Laboratory Rearing of Lesser Appleworm (Lepidoptera: Tortricidae)

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ABSTRACT The lesser appleworm, *Cydia prunivora* (Walsh), was reared successfully in the laboratory. Larvae of various instars were collected in the field from hawthorn fruit, *Crataegus* spp. Initially, immature apples served as the food source for the larvae in the laboratory. Rearing was conducted in a greenhouse and later in combination with a controlled environment room at 25°C, 50–60% RH, and a photoperiod of 18:6 (L:D) h. Under these conditions, a generation required ≈30 d. Fifty-six adult lesser appleworm moths emerged from the original field collected hawthorn fruits. After a decline in the number of the F_1 generation to 39 moths, the colony on mature apples, increased to in excess of 10,000 moths by the fifth generation with a mean survival rate to adult of 68.0%. When production on immature apples was compared with that on four artificial diets, the most promising of the artificial diets was the lima bean-based diet currently used to rear the oriental fruit moth, *Cydia molesta* (Busck), with a mean survival rate of 46.4%. The other bean-based diets tested were not as satisfactory. Pear foliage was the preferred oviposition substrate of those tested, including apple and hawthorn foliage. No eggs were deposited on plain waxed paper or glass microscope slides; however, large numbers of eggs were deposited on waxed paper treated with a water extract of pear foliage and immature apples.

KEY WORDS *Cydia prunivora*, lesser appleworm, rearing, hawthorn, *Crataegus*, diets

LESSER APPLEWORM, *Cydia prunivora* (Walsh), is widely distributed in North America and is probably a species native to eastern North America. The lesser appleworm was described by Walsh (1868) as infesting plums as early as 1867 in Illinois and was first reported to attack apples in British Columbia, Canada, in 1895 (Fletcher 1898). Lesser appleworm has not been a problem in most commercial orchards. Because the lesser appleworm's life history and habits are very similar to those of codling moth, the chemical control measures used to control codling moth also control lesser appleworm (Faurot 1912, Quaintance and Siegler 1922). Turmel and Fisher (1978) stressed the importance of not altering control programs for insects on apples in New Hampshire without considering the possible impact on lesser appleworm. With the increasing use of species specific biorational techniques for control of codling moth (Howell 1992) and the subsequent decrease in the use of pesticides, consideration of the impact of these techniques upon lesser appleworm becomes even more important.

The larvae of this species have been reported to attack the fruit of the family Rosaceae (Chapman and Lienk 1971, Brunner and Howitt 1981). The principal genera attacked are *Crataegus* (hawthorn) (Quaintance 1908, Wellhouse 1922), *Prunus* (prune, plum, cherry, peach, and apricot) (Brown 1953), *Pyrus* (apple, pear, and crab apple) (Fletcher 1898, Wellhouse 1920), *Rosa* (rose hips) (Mackie 1942), *Photinia* (toyon) (Keifer 1933), and *Amelanchier* (shadbush or serviceberry) (Heinrich 1926). Lesser appleworm larvae have also been reported on galls of the oak (Fagaceae) and elm (Ulmaceae) (Fletcher 1898). The larvae are internal feeders similar to codling moth, *Cydia pomonella* (L.), oriental fruit moth, *Cydia molesta* (Busck), and cherry fruitworm, *Cydia packardi* (Zeller). The larvae of all four species are similar in appearance. However, the codling moth larva lacks an anal comb, a structure that is present in the other three species (Garman 1918). Codling moth larvae are larger than the others and feed directly to the core of the fruit. Oriental fruit moth larvae are larger than lesser appleworm and cherry fruitworm larvae. Oriental fruit moth larvae do not feed on the seeds. Lesser appleworm and cherry fruitworm larvae are very close to the same size and the feeding habits are similar (Mackay 1959) (Chapman and Lienk 1971). Larvae of both species feed below the surface of the skin of the fruit causing surface blotches.

Lesser appleworm had remained relatively obscure until the Japanese government made it a quarantine issue for U.S.-produced apples in 1991 (H.R.M., unpublished data). Lesser appleworm is not reported to occur in Japan. Because lesser appleworm had been reported as a pest of stone and pome fruits in the Pacific Northwest in the Milton-Freewater area of Oregon (Brown and Jones 1953), the export of apples to Japan was delayed. This study was initiated to locate in the field and rear in the laboratory enough numbers of lesser appleworm to determine if the two component treatment, fumigation with methyl bromide followed by cold storage, (Moffitt 1988) devised to meet quarantine security requirements for codling moth would also meet requirements for lesser appleworm in apples destined for export to Japan.

Materials and Methods

Rearing on Thinning Apples. Larvae of various instars were collected initially in the field from fruit of the hawthorn, *Crataegus* spp. Hawthorn fruits were brought to the laboratory and placed in a plastic container (20.0 cm long by 14.0 cm wide by 10.0 cm deep) with a cheesecloth cover. The container was held in a greenhouse at 25°C, 50–60% RH, and a photoperiod of 18:6 (L:D) h for emergence. A greenhouse was selected to provide sunlight for the developing larvae and the subsequent mating of the emerging adults. An 18-h day was selected to prevent the larvae from entering diapause (Brown 1991). The emerging adults were collected and the sex of each adult was determined. The adults were then placed in a plastic container, identical to the original container, with hawthorn and apple fruits placed in the bottom for possible oviposition stimulation and ovipositional sites. The adults from this generation and those of subsequent generations were placed in mating and oviposition cages. The mating and oviposition cages were 30.0-cm cubes. The cage was constructed of a wooden frame with a wooden bottom. The frame was covered on three sides and the top with nylon organdy. The fourth side of the cage had an armhole opening for access to the inside of the cage. Ten thinning apples were placed on the bottom of each cage as a possible oviposition stimulant, an oviposition site and/or food source for any larvae that might hatch. A 250-ml Erlenmeyer flask of water with a cotton wick (15.0 cm dental roll) served as a source of moisture for the adult moths. Adult female moths from the first few generations were dissected for the presence of a spermatophore in the bursa copulatrix to determine if mating had occurred.

Oviposition Substrates. The search for an acceptable oviposition substrate included foliage of apple, pear and hawthorn, waxed paper, and glass microscope slides. Each of these possible oviposition substrates was presented to the female moths in the mating and oviposition cages.

Each of the three types of foliage was collected from the field and presented with the leaves attached. A 250-ml Erlenmeyer flask was filled with water, the shoots were wrapped with a 2.5 by 15.0-cm piece of rolled cotton to prevent the adults from drowning in the water, and placed in the flask. A 7.0-cm piece of plastic drinking straw was inserted through the rolled cotton into the flask to allow water to be added to the flask daily. Three of the flasks with shoots and one flask with the moisture wick were placed in each cage. Also, to supply pear foliage during the winter, pear shoots were collected from the field in the fall. The method of pear shoot collection and preservation was described by Fye (1981).

The waxed paper was presented plain and with an extract that was developed to enhance oviposition on the paper. The extract consisted of 250 ml water, eight thinning apples, and 16 pear leaves. The ingredients were blended for 1 min, forming a slurry. The slurry was filtered through qualitative filter paper, VWR grade 613. A cotton ball was dipped into the filtered solution and the liquid was spread across a 40.0 by 30.0-cm piece of waxed paper. The waxed paper was allowed to dry at room temperature. It was then cut into four 10.0 by 7.5-cm pieces. The waxed paper, both the plain and the extract enhanced, were crinkled and suspended along the sides of the cage. The glass slides were suspended from the top of the cage using tape and wire. Glass slides were also laid in the bottom of the cage.

A maximum of 500 adults was added to each cage. The conditions in the greenhouse were 25°C, 50–60% RH, and a photoperiod of 18:6 (L:D) h. After 5 d, each oviposition substrate with the eggs was removed from the cage and placed in a clear plastic container (35.0 cm long by 27.0 cm wide by 9.0 cm deep). A section was removed from the plastic lid of the container and organdy was glued over the hole for ventilation. Thinning apples, measuring ≈3.0 cm in diameter, were placed in the container to serve as the food source for the maturing larvae. The containers were transferred to a holding room at 25°C, 50% RH, and photoperiod of 16:8 (L:D) h. Corrugated fiberboard strips, (33.0 cm long by 1.3 cm wide) flute size 125BU (Tharco, Auburn, WA), were placed on the bottom and along the sides of the container. The flutes of the strips served as cocooning sites for mature larvae. The fiberboard strips containing the larvae were transferred to a clean plastic container of the same dimensions, where the adults emerged and were collected. The adults were collected by using a battery operated aspirator.

Rearing on Artificial Diets. Four artificial diets used for the rearing of other insects were evaluated for possible use as a food source for lesser appleworm larvae. The diets tested were as follows: the codling moth diet (CM diet) (Howell 1970), the oriental fruit moth diet (OFM diet) (Yokoyama et al. 1987), the McNeil diet with pinto beans (McNeil w/pinto) (Shorey and Hale 1965), and the McNeil diet with lima beans (McNeil w/lima). The ingredients for the diets are listed in Table 1. Thinning apples were used as the standard for comparison. The diets were mixed and poured into individual 30.0-ml plastic cups. Fifteen milliliters of the diet or one thinning apple was placed into each cup. There were 25 cups for each type of diet and each diet was replicated five times.

A single lesser appleworm egg was placed on each individual cup of diet or each thinning apple. The eggs were collected by placing pear foliage in an oviposition cage with 500 adult moths. The moths were allowed to mate and oviposit for a 48-h period. Foliage with eggs was removed from the cage and single eggs were isolated by cutting a piece of leaf with a single egg on it and piercing the leaf with a 2.0-cm long piece of wire. The other end of the wire was inserted into the diet or thinning apple, suspending the egg over the diet or apple to diminish the possibility of contamination through direct contact. After 9 d, the foliage with the egg was removed from the diet and the egg was evaluated for hatch. The larvae were allowed to develop and the number and sex of the emerging adult moths were recorded. Egg hatch, survival, and rearing

Table 1. List of ingredients and quantities for each artificial diet tested as a possible food source for lesser appleworm larvae

Ingredient	OFM diet	McNeil w/Pinto	CM diet	McNeil w/Lima
Agar	10.0 g	5.0 g	18.0 g	5.0 g
L-ascorbic acid	6.0 g	0.8 g	12.6 g	0.8 g
Brewer's yeast	60.0 g	8.0 g		8.0 g
Fructose	9.0 g			
MPHB	0.6 g	0.5 g	3.0 g	0.5 g
Soy protein	15.0 g			
Sorbic acid	1.5 g	0.25 g	2.7 g	0.25 g
Vitamin mixture	11.5 g	1.0 g	33.0 g	1.0 g
Wheat germ	30.0 g		180.0 g	
Water	900.0 ml	290.0 ml	3600.0 ml	290.0 ml
Lima beans	300.0 g			53.25 g
Formaldehyde		0.75 ml	8.1 ml	0.75 ml
Pinto beans		53.25 g		
Soybean meal			495.0 g	
Wheat starch			81.0 g	
Mineral salts			5.4 g	
Benlate				0.54 g
Propionic acid			8.1 ml	
Propylene glycol			30.0 ml	
Auremycin			8.0 g	
Sucrose			87.0 g	

weight data were subjected to analysis of variance (ANOVA) and the Tukey mean separation (SAS Institute 1989). Percentages were transformed by $\sqrt{\sin^{-1}}$ before analysis.

Results and Discussion

Fifty-six adult lesser appleworm moths emerged from the original field-collected hawthorn fruits. The ratio of male to female moths was 1.5:1. The frequency of mating among the native females that emerged was 42.9%. The F_1 generation declined in number and produced only 39 moths. The male to female ratio of this generation was 0.8:1 with 94.1% of the females mating. The total numbers of moths for the F_2 and F_3 generations were 228 and 697, respectively. Each successive generation continued to increase in numbers until space in the rearing facility became the limiting factor (Fig. 1). The typical size of each generation maintained is currently at 10–15,000 moths.

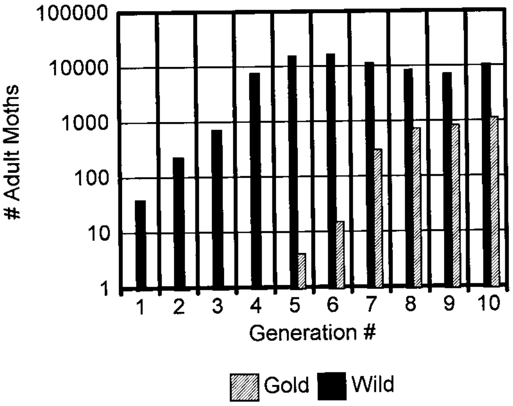

Fig. 1. Propagation of laboratory-reared wild and gold populations of lesser appleworm.

Table 2. Comparison of four artificial diets and thinning apples as a food source for lesser appleworm larvae

Food source	Mean % hatch	Mean % survival
Thinning apples	96.0a	68.0a
OFM diet	94.4a	46.4b
CM diet	96.8a	27.9b
McNeil w/pinto	94.4a	38.5b
McNeil w/lima	94.4a	27.7b

For mean percent hatch: $F = 0.20$, df = 4, P = NS. For mean percent survival $F = 6.77$; df = 4, 20; $P < 0.01$. Means followed by the same letter within a column are not significantly different (ANOVA, Tukey mean separation test).

A gold color variant was observed starting with the F_5 generation (Fig. 1). The color variant has the same markings as the normal lesser appleworm adult, but the colors are muted resulting in a moth that has a more pale appearance. A total of four gold adult moths (two males and two females) was collected. These four moths were held in a separate cage to form a subcolony of homozygous gold moths. This gold colony was not as prolific as the wild colony. A gold color variant has also been reported for codling moth (Hutt and White 1975). The results of their laboratory and field studies indicated similar results, that gold codling moth population was not as vigorous as the wild population.

Pear foliage was observed to be the preferred oviposition substrate. The female moths laid eggs singly on the upper and lower surfaces of the leaves as well as the stems, with most of the eggs being found on the underside of the leaf. Female moths did not lay any eggs on the plain, waxed paper or the glass slides. However, waxed paper treated with the extract residue was found to be an acceptable substrate for oviposition. When the waxed paper with the extract residue and the pear foliage was monitored for a year in the mass rearing facility, the treated waxed paper averaged 448 eggs with a range of 28–4039 eggs per oviposition cage. The average hatch was 75%. The pear foliage averaged 2,152 eggs per oviposition cage with a range of 262–3,248. The average hatch was 86.6%. Sunlight played a significant role in selection of oviposition sites. Pear foliage and waxed paper strips treated with extract that were oriented toward the sun had significantly more eggs than those not oriented

Table 3. Weights of lesser appleworm adults reared on thinning apples compared to weights of adults reared on OFM diet

Food source	Sex	Mean wt	Range	STD
Thinning apple	♀	4.6a	2.5–6.3	0.7
Thinning apple	♂	4.3b	3.0–5.7	0.6
OFM diet	♀	3.6c	1.4–5.6	0.9
OFM diet	♂	3.8c	1.6–5.5	0.9

For mean weight: $F = 34.95$; df = 3, 396; $P < 0.01$. Means followed by the same small letter within a column are not significantly different (ANOVA, Tukey mean separation test).

toward the sun. The procedure was modified and the mating and oviposition cages were rotated 90° daily.

As the eggs hatched, neonate larvae entered the apple and fed just below the surface of the apple, forming dark discolored areas on the fruit. Some of the larvae have been observed moving from one apple to another during the course of their maturation. Mature larvae left the fruit and spun cocoons in the flutes of the corrugated fiberboard. The efficiency of the fiberboard strip as a cocooning site was increased by placing the strips under the apples instead of on the top surface.

When the four artificial diets and the thinning apples were compared as food sources, the thinning apples proved to be a significantly better food source than any of the diets with a survival rate of 68% (Table 2). When the four artificial diets were compared, the OFM diet showed the most promise with 46.4% of the eggs surviving to adult. By the F_{37} generation after being established in the laboratory, and after 12 generations of rearing on OFM diet, approximately 500 lesser appleworm moths were produced per tray of OFM diet (3,600 ml of diet). When the weights of the adults collected from the thinning apples and the OFM diet were compared, the weights of the adults reared on thinning apples were significantly higher (Table 3).

Although the thinning apples appear to be the best choice for the rearing food source, there are some disadvantages. The availability of thinning apples is variable, the quality of the apple diminishes as the year progresses, aging thinning apples are a substrate for mold growth, and are a source and reservoir for mites, which can be vectors for lepidopterous diseases. The use of an artificial diet that produces ≈20.0% fewer surviving adults without the risk of disease vectors and mold contaminants may be satisfactory. However, development of an artificial diet more suitable for lesser appleworm is desirable.

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