An improved system for mass-rearing codling moths[1]

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ABSTRACT

Various modifications were made to a system for mass-rearing the codling moth, *Cydia pomonella* (L.), on formulated diets and immature apples to improve production efficiency and to reduce exposure of workers to formaldehyde and moth scales. The modifications included: an improved oviposition cage, an oviposition cabinet, an apparatus to surface-sterilize eggs with formaldehyde fumes, a moth scale removal system in the adult collection room, and disposable adult eclosion containers. This system is suitable for research requiring large numbers of selected stages of the codling moth.

INTRODUCTION

The codling moth, *Cydia pomonella* (L.), a serious pest of several deciduous fruits and walnuts, has been mass-reared at the USDA, ARS research facility at Yakima for over 20 years. In the past, it was reared primarily for use in studies of the sterile insect technique, population movement and suppression, and pheromones (Hamilton and Hathaway 1966). Current programs in postharvest quarantine treatment research require large numbers of selected stages of the codling moth for use in studies to evaluate the efficacy of proposed treatments, such as fumigation, irradiation, and cold or controlled atmosphere storage. For example, in order to have a 99.9% confidence level in quarantine security, 93,616 insects are needed per treatment (Chew and Ouye 1985). Through the years, many changes have been made in codling moth diet (Howell 1967, 1970, 1971, 1972) and rearing procedures (Hathaway 1967, Hathaway *et al.* 1972, Hutt *et al.* 1972) to meet the need for more safe, efficient and cost-effective rearing.

This paper describes further modifications made to the rearing system, particularly to reduce worker exposure to hazardous materials, such as moth scales and formaldehyde, and the current procedures used to mass rear the codling moth at this location.

EGG COLLECTION AND HANDLING

Eggs are obtained using the oviposition cage of Hathaway *et al.* (1972) with one change; the muslin cloth liner in the top portion is replaced with 16-mesh wire screen. The oviposition substrate is either waxed paper or polystyrene pellets (Dow Chemical Co., granulation number 451-27-35). Waxed paper sheets are first crumpled, then flattened, before they are fitted into the bottom of the cages. Moths prefer to oviposit on crease lines in the sheets rather than on flat, smooth surfaces. In the use of pellets, 250 g are placed in each cage. Adults are transferred from collection containers to oviposition cages in a hood located in the adult collection room maintained at about 3°C (see below). Each cage holds 250-300 unsexed moths, which produce about 6000 eggs.

The prepared oviposition cages are held in a plywood cabinet, which was designed to control environmental conditions and remove moth scales (Fig. 1). The cabinet measures 2.9 m wide, 0.6 m deep, and 2.0 m high, and houses a heater, air filters (5W512-D Extended Surface Air Filter 50.8 x 63.5 x 2.5 cm, Dayton Electric Mfg. Co., Chicago, IL), a blower, and humidifiers. Sliding glass doors give access to two sets of five open metal wire shelves, 25 cm apart, each shelf with a 30-watt fluorescent light overhead. Air from the room, which is maintained at 23 ± 3°C, enters the plenum through the blower, passes

Footnote

1. Mention of a proprietary product does not constitute an endorsement by the USDA.

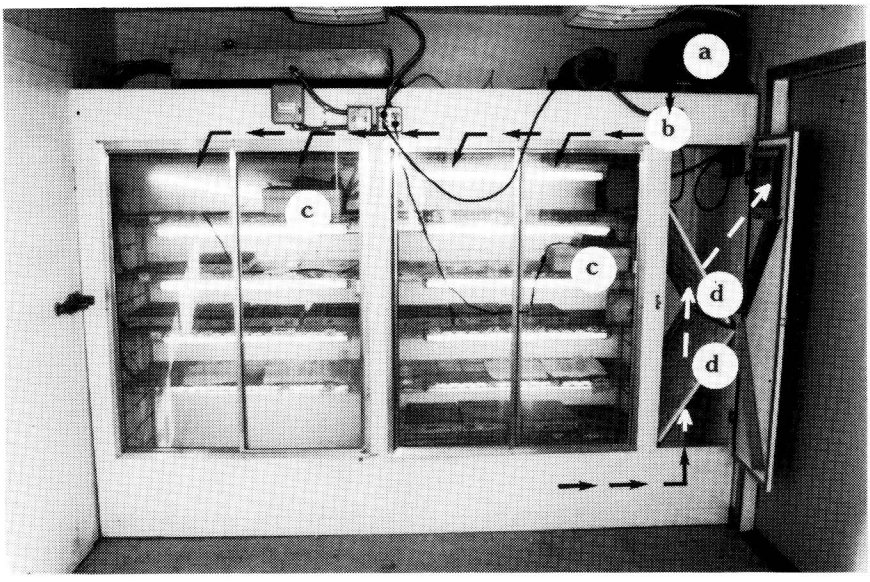

Fig. 1. Codling moth oviposition cabinet: (a) blower, (b) heater (hidden), (c) humidifier, (d) filters. Direction of air flow indicated by arrows.

over the heater, and enters the cabinet at the top, circulating in the cabinet at a rate of 0.15 m^3 per min. The air then enters the plenum at the bottom, passes through the air filters and is exhausted into the room. The cabinet is maintained at 24 ± 3°C, 62 ± 10% RH, and 16:8 (L:D) photophase.

After a 5-day oviposition period the cages are removed from the cabinet and transferred to the hood described above. After about 15 min in the cold room the adults become inactive, and are collected by vacuum and discarded.

Eggs used in mass-rearing need to be surface sterilized to eliminate surface contamination by pathogenic and non-pathogenic microorganisms. In the past, waxed paper sheets or pellets with codling moth eggs were dipped in a 0.1% sodium hypochlorite solution for 2 min (Hamilton and Hathaway 1966, Hathaway *et al.* 1972) or in formaldehyde solution (Howell 1970). These methods were time-consuming and hazardous. Furthermore, pellets tended to clump together when wet, and some eggs dislodged from waxed paper. We found that eggs tolerated formaldehyde vapor for 120 min without effect on hatching or the ability of neonate larvae to enter fruit. Tests showed that there were no codling moth larval deaths due to granulosis virus when eggs were fumigated with formaldehyde for 45 min (J. S. Tebbets and P. V. Vail, ARS Stored Products Research Laboratory, Fresno, CA, personal communication). Our procedure for the past 7 years to surface-sterilize eggs has been to fumigate them with formaldehyde vapor for 90 min at room temperature (23°C).

The fumigation apparatus, constructed of Plexiglas® and located in a fume hood, measures 41 cm deep, 26 cm wide, and 80 cm high (Fig. 2). It holds four removable wooden-framed trays of 0.36-cm mesh hardware cloth spaced 13 cm from the top of the chamber to the top tray and 13 cm between trays. A 10-cm diam exhaust fan is located at the top of the apparatus. Three 2.2-cm diam ventilation holes are located on each side, 5 cm from the bottom, for air to enter the apparatus during evacuation of formaldehyde fumes before the hinged door in front is opened. A 28 x 15 x 10 cm stainless steel container, with a lid, containing the undiluted formaldehyde sits on the floor of the chamber.

For fumigation, egg-laden pellets held on 18 x 16 mesh saran screen are placed on the trays in the fumigation chamber. Egg-laden waxed papers are placed directly on the trays. The lid to the formaldehyde container is removed and the door closed. At the end of the

Fig. 2. Fumigation apparatus in fume hood for surface-sterilizing codling moth eggs with formaldehyde fumes: (a) exhaust fan, (b) empty tray, (c) tray with egg-laden waxed paper, (d) tray with egg-laden polystyrene pellets, (e) stainless-steel container for formaldehyde.

fumigation period, the exhaust fan is turned on and formaldehyde fumes evacuated for 2-3 min. The formaldehyde tank lid is then replaced and the eggs removed.

LARVAL REARING

Formulated diet

Various formulated diets have been used to mass-rear the codling moth at this laboratory (Hamilton and Hathaway 1966; Howell 1967, 1970, 1971, 1972). However, we have been using a diet developed by Howell and Toba (unpublished) for the past 7 years because it has been the most satisfactory one for our purpose. To infest trays of diet a waxed paper sheet with eggs is cut into 20 equal squares, each generally having about 300 eggs. Five squares are placed on the diet in each stainless steel tray (45 x 26 x 7 cm) and the tray is covered with a muslin cloth lid with a wood frame. The weight of the frame, which hangsover the edge of the tray, holds the cloth snug against the lip of the tray to prevent larvae from escaping and to help control dehydration of the diet. The trays are placed on wheeled metal racks and maintained at 23 $\pm$ 2°C, 50 $\pm$ 10% RH, and 16:8 (L:D) photophase. Seven days later the waxed paper squares are removed. Fifteen days after egg infestation, 40.5 x 1.9 cm fluted fiberboard strips are placed in each tray to serve as cocooning sites for mature larvae. The strips are placed in spaces between the diet and the sides of the tray, plus three to four on the surface of the diet.

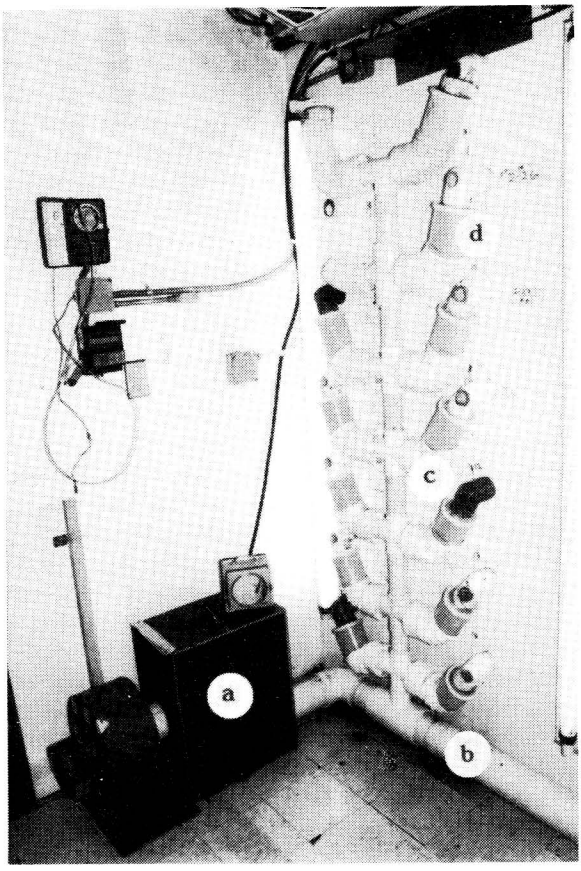

Fig. 3. Scale removal system in codling moth adult collection room: (a) filtering apparatus, (b) polyvinyl chloride tubing, (c) dryer duct sleeve, (d) moth collection container.

Immature apples

When rearing codling moths on apples, one layer of immature, thinning apples previously washed in water is placed in each disposable fiberboard tray (Hathaway 1967). Because water does not remove pesticides that are harmful to codling moths, apple samples are analyzed for residue before using. Each tray is inoculated with about 1500 eggs by sprinkling a given volume (usually 25 ml) of egg-laden pellets. The number of eggs is based on counts in a sample of pellets. The trays are then covered with 18 x 16 mesh saran screen, fitted with tops, and placed on wheeled metal racks. If mature cocooning larvae, pupae or adults are desired, fiberboard pupation strips are placed on the apples. The trays are maintained at 23 ± 2°C, 60 ± 10% RH, and 16:8 (L:D) photophase.

COCOON AND ADULT COLLECTION AND HANDLING

Fiberboard strips with cocooned larvae are harvested from trays of diet or immature apples. A system developed by Hutt *et al.* (1972) to automatically collect emerged moths utilizes two adjoining rooms: an unlighted eclosion room maintained at 24 ± 2°C and 70 ± 10% RH, and a lighted room maintained at about 3°C where the moths are collected. Moths emerging in the dark eclosion containers are attracted to light in the cold room through tubes attached to adult collection containers. Various modifications have been made to this system, primarily to control moth scales.

Eclosion containers made of galvanized sheet metal (Hutt *et al.* 1972) have been replaced with disposable fiberboard containers of the same dimensions. Cocooning strips

are loosely stacked criss-cross in the fiberboard containers instead of on racks used in the metal containers. The fiberboard containers are disposed of after use, thereby eliminating the need to clean and sterilize them and reducing worker exposure to moth scales and microbial contamination.

The problem of moth scales in the cold room where the moths are collected and handled has been decreased with a scale removal system (Fig. 3). One end of polyvinyl chloride tubing is attached to a box housing a blower and an air filter (TA Pinch Pleat 25.4 x 50.8 x 2.5 cm, Environmental Filter Corp., Santa Rosa, CA). Moth collection containers with screen bottoms are connected to the tubing by means of short pieces of flexible dryer duct sleeves. A small hood in the cold room used to transfer moths is also connected to the scale removal system by polyvinyl chloride tubing. The hood is not vented outdoors to conserve cold air in the room.

SANITATION

When mass-rearing insects, sanitation is essential to control contamination of diet, equipment and insect by microorganisms. Certain measures have been taken to minimize these problems in rearing the codling moth. After each use, moth collection containers and oviposition cages are cleaned in a dishwasher. Used diet trays are held in a freezer at about -18°C for 2 days to kill any insects present, then cleaned and autoclaved at 115.5°C and 18-20 psi for 1 hr. Diet tray covers are similarly cleaned and autoclaved for 0.5 hr. The autoclave opens at both ends, each end opening into a separate room. Dirty trays and covers are cleaned and placed in the autoclave in one room (dirty room), and removed and stored in the other room (clean room) after autoclaving. Used apple rearing trays and adult eclosion containers with cocooning strips are held in a room at about 49°C for 12 hr to kill any insects present before discarding them. Walls and floors are cleaned weekly with household ammonia or detergent.

Scavenger mites (family Ascaidae) sometimes become a problem when larvae are reared on immature apples. To prevent mite contamination, the rearing rooms are emptied after each use, cleaned with household ammonia, and heated to about 49°C for 2 days to kill the mites.

DISCUSSION

Various modifications made to improve on a system to mass-rear the codling moth at Yakima has resulted in improved production efficiency. The desired environmental conditions in the oviposition cabinet can now be controlled and maintained, and the moth scale hazard has been removed. The advantages of surface-sterilizing eggs with formaldehyde fumes over dipping them in sodium hypochorite or formaldehyde include not only a reduction of worker exposure to these hazardous chemicals, but also about a 50% reduction in handling time. Further reduction in worker exposure to moth scales has been achieved with the scale removal system in the adult collection room, and the use of disposable adult eclosion containers. The cost of disposable adult eclosion containers is about 35% of the cost of cleaning and sterilizing metal containers, resulting in about a 65% saving.

This system is suitable for obtaining large numbers of selected stages of the codling moth required for such research as quarantine treatments and studies on pheromones, attractants, biological control, and pesticides. Eggs can be readily obtained from egg-laden waxed paper or polystyrene pellets, larvae from the formulated diet, mature cocooning larvae or pupae from the pupation strips, and adults from collection containers. Large numbers of infested immature apples can also be produced by this system. Since 1983, we have successfully produced up to 32,000 larvae per week on the formulated diet.

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Compound eye of male *Stylops pacifica* (Strepsiptera; Stylopidae)

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INTRODUCTION

Few insect groups have greater sexual dimorphism than the Strepsiptera. With the exception of a single family (Mengeidae), the female is a completely passive endoparasite in a much larger insect, and nearly without the ususal external features of other insects (Gehrhardt 1939). These are reduced to a hint of segmentation on the abdomen and a few indeterminate pits and sutures on the sclerotized cephalothorax (Fig. 1). Copulation is said to be achieved *in situ* (Bohart 1941).

The male develops in a larval capsule similar to that of the female, but upon emergence is a small, unusually active, winged insect, about 3 mm long, already well sclerotized, short-lived and nervous, having many of its structures much modified. The antennae show development and variation between species and are well provided with large sensoria (Fig. 2).

The compound eyes of adult males appear to be somewhat primitive and are possibly of secondary importance to the insect. They resemble the eyes of thrips, collembolans, male coccids or the pupal eyes of some beetles (Pankrath 1890). There are no ocelli. Strohm (1910) suggested that each facet represents a lateral ocellus (ocellare komplexaugen), but Bohart (1941) pointed out that they may equally well have been reduced to their present form from normal compound eyes. Each optic unit resembles an ocellus rather than an ommatidium.

METHODS AND MATERIALS

Dr. J.W. McSwain, Instructor in Entomology at the University of California, Berkeley, caught and identified the insects as *Stylops pacifica* Bohart. He allowed me to accompany him into the hills above and behind the town on a fine afternoon in mid-March, 1951. We took eight bees (*Andrena complexa* Vier.) feeding on *Ranunculus*. All were parasitized with Strepsiptera, five with females including one bee with two, and three with males. One male was in the act of emerging. The material for study was put alive into Petrunkevitch fixer and held for a few weeks. The emergent male was the principal subject.