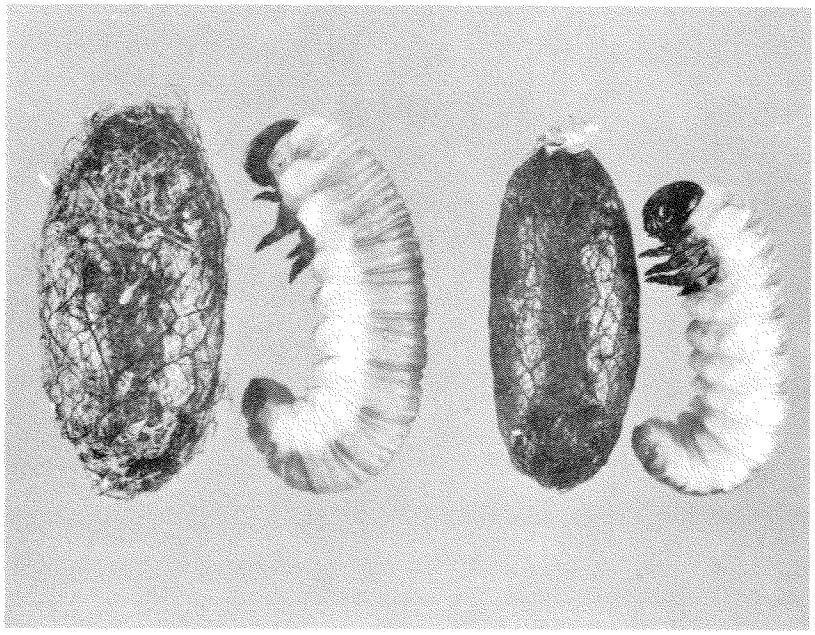

Fig. 4. Normal cocoon and eonymph of female larch sawfly (left) and cocoon and eonymph of female parasitized by Olesicampe benefactor (right).

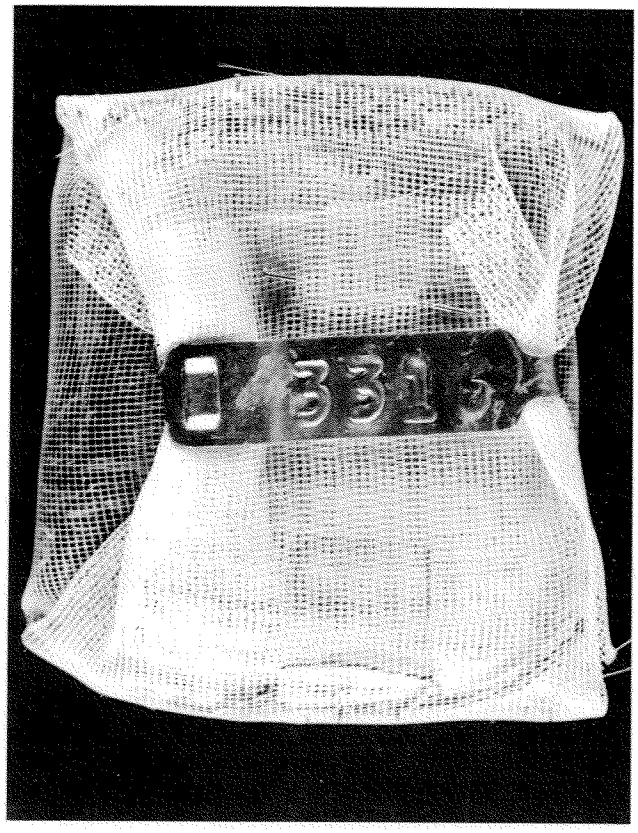

Fig. 5. Cocoons packed in moist sphagnum moss and wrapped in dacron marquisette for storage. Package has aluminum band with identifying number.

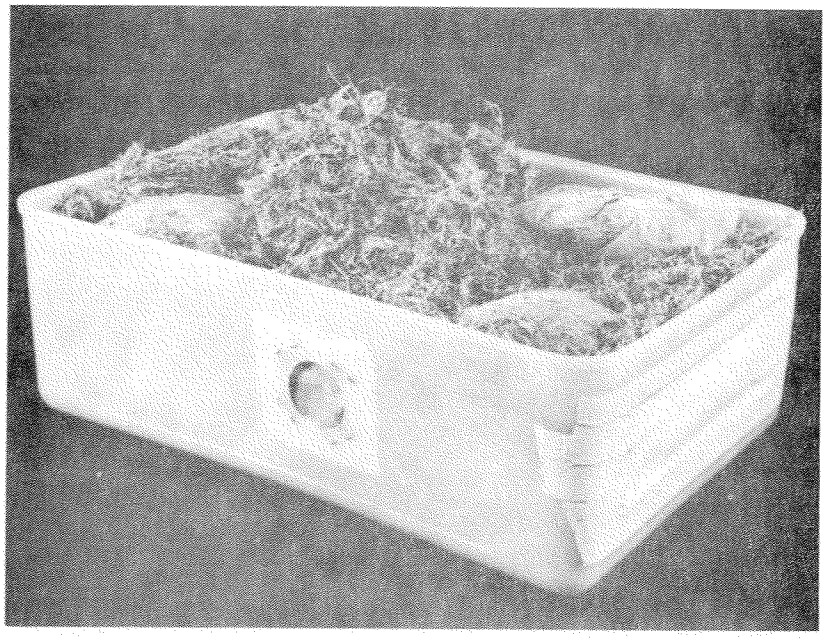

Fig. 6. Packages of cocoons layered in moss in plastic container for cold storage.

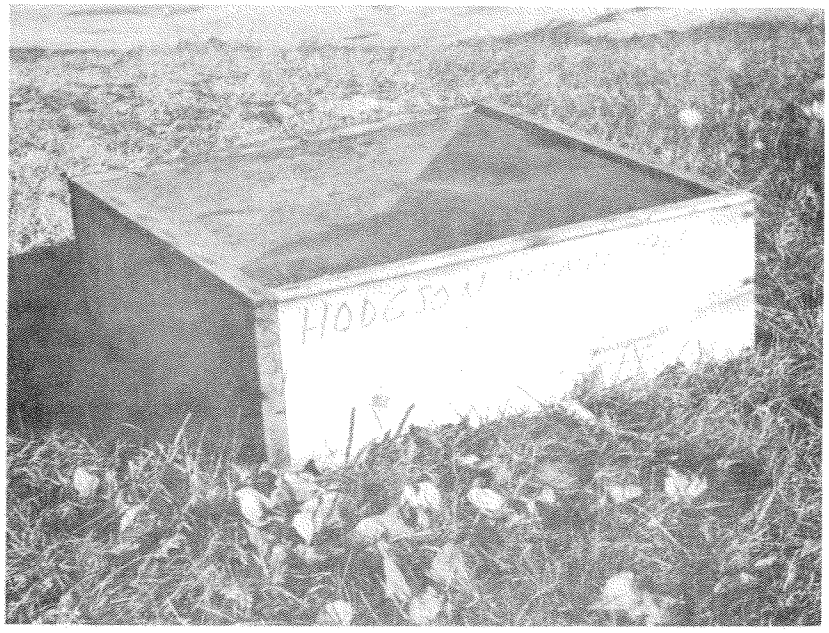

Fig. 7. Screened wooden container for outdoor winter storage of cocoons.

II SEASONAL LABORATORY AND INSECTARY REARINGS

Cocoon Storage, Incubation and Adult Emergence

After the larvae have formed cocoons they should be stored for a prolonged period at a low temperature prior to incubation and subsequent development. As mentioned in the foregoing section this can be achieved by storage in a suitable outdoor location or by artificial refrigeration. Cocoons should be subjected to about the same temperatures they would encounter under natural conditions. After formation do not hold them at normal room or outdoor air temperatures for more than 7 to 10 days. If they are to be stored under artificial refrigeration, they should be held at 10° to 15°C for a period of 3 to 5 weeks and then transferred to the holding temperature, 2°C. For outdoor storage they should be placed in a screened box (Fig. 7) to protect them from mammalian predators and buried beneath a foot or more of moist sphagnum moss in a shaded location not subject to flooding.

Removal of the cocoons from cold storage for incubation should be timed to synchronize the beginning of adult emergence with the development of the terminal shoots of larch required for oviposition. In southern and central Manitoba and Saskatchewan, the shoots are usually sufficiently developed by early June and the cocoons should be removed from cold storage during the latter half of May. Field emergence of adults extends over a period of 2 to 3 months (Fig. 8A) and even at uniform incubation temperatures, emergence is normally prolonged (Fig. 8B). If a more or less constant supply of adults is required throughout the summer, groups of cocoons can be removed from storage at weekly intervals between mid-May and mid-July.

After removal from storage, examine the cocoons and discard all that obviously contain dead larvae (see previous section). Several species of fungi infect the larch sawfly (MacLeod and Heimpel 1955; Smirnoff 1968) and cocoons with fructifications (Fig. 9) should be discarded.

For incubation, transfer the sound cocoons to clear plastic petri dishes containing a layer of moist sphagnum moss. Plastic petri dishes 50 mm in diameter and 9 mm deep will accommodate 10 cocoons and larger dishes, 70 mm in diameter and 19 mm deep will hold 25 (Fig. 10). The dish lids fit quite tightly and the moss will generally remain moist until the completion of incubation if properly handled.

The cocoons should be incubated at 10° to 15°C for post-diapause development. Higher temperatures are very detrimental and will result in appreciable mortality (Heron unpublished). In the field, suitable conditions can be provided in an insulated box buried in a sphagnum bog. An old metal icebox or refrigerator chassis is suitable for this purpose.

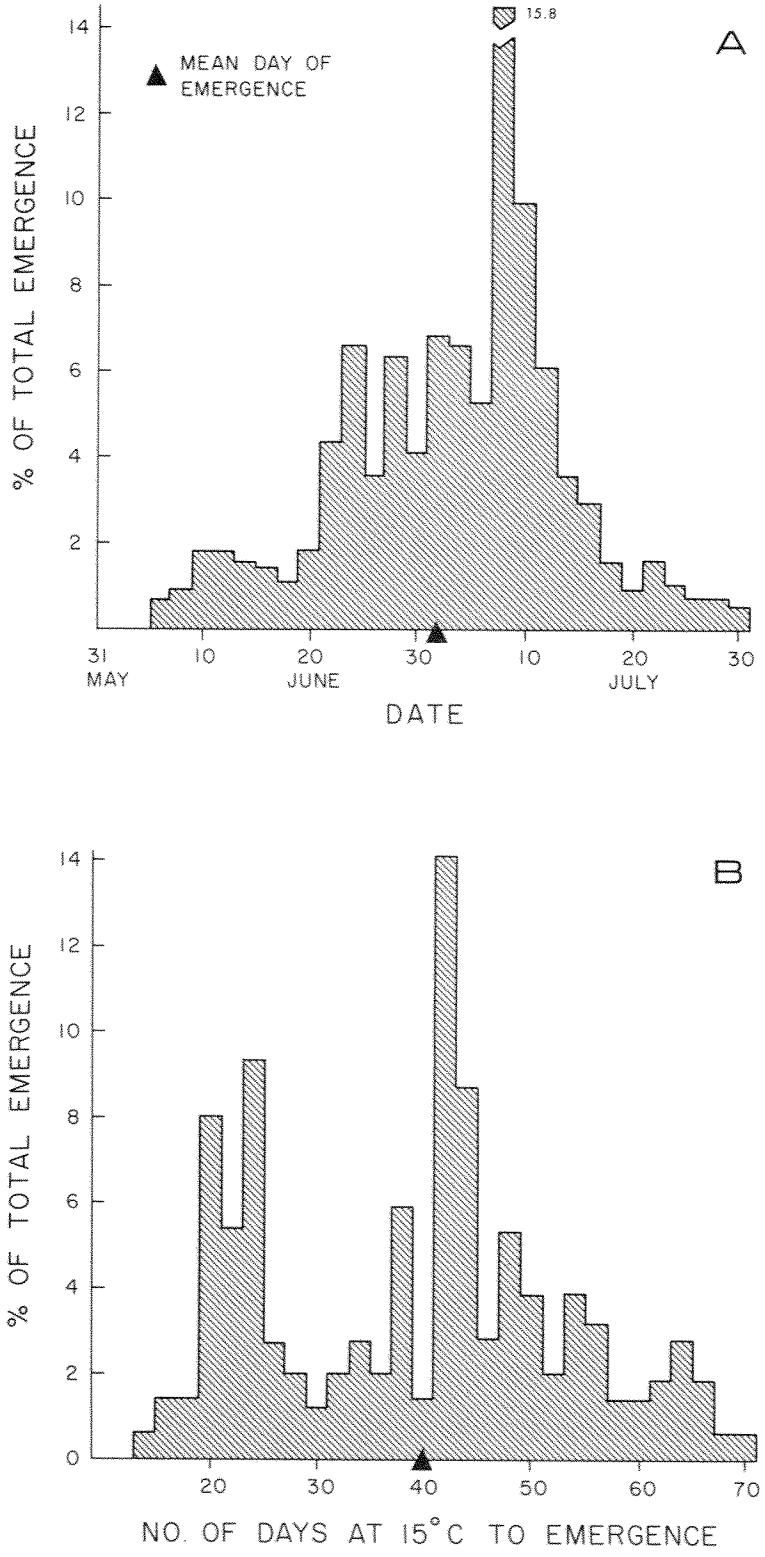

Fig. 8. (A) Frequency distribution of larch sawfly adult emergence in a field population in central Saskatchewan in 1953

(B) Frequency distribution of larch sawfly adult emergence from cocoons incubated at 15°C commencing May 6

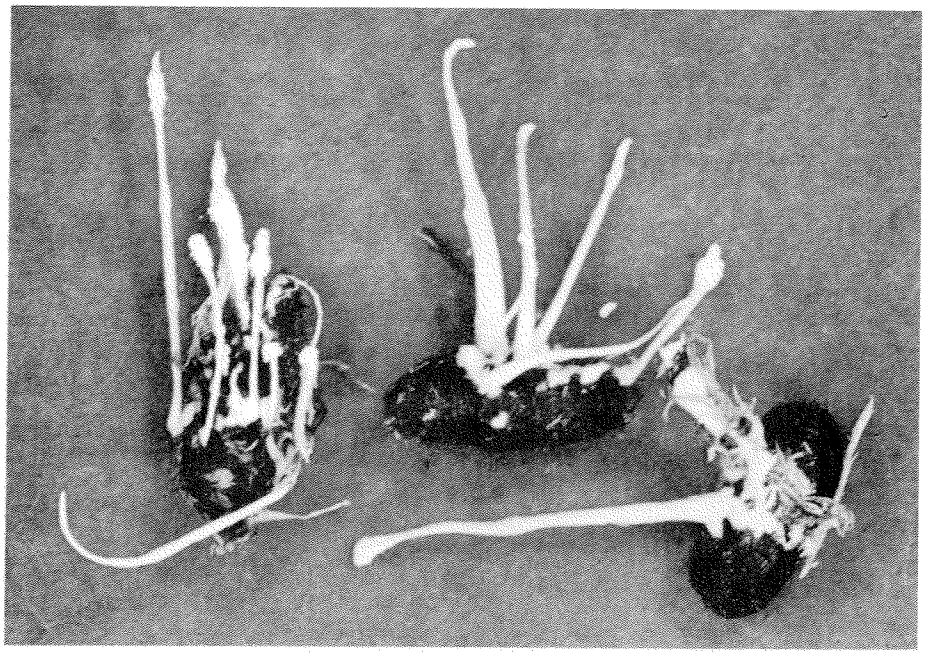

Fig. 9. Larch sawfly cocoons with fructifications of Spicaria farinosa

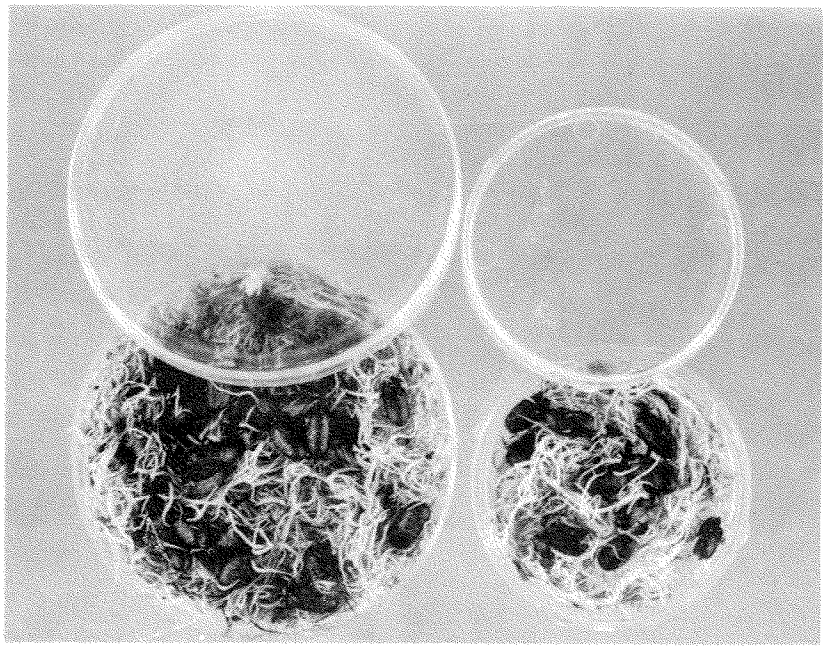

Fig. 10. Plastic petri dishes with cocoons on sphagnum moss for incubation. Left: 25 cocoons in 70 mm X 19 mm petri dish. Right: 10 cocoons in 50 mm X 9 mm petri dish

Examine the cocoons at weekly intervals until the first emergence is noted and thereafter remove the adults daily if time permits. The adults are not very active when recently removed from the incubation temperature and so are easily handled. After exposure to room temperature they become increasingly active and then will fly readily. If the adults are to be kept for a day or more prior to being allowed to oviposit they should be held at 10° to 15°C to minimize mortality. A screen sleeve cage is suitable for holding groups of adults. Water for drinking, sprinkled on the screen, may help increase longevity but adults should not be held for longer than a week after emergence.

Oviposition

The larch sawfly reproduces by parthenogenesis. Males normally constitute only about 2% of the population and although they produce active sperm and may mate it is not known whether fertilization of eggs occurs. In any case, unmated females will oviposit readily shortly after emergence if conditions are suitable. Successful egg laying is easily obtained in insectary and laboratory rearings.

Polyethylene plastic cages of the type originally described by Ives (1962) have proven very satisfactory for egg laying and subsequent larval rearing. These cages are illustrated and described in Appendix II.

The tamarack foliage used in the cages should be fresh. The branch stems should be kept in water from the time the branches are removed from the tree. If fresh foliage cannot be collected daily a stock should be stored at 2° to 5°C and removed as required. The supply should be renewed at intervals of three days or less.

Every cage should be supplied with one or two twigs each with 6 or more developing terminal shoots. Examine the foliage carefully before placing it in a cage. There are a number of insects and spiders found on tamarack foliage that prey on larch sawfly eggs and larvae (Ives 1967). In addition, several sawfly and lepidopterous larvae and aphids feed on the foliage. All these should be removed. Foliage infested with the spruce spider mite, Oligonychus ununguis (Jacobi), is unsuitable. The presence of these mites is usually easily detected because they cause the foliage to assume a grey-green appearance.

A temperature of 21°C or slightly higher is suitable for oviposition and larval rearing. If a room with humidity control is available the relative humidity should be maintained at approximately 55%. The rearing room or insectary should be well illuminated but the cages should be shaded from direct sunlight. Under artificial lighting conditions a photoperiod of 15 hours, from 0700 hr to 2200 hr, has been used routinely with good results.

One to three adult females can be released in each oviposition cage. Some adults will begin to oviposit immediately but egg-laying may extend over several days. At 21°C the eggs will hatch 6 to 8 days after being laid. The presence of the first-instar larvae can be detected by the fine frass that accumulates on the floor of the cage. The newly-hatched larvae should be left undisturbed in the cage for rearing.

Larval Rearing

The cages used for egg laying purposes are also used for larval rearing. After the eggs have hatched, the cage is left undisturbed except to add water to the reservoir occasionally and to examine the foliage every second or third day to determine whether it is wilting or drying. Normally the foliage should not need changing until about one week after the eggs hatch.

Transfer of larvae to fresh foliage should be done by cutting off the portions of the twig on which the larvae are feeding and laying these on the fresh foliage. This will minimize mortality due to handling.

The larch sawfly has 5 larval instars. . Larval developmental rate varies with temperature. The mean duration of the feeding stages at insectary temperatures in southern Manitoba is 20 days (Heron 1951). The average duration of the first and second stadia combined is 5 days, the third and fourth stadia average 3 days each and the fifth stadium averages 9 days. At a constant temperature of 21°C the larvae complete feeding about 12 days after hatching. Over 80% of the foliage consumption occurs during the final stadium.

The foliage, if it was originally fresh and in good condition, should not need renewing more than once or twice prior to the last larval stadium. Frequent changes of foliage are necessary during the last larval stadium because of the increased rate of larval feeding.

Very little larval mortality should occur if foliage changes are made when necessary and the cage is kept free of spiders and other predators. Losses due to disease are normally low and can be held to a minimum by thoroughly cleaning the cages after each rearing is completed. Wash in warm water with detergent, rinse in tap water, treat with a solution of 1 part chlorine bleach ("Javex") to 2 parts tap water for 10 minutes and rinse in tap water.

Cocoon Formation

When larch sawfly larvae complete feeding they drop from the foliage to seek a location for cocoon spinning. These mature larvae should be promptly transferred to a container supplied with a suitable medium for cocoon formation. A plastic refrigerator tray fitted with a screened lid (Fig. 11) filled to a depth of 3 inches with clean, moist sphagnum moss is suitable. Lay a few tamarack twigs on the surface of the moss and place the mature larvae on the foliage. The moss should be left undisturbed for a week after transfer of the larvae. If the moss begins to dry it should be moistened with a fine mist spray.

The foregoing procedure requires frequent examination of the rearing cage to remove the mature larvae. If this is not feasible an alternative method is to place a layer of moist moss in the bottom of the larval rearing cage to provide a medium for cocoon formation. The moss must be kept moist otherwise the cocoons that are formed will be abnormal and survival will be poor.

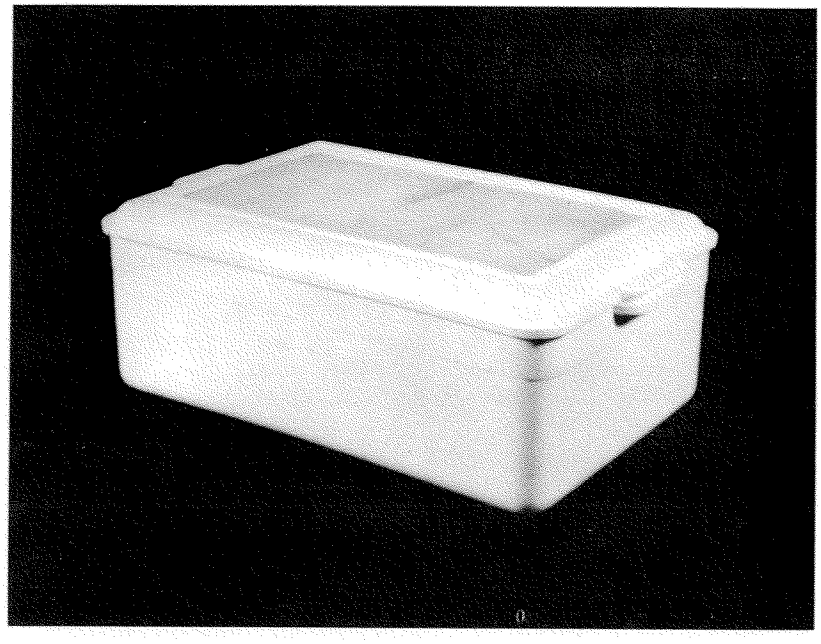

Fig. 11. Plastic container with screened lid for holding larch sawfly larvae during cocoon formation.

A week or so after the larvae have dropped to form cocoons, the moss should be examined and the cocoons removed. The cocoons should then be handled and stored as described earlier.

III EXTRA-SEASONAL REARING METHODS

For many types of experiments it is desirable to have insects available for use throughout the year. In Canada a major difficulty during the winter months is the unavailability of the food of most phytophagous species. This problem has been overcome for several insects by the use of artificial diets (House 1967). The specialized feeding behavior of sawflies however presents some as yet unresolved problems that prevent the use of such diets. Another problem with the larch sawfly is its requirement for developing vegetative shoots in which to lay its eggs. Heimpel (1957) gave a brief account of methods for winter rearing of the larch sawfly utilizing frozen tamarack foliage for larval feeding and tamarack shoots from saplings grown in the greenhouse, for oviposition. The rearing methods presently used for winter rearing of the larch sawfly in this laboratory are basically the same as those described by Heimpel but incorporate some modifications of handling and procedure that were found necessary to increase rearing success.

Collection and Storage of Foliage for Larval Rearing

There are a number of factors to consider in collecting foliage for freezing and storage. Obviously only healthy foliage from vigorous trees should be used for this purpose. Open-growing tamarack, in particular, are frequently infested with the spruce spider mite, Oligonychus ununguis (Jacobi) and foliage from such trees is unsuitable.

Heimpel (loc. cit.) suggested making foliage collections in the fall. Our experience indicates that mid-summer, during the period when larval populations are at their peak in the field or shortly following this (i.e., mid-July to mid-August in southern and central Manitoba and Saskatchewan) is the best time for foliage collection. The nutritional suitability of the foliage seems to deteriorate appreciably even during the initial stages of senescence. Larvae reared on fresh-appearing green needles collected late in the season have poor growth and survival. High mortality of larvae fed on foliage collected in mid-summer has also occurred occasionally. There is no conclusive evidence as to the cause of this mortality but it was apparently not due to pathogenic organisms. Since it may have been due to the nutritionally unsuitability of the foliage, it is recommended that foliage be collected from varied locations and that the sites be indicated on a label enclosed with the frozen material. It is probably inadvisable to make collections from trees growing on either excessively wet or dry sites.

The time between collecting the foliage and freezing it should be kept as short as is practical. During transportation from the field the cut ends of the branches should be kept in water to prevent wilting. If the foliage is not frozen and stored on the day of collection, the branches should be held in water overnight at 2° to 5°C and processed the following day. The twigs bearing the foliage fascicles should be cut into sections about 1 1/2 inches long and packed tightly into 2 lb. capacity polyethylene bags. The bags should be tightly sealed and stored in a deep freezer at -20°C until required.

Heimpel (1957) recommended treating the foliage with a mold inhibitor such as Mycoban (0.1% aqueous solution). We have found that this is not necessary as the food is changed too frequently for mold to be an important problem. In fact, soaking the foliage in the Mycoban solution may be detrimental because it probably affects the moisture balance of the foliage and excessive moisture has been found to be deleterious to larval survival. The frozen foliage should not be used after more than 10 months storage.

Maintenance of Tamarack in the Greenhouse for Shoot Production

Larch sawfly females normally oviposit only in developing vegetative shoots of larches, Larix spp. Eggs dissected from larch sawfly adults have been incubated to the point of larval eclosion with limited success but at least until this technique is perfected it will be necessary to have larch shoots available in order to rear the larch sawfly.

Greenhouse conditions and facilities: In greenhouses in northern latitudes shoot growth by tamarack during the winter months is stimulated by supplementing the natural light with artificial illumination. Banks of 8 ft, 215 watt, "cool white" reflector-type fluorescent tubes, whose height above the benches can be adjusted, give good results. The lighting circuit should include a time switch to control the periods of artificial illumination. Studies by Vaartaja (1957, 1959) have established that the growth of tamarack seedlings is strongly influenced by photoperiod. Under his "long day" conditions[1], tamarack seedlings were stimulated to grow continuously whereas under his "short day" conditions[2] the seedlings became dormant after the cotyledons and a few small juvenile needles developed. Shoot growth of transplanted saplings is also stimulated by the long-day lighting regimen.

To provide proper temperature conditions throughout the year the greenhouse should be provided with a thermostatically-controlled heat source, automatic louvres, adjustable shades and a cooling device such as an "Arctic cooler" for use during the summer months. If possible, the temperatures in the greenhouse should be maintained at about 21°C during the day and 15°C at night. Every precaution should be taken to prevent the temperature exceeding 32°C.

Transferring larch saplings from the field to the greenhouse: Tamarack saplings about 4 to 5 feet in height are suitable for transferring from the field to the greenhouse to provide shoots for adult oviposition. The saplings should be dug up in mid-October or later, after the needles have been cast, and planted in wooden boxes measuring about 2 ft x 2 ft x 16 inches. The bottoms of the boxes should be provided with drainage holes and covered to a depth

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1 Natural light was supplemented by continuous fluorescent light of 100 ft-C for one month after sowing and thereafter, 500 ft-C fluorescent lighting was provided for 13 hr during the day and 1 hr at midnight.

2 The natural photoperiod of approximately 14 hr was not modified during the first experimental month. Later natural illumination was supplemented with fluorescent light of 500 ft-C for 14 hr during the day.

of about 2 inches with coarse gravel. A soil mixture consisting of 2 parts compost, 2 parts top soil, 1 part peat and 1 part sand is suitable for planting. The sand should be of a silicaceous nature and not derived from limestone, as a high lime condition is unfavorable for growth. After planting, the saplings should be well watered and held outside until early November before transfer to the greenhouse.

Under the prescribed lighting and temperature conditions the first shoots for oviposition should be available in about 8 to 10 weeks after transfer to the greenhouse.

Growing larch from seed: Tamarack and other larch species can be grown from seed in the greenhouse to provide saplings for shoot production. When cultured in this way the growth of the saplings can be controlled by top pruning to give a bushy form that will provide numerous terminal shoots.

The seed should be treated with a fungicide such as "Captan" to control "damping-off". Good germination has been obtained by sowing the seeds in vermiculite contained in shallow clay pans and kept moist by judicious watering. Shortly after the sprouted seeds have shed the seed coat they can be transferred to flats containing the soil mixture described above. When they reach a height of 3 inches they can be transplanted to 6 inch diameter pots having good drainage. Once the saplings become pot-bound they should be transferred to larger containers. Three saplings can be grown in a box measuring 30 inches x 30 inches x 16 inches deep. When the photoperiod is controlled as previously described, growth will be continuous from time of germination. About 12 to 18 months after sowing the seed the saplings will have attained a suitable size and vigor to permit the harvesting of shoots. If pruning is not too severe they should remain vigorous for about three years before it is necessary to discard them.

By periodic planting it is possible to maintain a continuous supply of suitable saplings to provide shoots for oviposition.

Fertilizer treatments: Regular application of fertilizer is required to maintain healthy larch saplings with vigorous growth and shoot production when grown continuously under greenhouse conditions. Satisfactory results have been obtained in growing tamarack by monthly treatment with a fertilizer solution of the following composition:

21-0-0 (N:P:K)	-	40 grams
RX-15	-	40 grams
Iron chelate	-	2 tablespoons
Water	-	5 gals.

For the 21-0-0 component, any commercial fertilizer with the nitrogen in the form of ammonium nitrate (NH_4NO_3) is suitable. The "RX-15" is a proprietary product manufactured by Garden Research Laboratories Ltd., Toronto, Canada. This material has an N:P:K ratio of 15:30:15 and in addition contains sources of the elements magnesium, manganese, iron, copper, zinc, boron and molybdenum. The iron chelate used is "Sequestrene 330 Fe Chelate" produced by Geigy Agricultural Chemicals, Saw Mill Road, Ardaley, N.Y. The soil in the planting containers is soaked with this solution at each treatment.

Control of mites and aphids: Two types of arthropods commonly occur as pests on tamarack grown in the greenhouse. These are aphids of the genus Cinara and the spruce spider mite. Both the aphids and the mite overwinter in the egg stage and may be brought in to the greenhouse when the stock is transplanted from the field. Another source of infestation is from other conifers being grown in the greenhouse in the same or neighboring compartments. The aphids[3] are specific to tamarack but several species of conifers are natural hosts for the spruce spider mite. It is advisable to grow the tamarack in a separate compartment if possible and to keep the door to the compartment closed to minimize the opportunity for the introduction of these pests.

Both the mite and the aphids can be easily controlled by spraying with 50% malathion emulsifiable concentrate at the rate of 2 tablespoons in 2 1/2 gal of water[4]. A second spray should be applied about one week after the first. When spraying in the greenhouse it is advisable to wear a suitable respirator. Shoots from sprayed saplings should not be used for oviposition purposes until 2 weeks after the second application as this insecticide has quite a long residual toxicity for the larch sawfly when applied indoors.

Rearing and Handling of the Larch Sawfly

Cocoon storage: The importance of controlling moisture during cocoon storage has already been mentioned but it cannot be over-emphasized. Too much moisture at this stage is as detrimental to the larva in the cocoon as too little. Clean sphagnum moss that has been soaked in water and then wrung tightly in the hands until no water drips from it, will have a satisfactory content of moisture.

The cocoons can be stored in packets of moss wrapped in dacron marquisette and layered in moss in sealed plastic containers as described earlier or they can be placed on moss in a small, closed container such as a jelly jar.

Cocoons formed at rearing room or insectary temperatures should be transferred to 10°C within a week after they have been spun and held at this temperature for 3 or 4 weeks before being placed at 2°C for cold treatment and storage. When the cocoons are transferred to a lower temperature, moisture will condense on the walls of the container if it is tightly closed. Opportunity should be provided for the evaporation of this moisture and then the containers should be again tightly closed.

Cocoons stored in slightly moist clean moss will retain the original light brown color of newly-formed cocoons throughout the period of storage. If the moss is too wet the cocoons soon become darkened and many of the cocoons will eventually become turgid. The larvae in turgid cocoons will be dead.

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[3] Cinara laricifex (Fitch) and C. spiculosa Bradley both occur on twigs and small branches and produce copious amounts of honeydew.

[4] It is advisable to wear rubber gloves when handling malathion and to take the precautions indicated on the manufacturer's label.

Cocoons should be held at 2°C for 3 months or longer before being transferred to a higher temperature to complete post-diapause development. Development can be initiated after shorter periods of cold treatment but there will likely be increased mortality and the incubation time will be prolonged so that little advantage will be gained.

Cocoons can be held at 2°C for periods in excess of 12 months with little loss in viability. After prolonged storage an increasing proportion of the cocoons will contain larvae that have undergone some post-diapause development and have transformed to pronymphs or pupae. When storage at 2°C is extended beyond 18 months the numbers of viable adults that emerge decline appreciably.

Cocoon incubation: The optimum temperature for post-diapause development is in the range 10° to 15°C. Survival is generally better in the lower part of this range but the development rate is slower. The duration of the pre-emergence period is influenced by the length of the cold storage period as is shown by the following data for material that was placed in cold storage in September or early October and then incubated at 15°C after varying periods of storage. The data on numbers of adults have no significance with respect to survival as the numbers of cocoons in each treatment varied considerably.

Duration of storage at 2°C (days)	No. days at 15°C to emergence		No. of adults
	Range	Mode	
176	25-106	60	472
212	14-72	45	148
350 to 400	11-17	16	75
400 to 500	10-21	15	25

As the period of cold storage was extended the range and mode of the incubation period decreased. After about one year at 2°C most of the larvae had developed to the pronymph stage and very little decrease in the required incubation period occurred after that time.

Oviposition: Larch sawfly adults oviposit in the soft cortical tissues of developing larch shoots. To the present no substitute medium for oviposition or fully successful technique for incubating eggs dissected from adults has been developed. For extra-seasonal rearing it is therefore necessary to provide the adults with larch shoots grown in the greenhouse. Under the conditions already described it is possible to obtain these throughout the year although there is a period during December and January when shoot production is considerably reduced. Twigs 5 or 6 inches in length with 1 1/2 inches or more succulent terminal growth are suitable. The shoots developing under greenhouse conditions have a different form than those grown under natural conditions in the field. Because of their length, the needles on the greenhouse shoots tend to interfere with the oviposition behavior of the adults. This problem is overcome by trimming the needles off close to their bases.

Clear polystyrene-type plastic cages, of the type illustrated and described in Appendix II, are used for oviposition. Two twigs are placed in each cage along with two females. The cages should be examined daily to determine the date of oviposition. At 21°C eggs will hatch 6 or 7 days after being laid. When the larvae hatch they will crawl onto the needles of the shoot or those at its base and will begin to feed. If they are not transferred from the shoot to frozen foliage within a few hours after hatching they will soon die. For this reason it is necessary to make frequent examinations of the shoots once hatching becomes imminent. Eggs with well developed embryos can be detected by their darker appearance.

Larval rearing: Small (50 mm diam x 9 mm deep) disposable plastic petri dishes are suitable for rearing larvae. The fresh frozen foliage is removed from the deep freeze and placed on absorbent paper tissue for 15 or 20 minutes to thaw. Surface moisture is removed by blotting the foliage with the paper. A circle of blotting paper is placed in the bottom of the petri dish and sufficient water is added to moisten the paper but not enough to cause puddling. Two fascicles of the freshly thawed foliage are placed in the dish and about 10 larvae are transferred onto it. Larvae are transferred by removing the needles on which they are crawling with forceps. If necessary they can be manipulated using a fine camel hair brush but this is not recommended because of the susceptibility of the larvae to injury.

The petri dishes containing the larvae are placed in a desiccator jar charged with a saturated solution of NaCl to maintain the humidity at approximately 75% relative humidity at the rearing room temperature (21°C). The greatest hazard to rearing at this stage is moisture that may occur on the surface of the foliage or the petri dish. The small larvae become entrapped in small drops of water, swell and die.

After two days the larvae should be transferred to fresh foliage. Thereafter the foliage should be changed daily because progressive deterioration of the foliage has an adverse effect on the larvae if they are allowed to feed on foliage that has been thawed for more than two days. Larval growth is impeded and there is a loss of vigor often accompanied by discoloration of the larvae. Most of the larvae so affected, regardless of stage of development, will eventually die. With daily changes of food however, successful rearing and good survival usually results if the initial hazards of larval establishment have been overcome.

After the larvae molt to the fifth instar it is preferable to transfer them to slightly larger containers. Circular clear plastic boxes measuring 70 mm diam. x 19 mm deep are very suitable for this purpose.

When larvae are ready to cocoon they stop feeding and lie in the bottom of the dish. When touched or disturbed they twist about vigorously. These larvae should be provided with clean moist moss in which to spin their cocoons and left undisturbed at room temperature for a week to ten days. After that they can be removed from the moss and handled as described earlier for pre-storage and storage treatment.

ACKNOWLEDGEMENTS

In the preparation of this report we have drawn extensively on the work of our colleagues in larch sawfly research at this laboratory. We are indebted to them also for reviewing the manuscript.

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Fig. 1. Larch sawfly female laying eggs in tamarack terminal shoot.

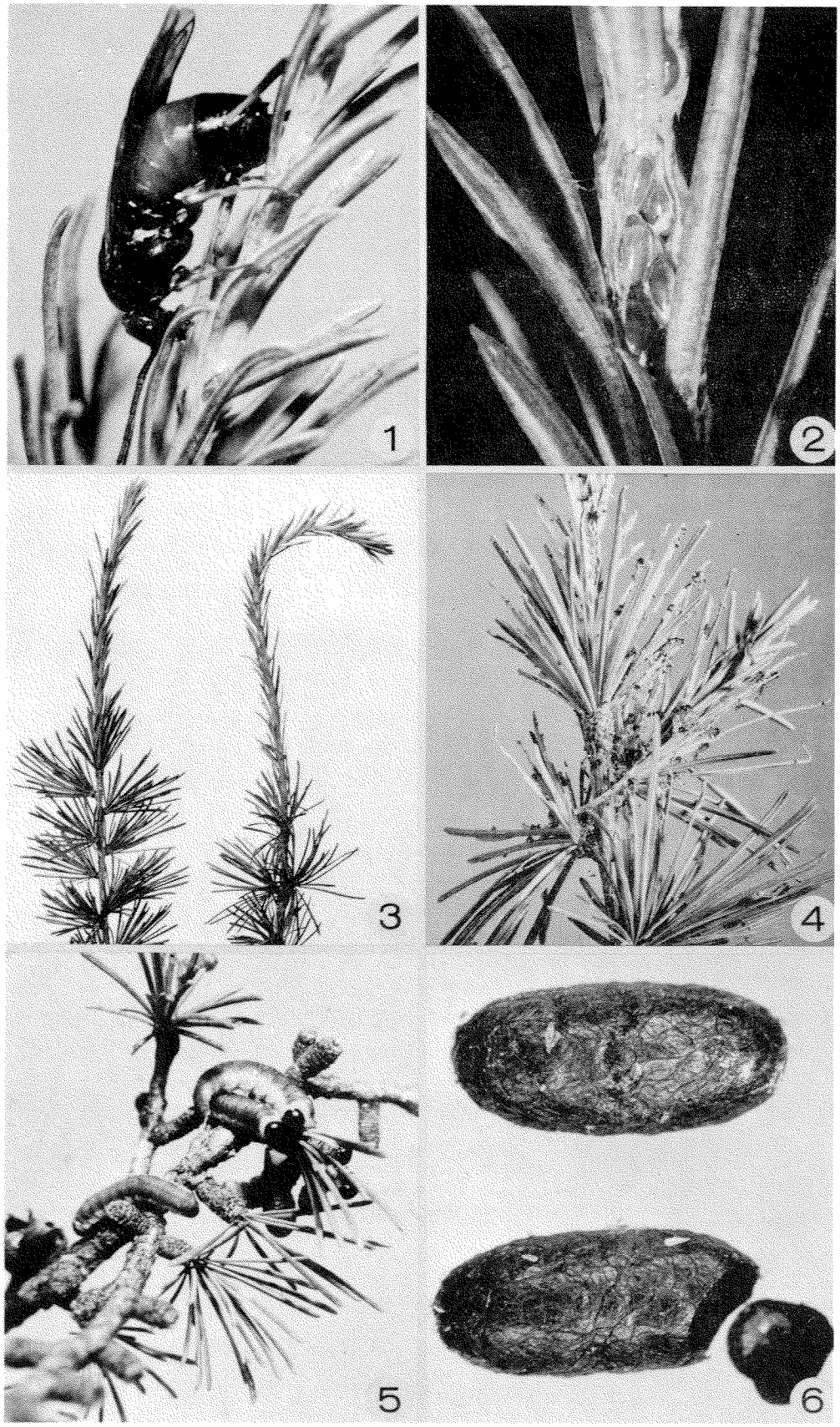
Fig. 2. Swollen eggs in terminal shoot.

Fig. 3. Normal tamarack shoot (left) and curled shoot (right) resulting from oviposition injury.

Fig. 4. First and second-instar larvae feeding on needle fascicles.

Fig. 5. Fifth-instar larvae feeding on tamarack.

Fig. 6. Cocoons before (top) and after (bottom) adult emergence.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APPENDIX II

DESCRIPTION AND FABRICATION OF CAGES

1. Cages for Mass Larval Rearing

These rearing cages (Fig. 1) measure 18 inches on each side. They consist of a 3/4 inch by 1 3/4 inch spruce or pine frame top and bottom, four side pieces as uprights with four 3/4 inch by 1 inch horizontal side stiffeners which also serve as a stapling medium for the screen and plastic sides. Glides are nailed to the top frame. The 3/4 inch by 7/8 inch glides are rabetted 1/8 inch by 1/4 inch to accommodate a piece of double diamond glass measuring 16 inches by 18 inches. Both the frame top and base can be assembled with 1/2 inch by 5 corrugations, corrugated fasteners. The base is then covered with 1/8 inch tempered hardboard. The four sides and frame top are then assembled and the side stiffeners applied 5 inches below the top frame. When applying the glides allow some leeway to prevent binding in damp weather. Heavy 6 mil plastic is then tautly stapled around the lower two thirds. Standard aluminum household screen drawn tightly and stapled around the upper portion completes the side covering. To prevent loss of insects which may crawl between the plastic and wooden pieces, wooden strips 1/8 inch by 3/4 inch should be stapled on all flat surfaces adjacent to the inside edges of the frame. A coat of white semi-gloss paint on the bottom hardboard helps in observing insects.

2. Plastic Cages Used for Oviposition and Larval Rearing

These four units are made from commercially available plastic containers that sell at moderate prices. With minor modifications they can be readily adapted for use as oviposition or larval rearing cages.

(a) Refrigerator tray type cage (Fig. 2a): This cage is used for oviposition and larval rearing. It is made from a polyethylene crisper or refrigerator tray[1]. It has a tight-fitting lid and measures 13 5/6 inches by 8 3/8 inches by 4 5/8 inches.

To provide for ventilation, 1 inch diameter holes are cut in each side, using a hole saw and drill press, and a 1 inch by 6 inch opening is made in the top. These openings are covered with "lumite" saran screening of the type known as "Chicopee natural"[2] in a 32 by 32 mesh. The screening is held in place by waterproof plastic tape placed along each edge on the inside of the cage. An additional 1 inch diameter hole is cut in the centre of the other end of the tray to accommodate the stems of the branches of foliage. A polyethylene stopper (1 inch diameter or #4) with the center cut out is used to clamp a 2 inch square of 1/4 inch thick plastic acetate foam[3] over the hole. The plastic foam provides a snug fit around the branch stems when they are pushed through it.

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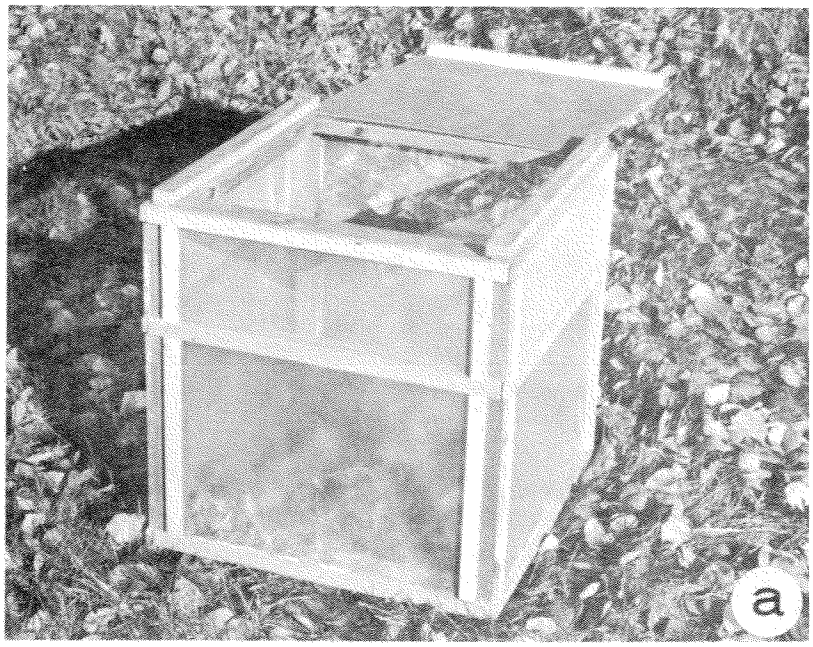
[1] Manufactured by Republic Molding Corporation, Chicago 31, Illinois, U.S.A. Item stock no. 137. A substitute plastic crisper made of opaque plastic that is not quite as satisfactory is available in Canada from Bow Plastics, Granby, Quebec.

[2] Manufactured by Lumite Division, Chicopee Mills Inc., 47 Worth Street, New York 13, N.Y.

[3] Available from most hobby craft supplies in sheets measuring 36 inches by 48 inches.



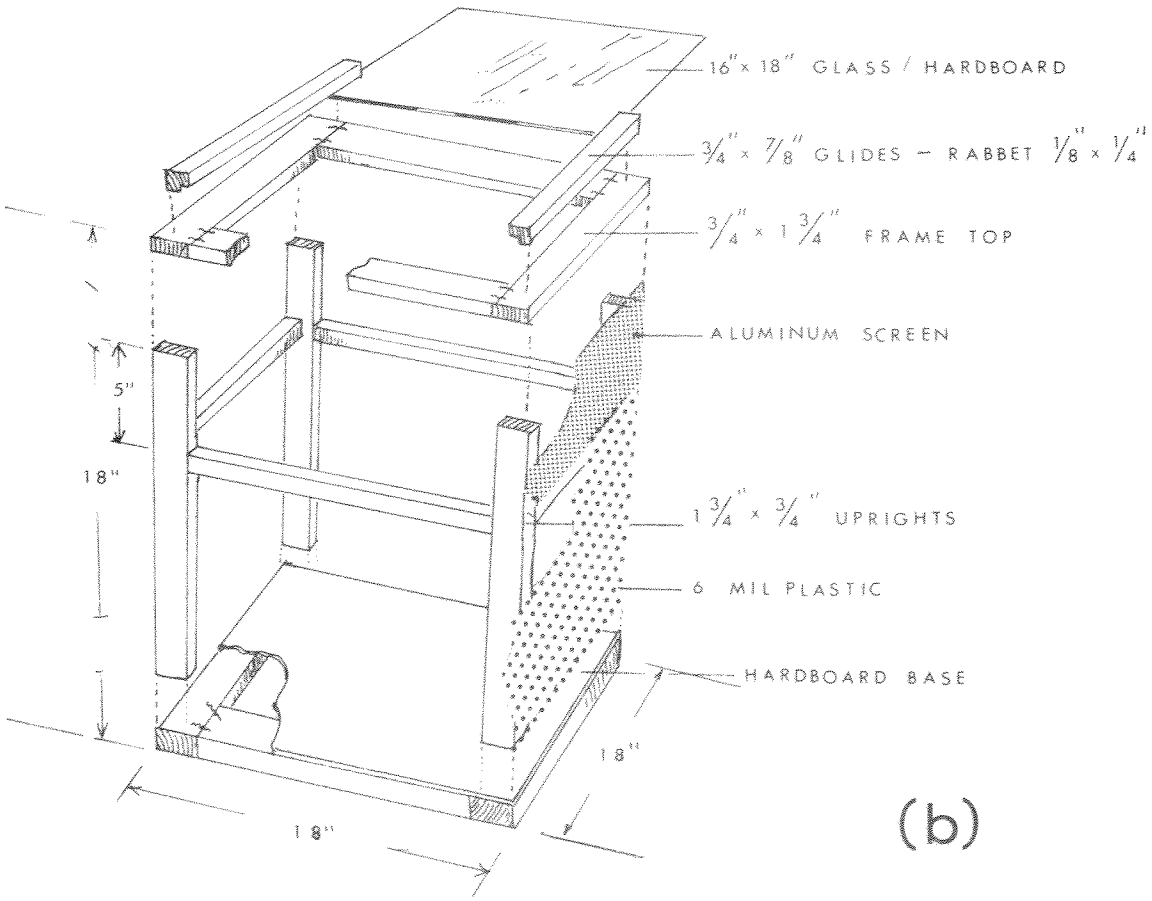


Fig. 1. (a) Cage used for ~~massing~~ rearing of larvae.
(b) Drawing showing details of construction.

The racks to support four refrigerator trays in an upright position (Fig. 2b, 2c) are constructed of 1/2 inch plywood and 1/4 inch hardboard. The plywood side pieces (7 1/4 inches by 18 inches) are held at the ends by hardboard (7 1/4 inches by 10 5/8 inches). The divider or base is made of plywood (9 5/8 inches by 18 inches) and is nailed 3 inches below the top edge. A separator strip of plywood (3 inches by 18 inches) further divides the upper portion. Four 3/4 inch holes are drilled in the base to accommodate the branches that protrude into the water-filled jars below. The lids of the jars are attached to the underside of the base by screws.

(b) Bread container-type cage (Fig. 3): This polyethylene cage is similar to the one described above but is slightly smaller. It has a tight-fitting lid and measures 14 inches by 5 7/8 inches by 5 3/16 inches[4]. To provide ventilation, holes 2 1/2 inches in diameter are cut in each side. These are covered with 32 by 32 mesh lumite plastic screen. As before a 1 inch diameter hole in the bottom is provided to accommodate the branches. The branch stems are inserted in a jelly jar filled with water and the whole assembly is placed on a shelf in the rearing room (Fig. 4).

(c) Jumbo polyethylene juice container-type cage: This cage is made from a juice container measuring 4 1/8 inches in diameter and 7 1/2 inches in height[5] (Fig. 5a). To provide ventilation a 2 inch by 2 inch hole is cut in the wall and covered with 32 by 32 mesh lumite plastic screen. The centre of the pouring spout lid is cut out leaving an outer retaining ring. This then serves as a snap to hold in place the piece of plastic foam through which the stems of the branches are inserted. The racks to hold these cages are made of two pieces of 3/4 inch plywood measuring 9 inches by 9 inches and four 1/2 inch diameter dowels (Fig. 5b).

(d) Polystyrene plastic cage for oviposition in extra-seasonal rearings: This cage is made from a clear plastic utility box measuring 5 3/4 inches by 3 1/2 inches by 1 1/4 inches[6]. A hole 5/16 inch in diameter is cut in the centre of each side and one end. A piece of fine mesh dacron marquisette is glued over each of the side holes to provide ventilation. The bottom hole accommodates the stems of the terminal shoots provided for oviposition. The shoots are fitted tightly in the opening by wrapping a small piece of acetate plastic foam around them. This prevents the escape of the insects from the cage. The cages are supported on racks made of 1/2 inch plywood ends measuring 6 inches by 7 inches and four 1/2 inch diameter dowels (Fig. 6).

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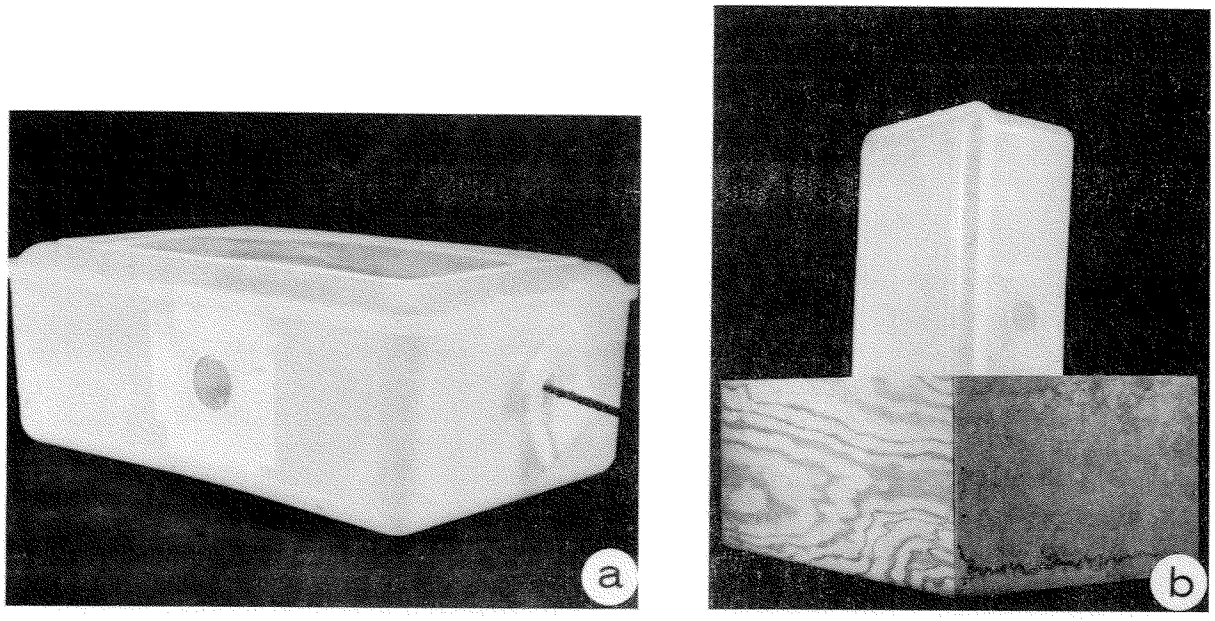
4 Manufactured by Midland Flambeau Ltd., Midland, Ontario.

5 Manufactured by Rogers Plastics Ltd., 1176 Sherbrooke St., West, Montreal, Quebec.

6 Manufactured by Bow Plastics Ltd., Granby, Quebec.



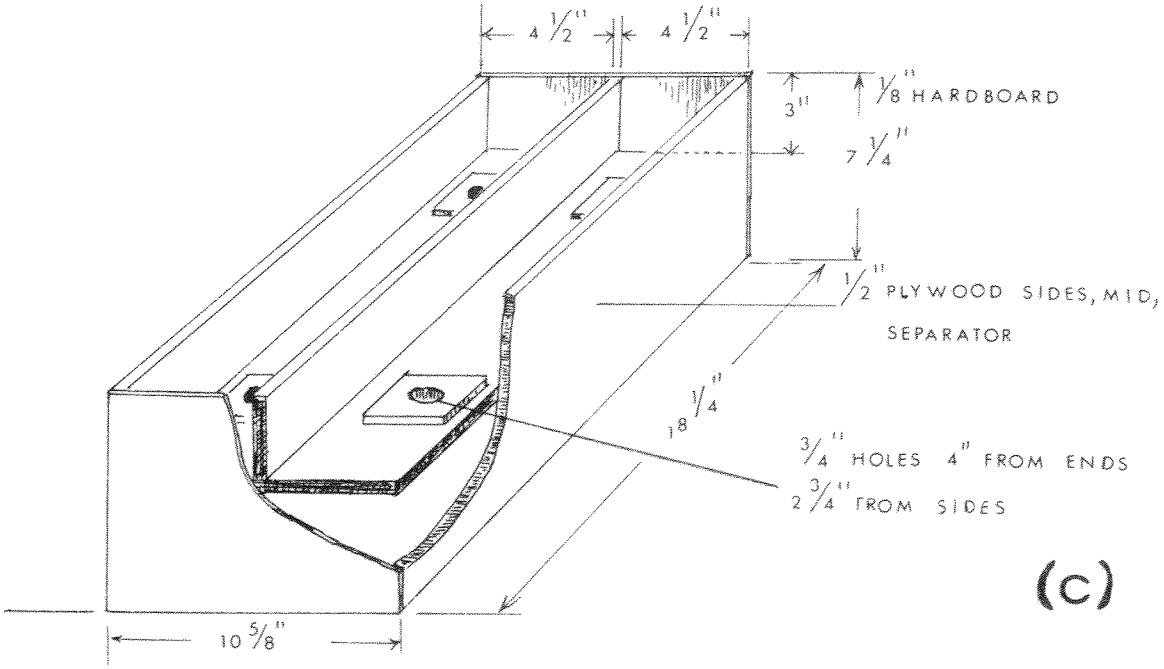


Fig. 2. Polyethylene refrigerator tray-type cage. (a) End view. (b) Supporting rack with one of four cages in position. (c) Drawing showing details of construction of rack.

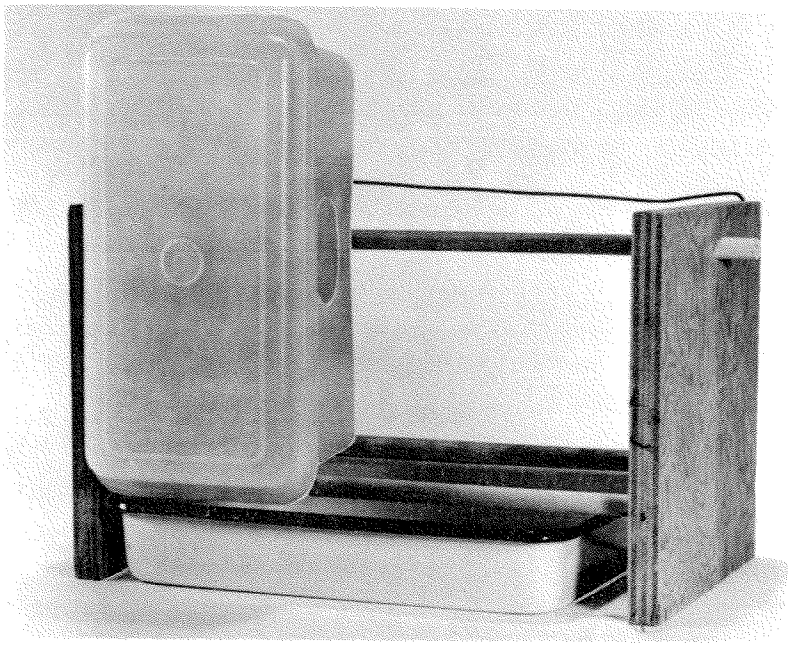


Fig. 3. Polyethylene bread container-type cage for oviposition and larval rearing.

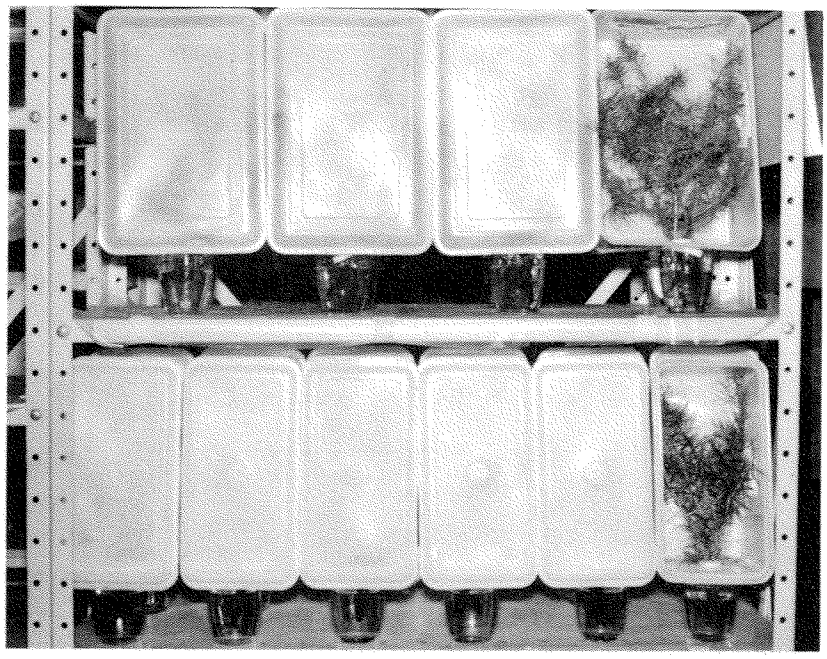
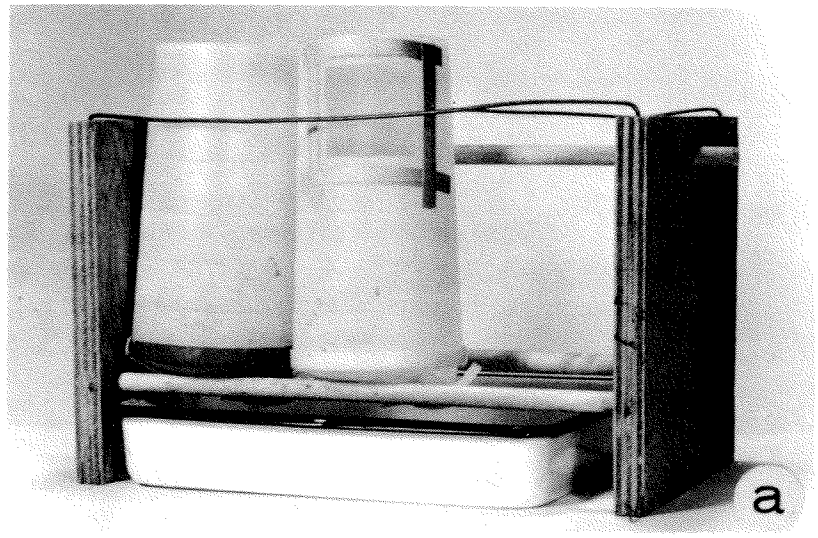


Fig. 4. Refrigerator tray-type cages (top shelf) and bread container-type cages (bottom shelf) on metal shelving in rearing room. One cage of each type open to show interior.



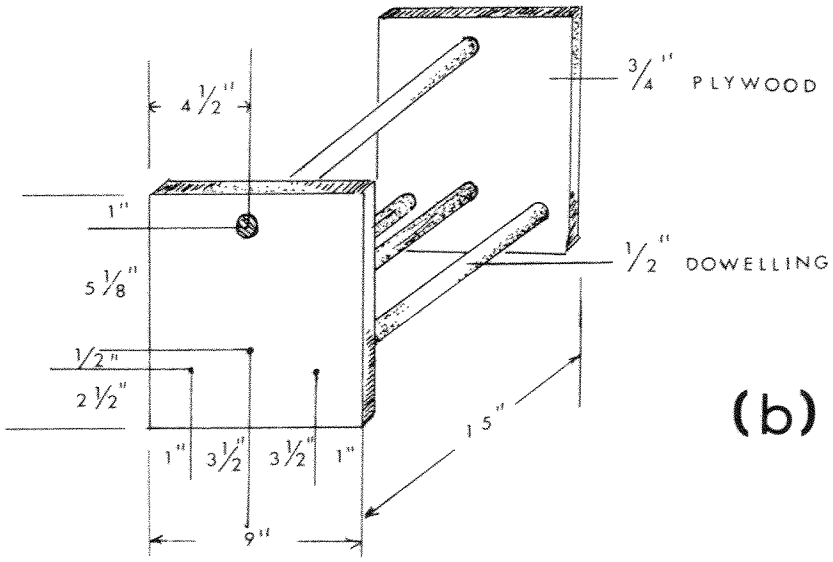
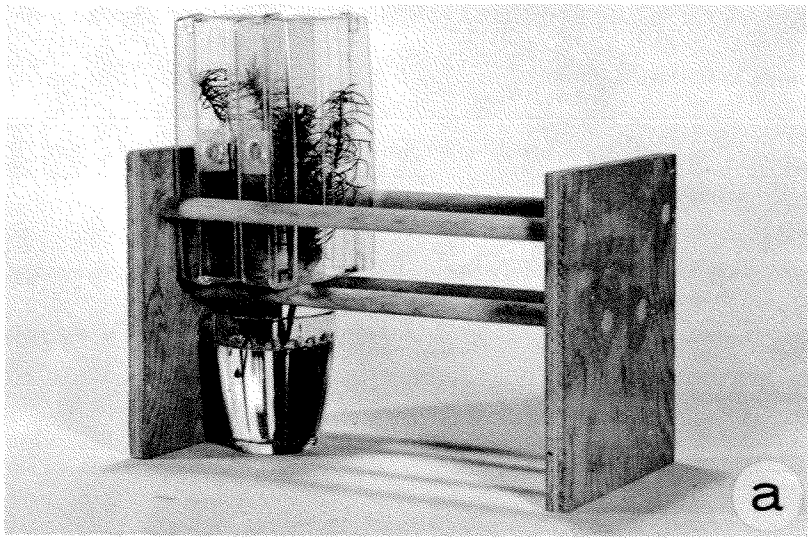


Fig. 5. (a) Polyethylene juice container-type cage on supporting rack. (b) Drawing showing details of construction of rack.



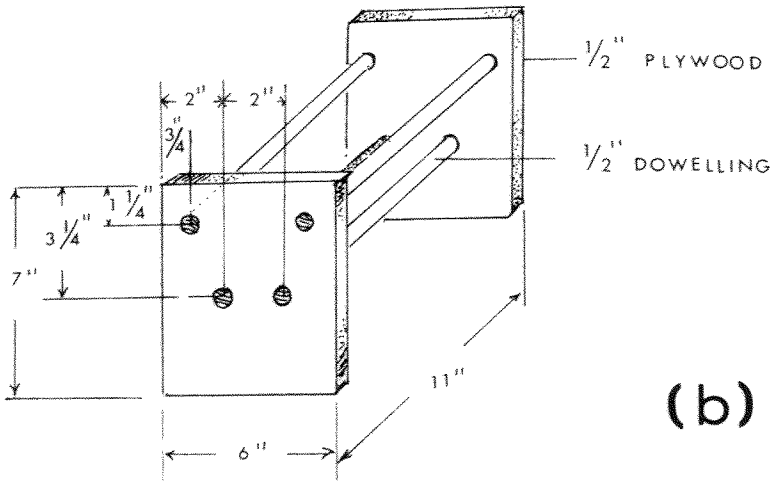


Fig. 6. (a) Polystyrene plastic oviposition cages on supporting rack.
(b) Drawing of construction details of rack.