Mass-rearing and storing codling moth larvae in diapause: a novel approach to increase production for sterile insect release

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ABSTRACT

A method that induces diapause, originally developed for individually reared codling moth (CM), *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), was tested on the open tray, sawdust-based diet system used in Canada for mass-rearing. The efficiency of the standard and diapause rearing systems are compared and the quality of the adults reared from the two systems is discussed. The benefits and economics of rearing and storing CM larvae in diapause are discussed and related to the ongoing CM eradication program.

Key words: *Cydia pomonella,* mass-rearing, diapause, insect quality, storing

INTRODUCTION

The Sterile Insect Release (SIR) Program to eradicate the codling moth, *Cydia pomonella* (L.), in British Columbia began rearing and release operations in 1994 (Dyck *et al.* 1993). Its goal is to eradicate this key pest of apples and pears from the orchards of south-central BC by 2005 (OKSIRP Strategic Plan 1996). Infestations of codling moth (CM) were first reported at Victoria, BC, in 1900 and by 1916 it was a serious pest in the Okanagan and Similkameen Valleys, some 600 km away (Marshall 1951). At this latitude (49°), CM typically has two generations per year (Madsen and Vakenti 1973), with peak flight activity of overwintered adults in early May and in late July-early August for the summer generation (Madsen and Procter 1982).

During the 1960's and 70's, scientists with Agriculture Canada in BC, and USDA-ARS in the state of Washington, investigated SIR to eradicate CM (Proverbs 1971; Proverbs *et al.* 1969, 1982; White *et al.* 1976a, 1976b). In BC, Proverbs and colleagues developed an open tray, sawdust-based diet (Brinton *et al.* 1969) to mass-rear CM, which proved to be much more efficient and economical than conventional lepidopteran diets. The diet substitutes shredded paper pulp for agar as the binding agent, significantly reducing costs, and uses coarse sawdust to regulate moisture and provide shelters for developing larvae, thus reducing cannibalism and allowing more insect production per tray. Brinton *et al.* reported in 1969 that the new diet consistently produced about 200 adult CM per tray. We now use a modified version of the Brinton diet, with yields of about 1200 adults at a cost of about $3.00 Cdn per tray.

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CM production at the SIR facility is seasonal and follows the phenology of the wild population. Moths (12-14 million per week in 1997) are reared from late March to mid-August and released into orchards at the rate of 2000/ha/week from May to September. In mid-August, rearing is cut back to a maintenance level of between 75000-100000 per week. Production for the next season is stepped-up in February, and peak production is reached in two generations (10-12 weeks).

The key to success with SIR is to produce and release so many competitive insects that the majority of matings in the field are with sterile moths. A 40:1 sterile to wild ratio is necessary to suppress CM populations and eventually lead to eradication. In the Okanagan and Similkameen Valleys the overwintering generation is the largest. This, combined with cool rainy weather, makes it particularly difficult to achieve good overflooding ratios during peak spring adult flight in May.

Codling moths diapause as mature 5th-instar larvae (Brown 1991). Diapause has been experimentally induced and successfully terminated in laboratory colonies, and has mostly been used to store larvae for post-harvest research (Singh and Ashby 1986; Ashby and Singh 1990). If storage in diapause was used as a year-round insect production strategy for SIR, emerging moths would have to compete successfully with wild ones for the system to be useful.

Here, we examine laboratory-induced diapause as a tool to mass-rear and store CM for use in the current eradication effort in BC. The efficiency of the diapause induction system, the timing of emergence from diapause and the quality of the diapaused adults are compared to moths from standard production and with some attributes of wild adults. The costs and benefits of the diapause rearing system are discussed.

MATERIALS AND METHODS

Rearing Comparisons. All rearing was done at the SIR CM mass-rearing facility in Osoyoos, BC, during the fall and winter of 1993. Thirty trays (45x29x2.5 cm) of fresh diet were obtained from standard SIR production. An egg sheet containing 2500-3000 CM eggs previously incubated at 27°C, 16L:8D, 70% RH for 2 days was placed on top of each tray. Fifteen trays were placed in a rearing room under standard conditions (27°C, 16L:8D, 55% RH) for 21 days to serve as controls. The other 15 trays were kept in a separate rearing room to induce diapause at 25°C, 12L:12D, 55% RH (Singh and Ashby 1986). Egg sheets were removed after 7 days and percent hatch calculated. Larval development in the diapause trays was compared with that in the controls once per week. The larvae normally pupate in the diet, beginning at about day 20. However, during our first attempt to induce diapause in open trays, large numbers of mature larvae migrated out of the diet and wandered through the room. In all subsequent rearing trials, 15-20 corrugated cardboard rolls (C- flute, 6 cm diam x 4 cm high) were placed vertically on top of each diet tray at day 18 to collect the wandering larvae.

At day 22, control trays (with late larvae or early pupae or both) were moved into an emergence room (27°C, 16L:8D, 30% RH), where adult eclosion began at day 30 and lasted about 2 weeks. Adult moths were attracted to ultraviolet lights in the ceiling and transported through vacuum ducts to a cold room (0-2°C). At day 45, 4 control trays were selected at random and pupal exuviae on the surface of the diet were counted on 1/4 of each tray. The counts were summed and used as an estimate of adults produced per tray under standard conditions.

The cardboard rolls were removed from the trays on day 30. The trays were then moved into an emergence room under long day conditions (27°C, 16L:8D, 30% RH) and

pupal exuviae were counted after 21 days as above. This number was used to estimate how many CM either did not enter diapause or entered diapause, spun cocoons in the diet and "broke" diapause when the trays were returned to long day conditions. The cardboard rolls containing diapausing larvae were placed in black polyethylene bags and room conditions adjusted to 15°C, OL:24D, 50% RH for 100 days (Ashby and Singh 1990). The bags were then moved to a cold room at 0-2°C, OL:24D, 50% RH, where they were stored for about 50 days (Ashby and Singh 1990). To terminate diapause, the rolls were placed in small emergence boxes at 27°C, 16L:8D, 30% RH (long day conditions). Date of first adult eclosion was noted and adult moths were collected and weighed daily until they had all emerged. The cardboard rolls were examined and total numbers of pupal exuviae, dead larvae and dead unemerged pupae were counted.

The experiment was replicated 5 times (30 trays per replicate, 15 control and 15 induced). The data from each replicate were first tested for homogeneity (means not significantly different at $p < 0.05$) and then pooled. The eclosion curve for adults emerging from diapause was plotted and compared with eclosion curves from standard rearing.

Assessment of Moth Quality. Sample moths from each replicate were collected and used to measure selected characters. Pupal and adult weights for both males and females, adult longevity (with water), female fecundity and fertility, and male mating ability were measured. For longevity, newly eclosed adults were placed individually in small plastic cups (29.5 ml) with a water-moistened cotton wick and kept at 25°C, 16L:8D, 50% RH. The cups were checked daily and the date of death recorded. For assessment of fecundity and fertility, individual newly eclosed females were paired with a virgin male from the colony in clear 200 ml plastic cups with lids; a wick through the lid provided moisture and was re-wetted daily. The pairs were allowed to mate and oviposit at 25°C, 16L:8D and 50% RH, until the female died. The cups were incubated at 25°C, 16L:8D and 50% RH for a further 7 days when the total eggs laid and number of hatched eggs were counted. To assess male mating ability, newly eclosed males were paired with virgin females from the colony in similar cups and allowed to mate for 48 hours. The males were paired with a new virgin female every 48 hours until death. The females were dissected and the total number of spermatophores produced by each male was determined. Data from each replicate were first tested for homogeneity (means not significantly different at $p < 0.05$) and then pooled. For comparison, a sample of cocooning larvae was assessed from corrugated cardboard bands placed around the trunks of 50 apple trees in Oliver, BC during July 1994. Statistical differences between means of laboratory data were compared by examining the 95% confidence limits around each value (Jones 1984).

Cost Comparisons. A comparison was made of the costs associated with rearing CM under standard and diapause conditions. The calculations took into account the cost of diet and all materials employed in rearing. The costs of electrical power and gas consumption, facility wear-and-tear, and labour associated with both rearing processes were estimated. Finally, the efficiency of each rearing system, *i.e.*, the number of adults produced, was included.

RESULTS AND DISCUSSION

In 1993, about 850 adults were reared per tray under standard conditions, which represents about 39% of the neonates that hatched (72.2% egg hatch and 3000 eggs/sheet). In 1997, standard production has increased to 1270 adults per tray and

percent hatch is currently 81%. Rearing efficiency (hatched egg to adult) has therefore increased to 52.3%, mainly because of better process control during mass-rearing.

Our current artificial diet loses moisture over the 3-4 week larval rearing period at 27°C, 16L:8D, 55% RH. When 5th-instar larvae are ready to spin cocoons and pupate, the diet provides a suitable dry site in which to do so. Most larvae reared under these conditions pupate inside their individual feeding tubes and eclose directly from the diet. Larval wandering, although normal in both generations in the wild (Madsen and Procter 1982), has largely been eliminated in the modified Brinton diet system. We expected that larvae induced into diapause would behave similarly and spin overwintering hibernacula inside the diet. A large number of mature diapausing larvae wandered out of the diet before spinning hibernacula. We captured most of the larvae in corrugated cardboard rolls placed on the diet at day 18 before they started to wander. Collecting the larvae into this inert cocooning material unexpectedly reduced the risk of pathogen infection and allowed for cleaner storage of the diapausing larvae.

A comparison of the efficiency of standard and diapause rearing methods is given in Table 1. In 1993, the mean number of adults per tray using standard rearing was 813. When rearing through diapause, the mean number of adults per tray recovered from the rolls after conditioning and chilling was 584. An additional 125 adult moths eclosed directly from the diet when it was returned to long day conditions. Diapause rearing was only 87.2% as efficient as the standard rearing system (584 + 125 vs. 813).

Table 1

A comparison of standard and diapause rearing methods for codling moths.

Stage	% of Individuals per tray of diet (Mean±SD)	
	Standard	Diapause
Egg hatch	77.2 (1923±205)	77.5 (2106±257)
Adults from non-diapausing larvae	100.0 (813±185)	15.2 (125±47)[a]
Larvae diapausing	0.0	84.8 (688±157)
Dead 5th-instar larvae/pupae	1.4 (9±4)	1.7 (10±4)
Adults from diapausing larvae	0.0	72.8 (584±80)
Larvae not accounted for	-	12.5 (94±9)

[a] Number that eclosed from larvae that did not diapause or that diapaused in the diet.

The 125 adults that eclosed directly from the diet came from larvae that either failed to diapause under our conditions or were induced into diapause but failed to wander and cocooned inside the diet. Ashby and Singh (1990) found that when larvae from a New Zealand colony were moved to long day conditions immediately after diapause was induced only 57.7% were able to eclose over 1 year. However, Peterson and Hamner (1968) reported that 97% of CM broke diapause after 28 days in long day conditions immediately after it was induced. We thoroughly examined the diet after 21 days in long day conditions and found no unemerged CM. Our colony responded similarly to that of Peterson and Hamner (1968). The SIR Program prepares 1-2 diet batches per week to maintain a stock colony of between 75000-100000 CM during the fall and winter. Because there is approximately a 10-fold increase per generation, only 10% of the adults produced per batch are used for breeding and 90% are discarded (during the release season, 10% of adults are kept for breeding and 90% are sterilized and released into the field). If diapause rearing were implemented at the SIR facility in fall and winter, the larvae collected in the cardboard rolls could be stored and the CM remaining in the diet

could be made to emerge under long day conditions and used as the stock colony, thereby eliminating any production waste.

With the diapause method, 94 of the 688 larvae per tray that entered diapause and wandered were lost. They probably spun hibernacula directly on the tray carts or room walls. Future research will focus on improving both the capture of wandering larvae and the response of colony CM to diapause induction cues.

The emergence curve for diapaused adults was remarkably similar to that from standard rearing, where adults begin to eclose from the diet around day 30, peak emergence occurs around day 35 and the diet is discarded after day 42. Diapaused adults began emerging 16 days after placement under long day conditions, peak emergence occurred on days 19-20, and by day 24 more than 95% of the adults had eclosed.

Comparisons of CM reared by standard and diapause methods and for wild CM are summarized in Table 2. Mean pupal weight of diapaused males was significantly higher than for males from standard colony or wild material. We cannot explain this significant difference. Typically, overwintered wild CM are lighter than those from the summer generation (Riedl 1983); however, this was not the case when colony adults were reared through diapause. The weights of female pupae or adults (male or female) were not significantly different.

Table 2

A comparison of various quality characters of codling moths reared by standard and diapause methods and collected in the wild (n=number of individuals measured).

Quality character	Rearing method		
(Mean ± 95% CI)	Standard	Diapause	Wild
Pupal weight (mg)			
males	30.93±1.15	36.66±0.79	31.09±0.77
(n)	(50)	(125)	(50)
females	39.22±1.68	40.59±2.09	40.47±1.26
(n)	(50)	(125)	(50)
Adult weight (mg)			
males	19.72±0.94	19.14±0.57	18.86±0.69
(n)	(50)	(125)	(50)
females	29.75±1.43	29.97±0.78	30.90±1.10
(n)	(50)	(125)	(50)
Longevity (days)			
males	14.36±0.69	11.54±0.77	10.32±1.61
(n)	(50)	(118)	(50)
females	11.22±0.66	10.71±0.60	9.34±1.22
(n)	(50)	(119)	(50)
Eggs per female	216.28±17.60	181.74±14.48	--
(n)	(50)	(50)	
% Egg hatch	88.90±3.28	84.15±3.27	--
Spermatophores per male	4.70±1.10	4.51±1.25	--
(n)	(25)	(25)	

Tauber *et al.* (1986) suggested that some developmental, reproductive or survival costs and benefits are associated with a physiological trait like diapause, and that for some species the reproductive costs may be direct, *e. g.*, reduced fecundity. In our experiments the fecundity of diapaused females was significantly lower ($p < 0.05$) than for standard

females, although the proportion of eggs that hatched was similar for both groups. We found males significantly ($p < 0.05$) longer lived from standard than from diapause or wild rearing. Females from standard rearing also lived longer, although the difference from the other groups was not significant. Induction into diapause requires that larvae use some resources while in hibernation (6-9 months) and this may influence adult longevity. The wild CM were also shorter lived than the standard colony insects, perhaps because they die sooner from the stress of being handled. Wild females also did not mate or oviposit in the laboratory. No significant difference was found in mating ability between diapaused and standard males as determined by the mean number of spermatophores produced. In general, moths reared through diapause and stored for 6 months appear to be similar in quality to those reared under standard conditions.

Costs associated with mass-rearing CM with standard and diapause methods are compared in Table 3. The higher cost of diapause rearing is due to the cardboard rolls ($0.05/roll 20 rolls/tray) and the cost of labour to place and remove them. When the efficiency of diapause rearing (87.2%) is factored in, the cost of producing 1000 adults increases from $3.69 to $6.06. Although the cost of rearing through diapause is higher than the standard method, this is partly offset by efficiencies associated with continuous production and the added security of having several million CM in storage.

Table 3

A comparison of costs between standard and diapause rearing methods in 1993.

Rearing method	Cost per diet tray ($Cdn)	Mean adults per tray	Cost per 1000 adults ($Cdn)
Standard	3.00[a]	813	3.69
Diapause	4.30[a,b]	709[c]	6.06

[a] Includes cost of diet ingredients, labour, and facility operation.

[b] Includes cost of cardboard rolls to collect wandering diapausing larvae, labour to place and remove cardboard rolls, and cold storage.

[c] Includes number of diapaused adults eclosed from cardboard rolls on a tray plus the number that eclosed from the diet in that tray.

We show that diapause induction, originally developed for individually reared CM, can be applied to the mass-rearing system used by the SIR eradication program in BC. The technique may increase the program's efficiency by allowing year-round insect production, and could provide additional sterile CM for use in emergencies and even for export.

ACKNOWLEDGEMENTS

We thank S.J. Fielding for her technical assistance and T. Lowery and L. Tomlin for advice on the manuscript. We also thank the SIR Program in Osoyoos, BC, for providing space, insects and trays of diet and Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland B.C., for providing laboratory and office space for S. Bloem. This research was supported by a grant from the Natural Resources and Engineering Research Council of Canada and with funds from the Okanagan Valley Tree Fruit Authority.

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