Mass rearing codling moths: improvements and modifications

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ABSTRACT

Current diet, oviposition cages, rearing containers, diapause induction and adult handling are described for a rearing colony of codling moth, *Cydia pomonella* (L.), maintained at the USDA-ARS facility in Wapato, WA , USA, for over 40 years for use in field, laboratory and postharvest research. Previous studies have found codling moth production to approach maximum efficiency at a density of one larva per 4.8 ml of diet. Since 2002, the current YARL rearing program has produced an average of 1 adult per 4.5 ml diet.

Key Words: *Cydia pomonella*, reproduction

INTRODUCTION

The USDA-ARS Yakima Agricultural Research Laboratory (YARL) in Wapato, Washington, USA, has maintained a colony of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), for about 40 years, comprising more than 480 generations. This colony has primarily been used to provide immature stages for postharvest treatments and distribution to other research facilities. Many improvements and modifications have been made since Toba and Howell (1991) described the rearing procedures at YARL and the purpose of this paper is to describe the current methods used for large-scale rearing of codling moth at the same facility.

EGG COLLECTION AND HANDLING

The plywood oviposition cages described by Toba and Howell (1991) have been replaced by wax paper-covered drums (Muchkhof Manufacturing, Oliver, BC) that rotate on rollers within a Plexiglas compartment, which forces moths to evenly distribute their eggs. To help control moth scales, air is circulated within the enclosure with a 1 hp 20 amp electric model (Model No. 4C447, Dayton Electric Manufacturing, Co., Niles, IL) and passes through a HEPA filter (Kenmore Model No. U28337, Sears, Roebuck & Co., Hoffman Estates, IL).

Adult codling moths are collected from emergence chambers in a cold room set with continuous lighting and held at 1.1 °C. Numbers of moths are estimated by weight (38.5 moths/g), with a maximum of 1,425 ± 75 moths put in a single oviposition cage. Moths are placed into the oviposition cage through a slit cut in the wax paper. The paper is then pulled tight and the slit moved through the rollers so that the chamber is thoroughly enclosed by the wax paper.

Oviposition cages are held in an environmentally controlled room at 25 ± 0.5 °C, 77% RH, and a photoperiod of 16:8 h, L:D. Each female adult can produce 80 eggs (Howell 1971). The paper is cut into strips of (2.5 x 10 cm) to facilitate handling before the sheets are dipped into a 32 °C solution of 0.06% sodium hypochlorite for two min, rinsed in 32 °C water for 4 min, and allowed to dry.

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FORMULATED DIET AND REARING CONDITIONS

The YARL diet has been continually improved over three decades (Howell 1970, 1971, 1972). Diet ingredients are pre-measured before mixing (Table 1). The soybean meal is soaked for 1 h in 1000 ml of hot water (52 ± 3 °C) and then placed in a blender containing 1900 ml of hot water (52 ± 3 °C). Next, wheat germ, sucrose, wheat starch, agar, and mineral salts are added. The vitamins and aureomycin are mixed separately then, together with methyl p-hydroxy benzoate and sorbic acid, are dissolved in a sufficient amount (ca. 50 ml) of ethanol. In a third container, 30 ml of propylene glycol are dispensed into 600 ml of hot water (52 ± 3 °C), then the fungicide (Benlate ® SP Fungicide, E. I. du Pont de Nemours and Co., Wilmington, DE.), ascorbic acid and propionic acid are added. Finally, all the ingredients are mixed with the soybean meal in the blender, whose speed is gradually increased to high and then maintained for 1 min. After blending, 4.3 litres of the mixture (density = 1,080 g/ litre is poured in an aluminum pan (31.8 w x 50.8 l x 8.3 d cm). Pans filled with diet are sterilized in 121 °C for 22 min in an autoclave (Castle® M/C3522 Laboratory Sterilizer, Rochester, NY).

A wax film over the top surface of the diet prevents dehydration (Howell 1967). Following autoclaving, the pans of diet are allowed to cool overnight before they are waxed using a Dynamini Adhesive Supply Unit (ITW Dynatec, Henderson, TN). The 5 sec application spreads a thin coat (ca. 1 mm thick) of paraffin wax (2391 Wax, Dussek-Campbell Applied Wax Technology, San Francisco, CA) on the top surface of the diet. After hardening, holes (2 mm diam) are made in the wax using an edge of folded hardware cloth with 1.3 x 1.3 cm wire squares, which facilitate entry of neonate larvae into the diet.

Typically, codling moth production declines during the winter (Howell 1971). In the non-winter months (March to September), eight egg strips (nine strips during winter) are evenly spread out on top each of pan of diet, then placed on racks in incubation rooms set at 24 to 26 °C, 35 to 50% RH, and a 16:8 h L:D photoperiod to allow for egg hatch and larval development.

After 28 d, the eggs hatch and larvae develop through five instars. Larvae normally feed vertically into the rearing diet. When larvae reach mature fifth instar, they exit the rearing diet in search of pupation sites. Strips (14 per pan) of double-sided corrugated card board (2 x 40 cm) are placed vertical to the diet surface to facilitate cocoon formation. The average monthly number of pupae produced per pan is estimated from three pans (biweekly before 2005, weekly after 2005) by examining the pupal strips. In 2005, the average (± SEM) monthly pupal production was 952.8 (± 34.5) and increasing the number of egg strips in the winter allowed for stable production of pupae year round. The lowest production was in late summer-early fall when research activities slowed. Since 2003 the monthly production of pupae has increased by 19%.

Howell (1971) reported that a codling moth larva needed at least 1.3 ml of diet to complete development, but did not mention the effects on size and reproductive potential. Siegal *et al.* (2001) reared disease-free codling moth larvae on 1.8 ml of diet in individual tubes, one larva per tube. Howell (1971) found that the best initial larval density to obtain maximum adult yield per pan was 1 larva per 4.8 ml. Since 2002, the current YARL rearing program produces an average of 1 adult per 4.5 ml diet.

Because codling moth larvae are cannibalistic and intolerant of crowding, production efficacy decreases with increased larval density. Also, crowding increases the likelihood of pathogen transmission. A compromise must be made to produce the maximum number of insects with a limited amount of resources, yet avoid high densities that increase the likelihood of disease.

Table 1.
Ingredients for one pan of rearing media.

Item	Amount
Soybean meal	600.0 ml
Wheat germ	180.0 g
Sucrose	87.0 g
Wheat starch	81.0 g
Agar	18.0 g
Mineral salts	5.4 g
Benlate	0.45 g
Methyl p-hydroxy benzoate	3.0 g
Sorbic acid	2.7 g
Vitamins	33.0 g
Aureomycin	3.2 g
Ethanol	50.0 ml
Ascorbic acid	12.6 g
Priopionic acid	8.1 ml
Propylene glycol	30.0 ml
Heated water for mixing propylene glycol	600.0 ml
Water for meal soaking	1000.0 ml
Water for mixing	1900.0 ml

DIAPAUSE INDUCTION

Eggs strips are placed on pans of diet and held for five d in the rearing room (24 to 27 °C, 16:8 h L:D , 40% RH) to allow for eclosion. Then the pans are transferred to the diapause room (16 to 17 °C, 8:16 h L:D , 50 to 70% RH). This differs from Bloem *et al.* (1997) who described diapause induction at 25 °C, 12:12 h L:D, 55% RH. Three wk later, the corrugated cardboard strips are placed on the diet as described above and diapausing larvae are collected in the strips the following four wk. Diapausing larvae differ from non-diapausing late fifth instars by becoming more inactive, having lower respiration rates and thicker-walled cocoons, and assuming a paler cuticular color (Hansen and Harwood 1968). Diapausing larvae are only used for experimental purposes at YARL, are rarely allowed to develop to adulthood and are not intended for maintaining the colony.

ADULT COLLECTION AND HANDLING

After pupation, the strips are removed and the diet pans are placed in a 50 °C room for 24 h to destroy any remaining larvae and then disposed in commercial garbage. In the laboratory, the strips containing pupae are placed in a cardboard box (25 x 50 x 50 cm) sealed except for an exit hole to allow for adult emergence (the interior is dark except for the exit hole). The room containing the boxes is held at 24 - 25 °C. The exit hole in each box is connected by a PVC tube to a vertical clear Plexiglas shaft with continuous air flow (222.5 m/min and 1.1 °C) in the adjoining eclosion room. Adult moths in the boxes are attracted to the light in the next room, move through the connecting tube, then become inactive when they reach the cold air and drop to the

bottom of each shaft where they are collected on a sieve (Hutt *et al.* 1972). This system replaces the adult moth collection apparatus described by Hutt *et al.* (1972) and the version modified by Toba and Howell (1991).

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REFERENCES

Bloem, S., K.A. Bloem and L.S. Fielding. 1997. Mass-rearing and storing codling moth larvae in diapause: a novel approach to increase production for sterile insect release. Journal of the Entomological Society of British Columbia 94: 75-81.

Hansen, L.D. and R.F. Harwood. 1968. Comparisons of diapause and nondiapause larvae of the codling moth, *Carpocapsa pomonella*. Annals of the Entomological Society of America 61: 1611-1617.

Howell, J.F. 1967. Paraffin films to control dehydration of an artificial rearing medium for codling moth. Journal of Economic Entomology 60: 289-290.

Howell, J.F. 1970. Rearing the codling moth on an artificial diet. Journal of Economic Entomology 63: 1148-1150.

Howell, J.F. 1971. Problems involved in rearing the codling moth on diet in trays. Journal of Economic Entomology 64: 631-636.

Howell, J.F. 1972. Rearing the codling moth on a soya, wheat germ, starch medium. Journal of Economic Entomology 65: 636-637.

Hutt, R.B., L.D. White, L.G. Schoenleber and R.E. Short. 1972. Automatic collection of mass-reared codling moths by phototaxic response and a chilled environment. Journal of Economic Entomology 65: 1525-1527.

Siegel, J.P., L.A. Lacey and C.R. Vossbrinck. 2001. Impact of a North American isolate of the microsporidium *Nosema carpocapsae* on a laboratory population of the codling moth, *Cydia pomonella*. Journal of Invertebrate Pathology 78: 244-250.

Toba, H.H. and J.F. Howell. 1991. An improved system for mass-rearing codling moths. Journal of the Entomological Society of British Columbia 88: 22-27.