

## Success of *Grapholita molesta* (Busck) reared on the diet used for *Cydia pomonella* L. (Lepidoptera: Tortricidae) sterile insect release

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### ABSTRACT

The survival and development of *Grapholita molesta* (Busck) reared from egg to adult on the synthetic diet currently used by the Okanagan–Kootenay Sterile Insect Release Program to mass rear *Cydia pomonella* L., in Osoyoos, British Columbia, was compared to those of *G. molesta* reared on the synthetic diet normally used to rear *G. molesta* in the laboratory. Survival and development on a diet of crab apples were tracked as a control. The fitness of resulting moths was compared using metrics of survival to pupation and pupal weight, survival to adulthood, and female fecundity. More *G. molesta* reached the pupal stage and had significantly greater mass when reared on the *G. molesta* diet than on either the *C. pomonella* diet or the crab apple diet. Numbers of moths surviving to adulthood were similar for all three diet types. Although larval diet affected pupal mass of *G. molesta*, the resulting females produced a statistically similar number of offspring, regardless of diet. This study suggests that *Grapholita molesta* can be reared successfully on the diet currently used to rear *C. pomonella* for sterile insect release, but mass production of *G. molesta* will require modification, as well as a period of adaptation to this novel food source.

**Key Words:** Oriental fruit moth, *Grapholita molesta*, Codling moth, *Cydia pomonella*, Sterile Insect Release

### INTRODUCTION

The Oriental fruit moth (OFM), *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), is an economically destructive pest of stone and pome fruits, including peaches and apples (Rothschild and Vickers 1991), and is considered a key pest in many fruit-growing regions (Rothschild and Vickers 1991, Bellutti 2011). Female OFM lay eggs on or adjacent to young shoots and fruits. Upon hatching, neonate larvae usually penetrate the host within 24 h (Dustan 1960). Early in the season, they feed on new growth of twig terminals; after twigs mature, larvae feed internally on the host's fruit (Rothschild and Vickers 1991, Notter-Hausman and Dorn 2010).

The OFM originated in Central Asia (Roehrich 1961) and has spread to most of the world's temperate fruit-growing regions. It was first brought to North America on

ornamental fruit trees in the early 1900s, and now occurs in most stone-fruit growing regions of Canada and the USA (Rothschild and Vickers 1991). British Columbia (BC) remains the only temperate fruit-growing region in the world free of this pest and, as such, its orchard industry is at constant risk of inadvertent introduction of this internally feeding insect.

An absence of OFM and other pests of apples in BC justified attempts to eradicate the only key pest, codling moth (CM), *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), from the province's montane fruit-growing valleys (Dyck *et al.* 1993). In 1992, the Okanagan–Kootenay Sterile Insect Release Program was launched to eradicate CM from southern BC's Okanagan and Similkameen valleys (Dyck *et al.* 1993). Although eradication has not yet been achieved, a

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combination of sterile insect release (SIR) and other integrated pest management (IPM) tactics (Judd and Gardiner 2005) has provided area-wide suppression of this key pest of pome-fruit production in BC (Bloem *et al.* 2007).

Success of SIR against CM may eventually lead to the underutilization of the program's rearing facility in Osoyoos, BC, and additional uses for the facility are being considered. Possible alternative uses include the rearing of biological control agents that could supplement IPM programs for other BC crops or the rearing of other pest insects that might be controlled by SIR programs.

If OFM is introduced to BC, the insect may be a potential target for control by SIR technique. Preliminary trials in Bulgaria have shown that population densities of OFM on peaches were reduced during a pilot program that combined classic SIR with F<sub>1</sub> male sterility (Genchev 2002).

Development and reproductive output in OFM are significantly affected by the nutritional quality of their host plant (Meyers 2005, Meyers *et al.* 2006a). Low-quality larval food negatively affects OFM body size and flight capacity (Gu and Danthanaraya 1992), and larger OFM are more fecund (Hughes *et al.* 2004).

The first step in developing the SIR technique to control any insect pest species is the development of a diet and mass-rearing system that are inexpensive and produce good-quality insects. An objective of this study is to assess the success, as defined by survival, development time and fecundity, of OFM when reared on the diet currently used to mass rear CM for SIR in the BC Interior. The study is designed to also provide preliminary information on whether rearing OFM in the SIR facility currently used to mass rear CM is possible and feasible.

## MATERIALS AND METHODS

**Insects.** The OFM eggs used in this experiment were obtained from a colony reared under laboratory conditions (16:8 h light:dark, 24° C) for ~ 8 y (~12 generations per year) on a lima bean-based diet (Shorey and Hale 1965). The colony was originally obtained from Dr. Mitch Trimble at Agriculture and Agri-food Canada, in Vineland, Ontario. The eggs were collected from a 15 × 15 cm wax-paper roll that served as an oviposition substrate within a wooden mating chamber (31 × 18.5 × 18.5 cm). Viable eggs were determined by egg colour and were counted using a dissecting microscope. Groupings of 10–25 eggs were cut away from the wax paper. Sterile pins were used to arrange a total of 200 eggs on each diet, so that newly hatched neonate larvae could balloon directly onto the diet treatment (as described below). Three replicates of each diet type—codling moth diet (CMD), Oriental fruit moth diet (OFMD), and crab apple diet (ApD)—were conducted, for a total of 600 eggs per treatment.

**Codling Moth Diet.** The codling moth diet (CMD) is a sawdust-based larval diet modified from a diet originally developed by Brinton *et al.* (1969). The CMD currently contains a sawdust mixture obtained from

mills processing Douglas-fir and larch logs (Scott Arthur, Facilities Engineer, Sterile Insect Rearing Facility, Osoyoos, BC, personal communication). The CMD was obtained from the Okanagan–Kootenay Sterile Insect Release Program, in Osoyoos, BC. The ingredients (per kilogram of diet) were as follows: 717.00 ml distilled water; 12.40 g paper/wood pulp; 26.90 g casein; 9.00 g wheat germ; 18.00 g wheat bran; 26.90 g sucrose; 98.60 g whole wheat flour; 11.00 g ascorbic acid; 6.10 g vitamin mixture; 6.20 g Wesson's salt mixture (Cohen 2004); 4.94 g Aureomycin®; 2.70 g sorbic acid; 68.90 g sawdust; and, 9.00 g citric acid. The vitamin mixture was as follows, in grams per kilogram: 5.00 niacinamide, 5.00 calcium pantothenate, 1.30 thiamin hydrochloride, 1.30 folic acid, 0.10 biotin, 1.01 Vit. B12, 1,804.00 ascorbic acid, 449.00 sorbic acid. Five-hundred millilitres (500 ml) of CMD were poured into a plastic rearing box (21 × 22 × 7 cm) and allowed to reach room temperature for 60 min prior to experimental use.

**Oriental Fruit Moth Diet.** The OFM diet (OFMD) is the same lima bean-based diet adopted from Shorey and Hale (1965), and used to maintain the laboratory colony

described above. The ingredients include, per 1.30 litres: 400.00 g organic lima beans, *Phaseolus lunatus*; 80.00 g brewer's yeast; 8.00 g ascorbic acid (coated with fibre); 5.00 g methylparaben (USP); 2.00 g sorbic acid (FCC); 25.00 g vitamin mix (Vandersant-Adkission); 18.00 g carrageenan (Irish moss); and, 2,500.00 ml distilled water. The lima beans were purchased from Planet Organic, Edmonton, Alberta; all other ingredients were purchased from Bio-Serv®, New Jersey, USA. Five-hundred millilitres (500 ml) of OFMD were poured into a plastic rearing box (21 × 22 × 7 cm) and allowed to dry for 60 min.

**Apples.** The apples (ApD) used as a control diet were 'Dolgo' crab apples (*Malus* spp.) collected in Edmonton, Alberta, in late August, 2011. The apples were rinsed first with 1.5% bleach solution, then with distilled water. The apples were left to dry for 10 min on paper towel before being placed into plastic rearing boxes (21 × 22 × 7 cm) to ≈ 500 ml volume.

After being placed into rearing containers, all three diets were sterilized with ultraviolet radiation for 30 s (Kowalski 2009).

**Experimental Procedure.** Each rearing box contained 200 OFM eggs that were evenly distributed across the diet surface. The boxes were sealed with screened lids and held in a growth chamber at 24 °C, for 16:8 (light:dark) h. Initial hatch success of eggs positioned on diet was determined by number of unhatched and hatched eggs. Larvae were allowed to develop until the wandering stage, at which point two 5 × 12 cm cardboard strips were placed into each rearing box to provide pupation sites.

Every 2–3 days, fine forceps were used to remove pupae from their cocoons. The pupae were weighed on a microbalance (Mettler Toledo XS105) to the nearest 0.01 mg, and

each was placed into its own 30-ml plastic cup until adult eclosion.

Adult moths were separated by sex, and the first 10 male and female moths per replicate from each diet were established as mating pairs (N = 30 pairs per diet treatment). Each moth pair was placed in its own 30-ml plastic cup that was lined with wax paper as an oviposition substrate. Plastic mesh was glued to the inside of the lid of each cup to deter oviposition on the lid. Moths were provided with a 10% sucrose solution via a dental wick inserted through the cup lid. Mating pairs were maintained under the same conditions as those provided for larval rearing. Upon mortality of the females in each mating pair, the number of hatched and unhatched eggs laid per female was counted.

**Statistical Analyses.** Response variables were visually assessed for assumptions of normality. Initial hatch success was determined by number of hatched and unhatched eggs found on the egg sheets, and a mixed-effects model, with replicate serving as the random variable, was used to determine differences in initial hatch success among the diets. A mixed-effects model was used to determine effect of diet type and sex on pupal mass, with replicate designated as a random effect. Multiple comparisons were conducted using t-values from the summary output ( $\alpha = 0.05$ ). A logistic regression model was used to analyze effect of diet and pupal mass on survival to adulthood. Replicate was included as a blocking factor in the logistic regression model. We used a mixed-effects model, with replicate as the random effect, to determine relationship between female pupal mass and diet on fecundity (total eggs laid), and a square-root transformation to normalize data. Statistics were conducted using R (R Core Team 2012). Differences were deemed significant at  $P < 0.05$ .

## RESULTS

**Initial hatch success.** The emergence of OFM neonate larvae from eggs did not differ by diet type ( $F = 2.69$ ;  $df = 2$ ;  $P = 0.182$ ; Table 1).

**Pupation.** Numerically, but not statistically, more larvae developed to pupae on the OFMD than on either the CMD or ApD (Table 1). The interaction between diet and

sex ( $F = 6.22$ ;  $df = 2$ ;  $P = 0.0022$ ), and the main effects of diet ( $F = 116.82$ ;  $df = 2$ ;  $P < 0.0001$ ) and sex ( $F = 126.81$ ;  $df = 1$ ;  $P < 0.0001$ ), had significant effects on pupal mass (Fig. 1). Larvae reared on the OFMD and the CMD emerged from the diet in search of a pupation site within the same 24-h period. Pre-pupal wandering in the ApD occurred >

**Table 1**Mean ( $\pm$  SE) numbers of Oriental fruit moth surviving to various life stages on different diets.

Diet	Initial hatch success <sup>a</sup>	Pupae	Adults
Codling moth	188.67 $\pm$ 6.39	48.33 $\pm$ 4.74	34.00 $\pm$ 4.19
Oriental fruit moth	186.00 $\pm$ 4.36	67.33 $\pm$ 2.84	48.33 $\pm$ 2.88
Apples	181.67 $\pm$ 9.33	48.67 $\pm$ 5.10	30.33 $\pm$ 5.81

<sup>a</sup>Includes three replicates of 200 eggs reared on each diet.

48 h later than it had on either the CMD or the OFMD.

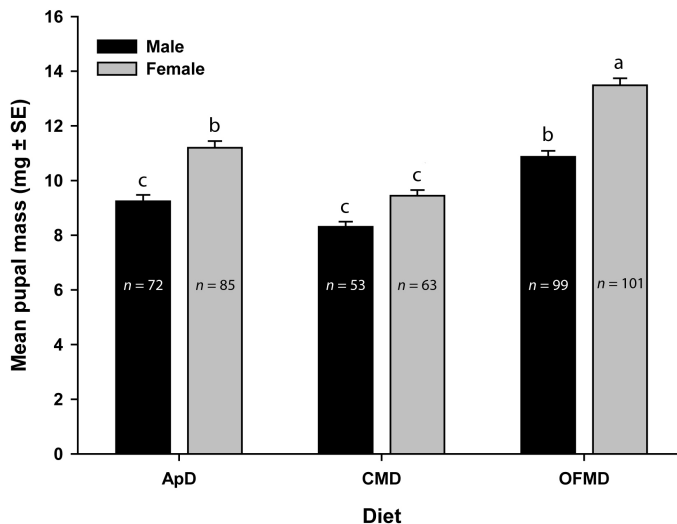
**Survival to adulthood.** The proportion of individuals that survived to adulthood was low on each diet treatment (Table 1). Survival to adulthood was not dependant on pupal mass ( $df = 1$ ;  $P = 0.79$ ), diet type ( $df = 2$ ;  $P = 0.91$ ), nor the interaction between pupal mass and diet ( $df = 2$ ;  $P = 0.91$ ).

**Female fecundity.** Females reared on OFMD produced the most offspring numerically (Table 2). However, diet treatment ( $df = 2$ ;  $F = 0.40$ ;  $P = 0.67$ ), female pupal mass ( $df = 1$ ;  $F = 1.18$ ;  $P = 0.28$ ), and the interaction between diet and pupal mass did not significantly influence female fecundity ( $df = 2$ ;  $F = 0.80$ ;  $P = 0.45$ ).

## DISCUSSION

Our results indicate that the CMD can be used to rear OFM, but the condition of moths reared on the CMD was poorer than that of the moths reared on the OFMD. The initial hatch of OFM eggs on each diet was high and did not differ by diet type. Diet type influenced the mass of pupae, which may reflect

differences in the nutritional quality of the diets. The larvae reared on the OFMD were the largest, followed by larvae reared on the ApD, with the CMD producing the lowest pupal weights. Yokoyama *et al.* (1987) found that OFM reared as larvae on apples were significantly smaller in mean pupal mass than



**Figure 1.** Mass of male and female Oriental fruit moth pupae on the codling moth diet (CMD), the Oriental fruit moth diet (OFMD), and crab apples (ApD). Bars marked with different letters are significantly different ( $\alpha = 0.05$ ).

**Table 2**

Mean ( $\pm$  SE) fecundity of female Oriental fruit moth in each of three replicates when reared on different diets.

Diet <sup>a</sup>	Replicate	Female fecundity <sup>b</sup>	No. of mating pairs ( <i>n</i> )
Codling moth	I	73.33 $\pm$ 7.68	8
	II	52.13 $\pm$ 5.59	10
	III	38.00 $\pm$ 6.16	10
Oriental fruit moth	I	90.5 $\pm$ 8.90	10
	II	78.5 $\pm$ 8.78	10
	III	39.2 $\pm$ 6.52	10
Apples	I	88.25 $\pm$ 8.51	8
	II	62.40 $\pm$ 8.02	7
	III	41.20 $\pm$ 5.76	9

<sup>a</sup>Pairs of virgin males and females reared from each diet type.

<sup>b</sup>Mean eggs laid per female. Female fecundity defined as total eggs laid.

those resulting from larvae reared on the lima bean diet. High survival occurs when OFM are reared on fresh thinning apples, but both survival and pupal mass decline as apple quality degrades over time (Vetter *et al.* 1989). Our experiment showed a decline in female fecundity among all three diet types in the latter replicates, particularly in replicate III. In our experiment, each diet resulted in 20–40% survival, which is comparable to larval survival on apple and peach twigs and fruit under field conditions in the eastern USA (Myers *et al.* 2006b), but is lower than that on other synthetic diets (Genchev 2002). More larvae reached pupation when reared on the OFMD than on the CMD or ApD. This may indicate that the insects used in this study were adapted to the diet the laboratory colony had been reared on for many preceding generations. In the Bulgaria study, emergence of adult female OFM was consistent, but fecundity increased with number of generations reared on two synthetic diets (Genchev 2002). Further research should determine if OFM survival increases on the CMD after multiple consecutive generations are reared on this new food source.

Both male and female pupae reared as larvae on the OFMD were significantly larger than pupae of larvae reared on the CMD or the ApD. Oriental fruit moth larvae deprived of food have an increased rate of pre-imaginal

development, with smaller pupae resulting (Hughes *et al.* 2004). Laboratory flight bioassays indicate OFM adults that eclose from small pupae do not fly as far as those that eclose from large pupae (Hughes *et al.* 2004). The suboptimal pupal weights attained by OFM reared on the CMD in the current study may reduce the flight capacity of the resulting moths.

Larval development time varied among the diets tested. The pre-pupal wandering stage on the ApD occurred 48 h later than on either synthetic diet tested. This may be due to differences in penetration of light into the natural versus the synthetic diet types, which can affect behaviour of last-instar larvae (Nylin *et al.* 2008). Apple skin contains phenolics that protect the fruit from UV damage (Solovchenko and Schmitz-Eiberger 2003). This may have decreased the amount of penetration of light radiation into the ApD, as compared to the CMD or OFMD. Short photophase duration correlates with increased pre-imaginal mortality and delayed reproduction in OFM (Hughes *et al.* 2004).

Larval development of OFM is faster on peach than on any other host, including apple (Bellutti 2011). Oriental fruit moth females prefer peach trees over apple trees for oviposition in close-range controlled tests (Meyers 2005). This preference may involve nutritional components found in peach that are

not present in apple. The crab apples used in the current study were reaching full maturity at the time of picking. Sugar levels and the allelochemistry of apples can vary as the fruit matures (Meyers *et al.* 2006b); this variation may change the fruit's suitability for larval development. For instance, larval CM survival is optimal as pear fruit approach maturity, but decreases as the fruit loses firmness with ripening (Van Steenwyk *et al.* 2004). Decreased penetration of the crab apple skin might also slow development and explain the 48-h delay in the pre-pupal wandering on the ApD.

Although diet type significantly influenced pupal mass in this study, females were similarly fecund, regardless of diet type. Body size is a good indicator of fitness in many moths (Tammaru *et al.* 2002), including OFM (Hughes *et al.* 2004). Compounds obtained from fruit by OFM larvae are used in the synthesis of courtship pheromones by male moths (Baker *et al.* 1981). Males reared on formulated synthetic diets lack trans-ethyl cinnamate, a compound sequestered from apples and peaches (Baker *et al.* 1981) that dictates mate acceptance by females (Loftstedt *et al.* 1989). Other diets used to rear OFM for SIR in Bulgaria incorporate peach and apple purée into the synthetic diet (Genchev 2002). The incorporation of trans-ethyl cinnamate or fruit purées into synthetic diets for OFM should be examined before mass rearing is considered.

The Okanagan–Kootenay Sterile Insect Release Program, in combination with other tactics (Judd and Gardiner 2005), has provided area-wide suppression of CM populations in

BC's Okanagan and Similkameen valleys (Bloem *et al.* 2007). This multi-million-dollar facility may soon be underutilized, but could be used to rear other insects for SIR. Adaptation of the facility to house another closely related species would be most cost effective if minimal changes to the rearing techniques currently used for CM were required.

Wood-based diets such as the CMD are more effective for mass insect rearing, because they are less susceptible to mould growth than agar-based diets are, and wood is a less costly binding material than agar (Brinton *et al.* 1969). The first step in developing the SIR technique for any species is the development of an inexpensive mass-rearing system, including diet. Although the agar-based diet used to rear OFM in this study produced good-quality moths, the diet would be too expensive for large-scale mass production of moths in the SIR facility. Our results show that OFM can be reared on the diet currently used for CM SIR. The quality of mass-reared insects is critical to the success of a SIR program (Calkins and Ashley 1989). Therefore, further research into specific nutritional requirements for OFM should be investigated to determine if the CM diet can be modified to improve reared-insect quality. Our study's findings indicate that, based on pupal size as a measure of insect condition, the CMD is inferior to the OFMD, but that both diets produced equally fecund female moths. Further research would determine if OFM can readily adapt to the CMD after several generations of rearing.

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