Mass Rearing of the Oriental Fruit Moth
(Lepidoptera: Tortricidae)

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ABSTRACT Oriental fruit moth, *Grapholita molesta* (Busck), was reared on modifications of a previously developed small lima bean diet. Initial experiments showed that different types of beans substituted for lima beans produced similar yields of oriental fruit moth pupae. Fresh, thinning apples provided the best diet for high yields and high pupal weights, but apple quality degenerates with time, producing poorer quality pupae. Modifications of the bean diet resulted in quality production of >1,000 pupae per 4-liter jar, allowing year-long maintenance of the colony that could not be accomplished on apples alone. Tolerance of oriental fruit moth to methyl p-hydroxybenzoate, sorbic acid, and benomyl (Benlate) preservatives in the diet was determined.

KEY WORDS Insecta, *Grapholita molesta*, formulated diet, legume diet

YOKOYAMA ET AL. (1987) developed a formulated diet using small lima beans for rearing the oriental fruit moth, *Grapholita molesta* (Busck). The diet is relatively inexpensive, easy to formulate, and its ingredients are readily available from commercial suppliers. Previous diets for mass rearing this insect have been deficient in some of these qualities (see references in Yokoyama et al. 1987).

Our objective was to determine if the small lima bean diet could be used in mass rearing where production of pupae would be prolific and efficient without losses caused by mold or fungus. Although Yokoyama et al. (1987) developed the lima bean diet and showed it to be sufficient to rear healthy and fertile *G. molesta* adults, their study was performed primarily on insects reared individually in small, plastic cups on 4 ml of diet. In preliminary work, they showed that the insects could be reared on pinto beans and peas. Using the lima bean diet as a basis, we attempted to mass rear *G. molesta* in large containers on a variety of bean diets. In addition, we tested different diet modifications that might reduce production costs and tested the tolerance of *G. molesta* to chemical preservatives incorporated into the bean diet to control microbial growth.

Materials and Methods

Insects. The colony of oriental fruit moth was started from wild populations and was reared for >8 yr on small green thinning apples. No infusion of wild type insects was made during this time, however, occasionally pupae from other lab colonies (similar to ours) were added.

Eggs were collected from a cheesecloth-lined wooden mating chamber (30 by 16 by 17 cm). A piece of Plexiglas slid into grooves running along its length and was positioned on the front open side. A continuous-feed 15-cm roll of wax paper was stretched across the chamber's open face between the Plexiglas and the interior. The moths, attracted to the light, rested and laid eggs on the wax paper. Eggs were removed from the chamber interior daily by pulling the wax paper, which simultaneously replaced the eggsheet for the next day. Eggsheets were washed in a weak formaldehyde solution (approximately 1%) for 30 s, rinsed, air dried, and stored at 25°C.

Apples. Small (3 cm diameter), green thinning apples, *Malus sylvestris*, were obtained from Oak Glen, Calif. in mid-June 1987 and transported to the University of California—Riverside campus. These apples were a mixture of 'Granny Smith' and 'Red Delicious' varieties. The apples were soaked in 12 g of 50% benomyl (Benlate, E. I. du Pont de Nemours and Company, Wilmington, Del.) per liter of water for 10 min in a large vat, removed, stored in the shade, allowed to dry outdoors overnight at approximately 25°C and then placed in cold storage (10°C). Apples were removed from storage as needed, and soaked in 50% benomyl (6 g/liter) for 1 h in the lab. The apple skins were then perforated with a board of nails, resoaked in the benomyl solution for 10 min, and then set out to dry.

Diet Ingredients. The following market classes of beans were used: great northern, pink, pinto, red (all varieties of *Phaseolus vulgaris* L.); small (baby) lima, *Phaseolus lunatus* L.; and garbanzo, *Cicer arietinum* L. Green split peas, *Pisum sativum* L. were also used. Legume types were determined by availability and were obtained through local grocers in 454-g bags (Evans brand, Chino,

Calif.) Our ingredients (quantities per 300-g batch of dry beans) and their sources were as follows: 10 g agar, 6 g L-ascorbic acid (Bioserv, Frenchtown, N.J.), 60 g Brewer's yeast, 9 g β-D(−)fructose, 0.6 g methyl p-hydroxybenzoate (MpH), 15 g α-protein, 1.5 g sorbic acid, 11.25 g Vanderzant Modification Vitamin Mixture (VMVM) (ICN, Cleveland) and 30 g wheat germ (local grocers). We also used Vitamin Diet Fortification Mixture (VDFM) from ICN in later experiments as a substitute for VMVM. VDFM is used in other lepidopteran diets (Shorey & Hale 1965) and is less expensive. L-ascorbic acid, wheat germ and the vitamin mixtures were stored at 10°C; all other ingredients were stored at room temperature. Any diets that deviated from this formulation are designated as such.

Formulation. All legume diets were made in batches of 300-g dry bean quantities as described by Yokoyama et al. (1987). We used distilled water throughout the experiment. Garbanzo beans required 100 ml additional water for the first 1-h presoak to cover all the beans. However, total water added to that diet was equal to the others.

In the first experiment, we tested seven types of legumes in comparison with fresh apples to determine which would rear the greatest number of pupae. Approximately 175 ml of diet was poured into the bottom of a clear plastic box (19 by 13 by 7.5 cm). The diet spread to a depth of 1 cm. One batch of diet produced four replicates in these small boxes; 12 replicates were tested for each legume type. The medium in the box was allowed to dry uncovered for 2 h before it was weighed (approximately 180 g), and its surface was perforated with a fork, to make >600 holes to allow easy access for first instars. (Although this is not the most efficient system for mass rearing, it was done here for comparison to procedures of Yokoyama et al. [1987].)

Twelve replicates were performed with apples. A replicate consisted of 13 to 15 apples, which weighed approximately 180 g, and the apples were placed in boxes described above.

Rearing. Legume diet was infested by placing strips of eggsheets containing 100 3-d-old eggs on top of bent paper clips positioned on top of the media. (Mold forms more easily when eggsheets touch the moist surface and eggs that contact the surface occasionally do not hatch.) Apples were infested with similar eggsheets laid on top of the skin; no paper clips were necessary because mold is less of a problem with fresh apples. Apple and legume boxes were covered with lids and placed in an environmental chamber with a photoperiod of 16:8 L:D. The boxes were covered with black cloth because larvae are attracted to light; this maximized the chances of first instars burrowing into the diet. Humidity was 50 ± 5% in the cabinet and 90 ± 2% in the diet boxes and the temperature was 25 ± 1°C.

After 2 d, eggsheets were removed and egg hatch was determined. Five days later, the contents of each box were transferred (one box per jar) to 1.9-

liter glass jars with brass screen lids; the legume diet was transferred with a putty knife and the apples by hand. Mold and fungus were removed at this time, and the infected surface area was recorded for each box. A roll of corrugated cardboard (3 cm thick, 7.5 cm diameter, 1.7 corrugations per cm), into which the larvae crawl and pupate, was placed in the top of the jar, and the lid was secured. After 11–13 more days, the rolls were removed, the pupae were counted and recorded as pupae reared per 100 hatched larvae. The pupae were segregated by sex, counted again for sex ratio determination, and five pupae of each sex from each replicate were weighed individually.

The cost of rearing 100 pupae from each diet was calculated. Only the ingredient costs were considered because the amount of labor was similar for apple and legume diets.

Mass Rearing. From the first experiment, small lima beans proved to be the best type for mass rearing G. molesta. A second experiment attempted to incorporate this legume into a mass-rearing regime currently used with apples for our program. The ranges of yields from the seven legume types were large in the first experiment, possibly because of differences between lots of beans purchased. To avoid this problem, a large quantity of small lima beans was purchased and thoroughly mixed; beans were removed as needed.

Beans were formulated in 300-g batches and approximately 875 ml diet (900 g) was poured into a large plastic box (32 by 25 by 10 cm) and spread to a depth of 1 cm. For the apple diet, similar weights of apples were used and totaled about 60 apples per box. The top half of these plastic boxes and their lids were spray painted black on the outside. After 2 h air drying, the legume diet was weighed and its surface was perforated with approximately 2,000 holes. Eggsheets containing 1,000 3-d-eggs were placed directly on apples or on bent paper clips on top of the perforated diet. Lids were placed on the boxes and boxes were then placed in the environmental chamber previously described. Egg hatch was determined after 2 d. After 5 more d, each box of infested diet or apples was transferred to a 4-liter glass jar. A 9.5-cm-diameter roll of corrugated cardboard was placed in the top of the jar, and the lid was secured. After 11–13 more d, pupae were collected, counted as before, and recorded as pupae per 1,000 hatched larvae. For each replicate, 100 pupae were segregated by sex, counted to determine the sex ratio and 10 of each sex were weighed individually.

Two treatments designed to reduce costs or labor were also included in the test. First, bean diet was formulated just as above but with VDFM instead of VMVM. Second, in diet formulated with VDFM, the diet surface was not perforated. Six replicates were performed on each diet variation.

Preservatives. In preliminary unpublished tests, >50% of boxes containing black bean (Phaseolus vulgaris var. Black Turtle Soup) formulations were

Table 1. G. molesta ($\bar{x}$ ± SD) reared on fresh thinning apples and formulated diet in small plastic boxes when infested with 100 eggs

Formulation[a]	No pupae per 100 hatch	Male wt, mg	Female wt, mg	Sex ratio, ♀:♂	Egg hatch
Apple	64.4 ± 10.8a	13.5 ± 2.2a	17.8 ± 2.8a	0.96 ± 0.21a	94.7 ± 5.7ab
Small lima bean	49.1 ± 14.3b	11.9 ± 1.7bcd	15.9 ± 2.0b	0.87 ± 0.29a	96.6 ± 1.7a
Garbanzo bean	45.3 ± 7.6bc	12.2 ± 1.6b	15.9 ± 1.9b	1.06 ± 0.31a	91.9 ± 4.1b
Great northern bean	43.5 ± 14.6bc	10.9 ± 1.5cd	13.4 ± 2.5c	1.02 ± 0.30a	95.2 ± 2.9ab
Green split pea	37.6 ± 10.5bc	10.7 ± 2.1d	13.4 ± 2.4c	0.88 ± 0.26a	93.9 ± 2.8ab
Pink bean	44.2 ± 8.0bc	11.0 ± 1.4bcd	14.9 ± 2.0b	0.91 ± 0.30a	93.8 ± 2.6ab
Pinto bean	36.8 ± 11.2bc	11.8 ± 1.3cd	15.3 ± 2.2b	0.94 ± 0.16a	91.9 ± 4.3b
Red bean	32.0 ± 16.4c	12.0 ± 2.0bc	15.1 ± 2.1b	1.00 ± 0.46a	94.7 ± 3.7ab

Values within a column that do not share the same letter are statistically different ($P < 0.05$) as determined by Tukey's studentized range test.

[a] Twelve replicates of 100 eggs per replicate.

quickly overgrown with mold that deleteriously affected larval growth. Therefore, black beans were chosen for an experiment using varying amounts of preservatives in the diet to test the tolerance of G. molesta to preservatives, while also inhibiting microbial growth.

The original diet formulation was the control. Six treatments were tested in which diet differed from the control in increased amounts of one of the following preservatives: MpH (1.2, 2.4 g), sorbic acid (3, 6 g) and 50% benomyl (1, 2 g). To eliminate quality differences among lots of black beans, a large quantity was purchased, thoroughly mixed, and removed as needed. Procedures and data collection were similar to the first experiment. Eight replicates were performed for each treatment.

Sterilization Procedures. Plastic boxes were emptied, cleaned, submerged for 16 h in a 1:4 solution of 5% sodium hypochlorite/water and then air dried. Glass jars were emptied, cleaned, filled with an approximately 1:20 solution of 5% sodium hypochlorite/hot water for 1 h, washed in hot, soapy water and then held overnight in an oven at >100°C.

Data Analysis. A one-way analysis of variance (ANOVA) was used to analyze the data. Tukey's studentized range test was used for determining differences among treatments (SAS Institute 1982). Differences were deemed significant at $P < 0.05$.

Results

In the first experiment comparing fresh thinning apples with seven legume types, significantly more G. molesta pupae were reared on apples than any of the legume diets ($F = 8.11$; df = 7, 88; Table 1). In addition, the male and female pupae reared on apples were significantly larger than any of their conspecifics reared on legume diet (males: $F = 23.02$; df = 7, 467; females: $F = 17.57$, df = 7, 469). Among the legumes, the largest pupal yield was produced from small lima bean diet but it was only significantly greater than red bean diet, which produced the lowest yield. There were no clearcut differences among weights of pupae reared on legumes, although pupae from small lima beans

and garbanzo beans tended to be heavier and those from green split peas and great northern beans tended to be lighter. There were no significant differences in sex ratio among the diets ($F = 0.60$; df = 7, 88). Eggs hatched with >90% success although there were some differences among the diets ($F = 2.20$; df = 7, 88).

Mold and fungus developed in 23 of 84 boxes of the formulated diet. The average amount of contaminated media removed in area was 3% and never amounted to more than 8% of the total area of the diet surface. The percentage of legume boxes developing mold were as follows: small lima (0%), garbanzo (0%), green split pea (0%), great northern (17%), pinto (58%), pink (58%), and red (58%).

The cost per 100 pupae for each diet was as follows: apples, $0.34; small lima, $1.23; pink, $1.34; great northern, $1.38; garbanzo, $1.41; green split peas, $1.53; pinto, $1.58; and red, $1.89.

Based on the results of the first experiment, small lima beans were used in the mass-rearing experiment because they produced the greatest yield of any formulated diet, they produced the least expensive pupae of any legume diets tested, and we could make comparisons with the data of Yokoyama et al. (1987).

In the mass-rearing experiment, significantly more pupae were reared on fresh apples than any permutation of the small lima bean diet ($F = 32.95$, df = 3, 18; Table 2). Among the bean diets, significantly greater production occurred in boxes of diet with a perforated surface than in a nonperforated one. The VDFM diet decreased pupal yield by 12% compared with VMVM, but this difference was not significant. In this test, pupae from apples were still larger than all bean-reared pupae; however, not significantly so (males: $F = 0.68$, df = 3, 114; females: $F = 3.10$, df = 3, 114; Table 2). There were no statistical differences in the sex ratio ($F = 0.78$, df = 3, 19), but egg hatch on apples was significantly greater than on two of the bean diets ($F = 3.98$, df = 3, 18).

In the preservative experiment, significantly more pupae were reared on the control black bean diet than in diets containing increased amounts of sorbic acid or benomyl ($F = 46.82$, df = 6, 49; Table

Table 2. *G. molesta* ($\bar{x}$ ± SD) reared on fresh thinning apples and formulated diet in large plastic boxes when infested with 1,000 eggs

Formulation[a]	No. pupae per 1,000 hatch	Male wt, mg	Female wt, mg	Sex ratio, ♀:♂	Egg hatch
Apples	699.9 ± 32.6a	12.0 ± 1.8a	16.1 ± 2.5a	1.09 ± 0.32a	964.9 ± 12.0a
Small lima bean	477.4 ± 15.9b	11.3 ± 1.4a	15.3 ± 1.8ab	1.04 ± 0.06a	925.2 ± 32.6b
Small lima bean with VDFM	420.3 ± 78.1bc	11.7 ± 1.7a	15.2 ± 2.2ab	1.00 ± 0.17a	924.8 ± 24.1b
Small lima bean without perforations	364.5 ± 88.5c	11.3 ± 1.6a	14.6 ± 1.5b	0.86 ± 0.24a	934.7 ± 12.8ab

Values within a column that do not share the same letter are statistically different ($P < 0.05$) as determined by Tukey's studentized range test

[a] Six replicates of 1,000 eggs per replicate.

3). However, yields from diet containing additional amounts of MpH did not differ significantly from the control. Mold formed only in the control (four of eight boxes). Pupal sex ratio did not differ among treatments ($F = 0.73$, df = 4, 29). All diets produced similar sized pupae except those with benomyl, which produced significantly smaller *G. molesta* (males: $F = 4.01$, df = 4, 154; females: $F = 6.53$, df = 4, 151). Increased amounts of sorbic acid caused a significant decrease in the number of larvae that successfully hatched ($F = 9.20$, df = 6, 49).

Discussion

Yokoyama et al. (1987) developed a lima bean diet for rearing *G. molesta* that has by far surpassed any previous attempt to rear this insect on formulated diet. Our study has provided evidence that *G. molesta* will readily accept several substitutes for the small lima beans (which are only seasonally available in our region) and that the insect readily adjusts to mass-rearing techniques on the formulated diet. Attempts to make the rearing procedures less expensive or less time-consuming adversely affected pupal yields.

In experiments with small and large amounts of diets, fresh thinning apples were the best choice for rearing *G. molesta* (Tables 1 and 2). Of the legume diets tested, no single type consistently produced good results for the *G. molesta* characteristics we evaluated. However, small lima beans were

judged to be best because they produced the least expensive bean-reared pupae and the pupae were relatively robust.

Perforation of the diet surface appears critical for better yields; however, a device that is more efficient than a fork would be necessary for making holes. Currently, we rake a fork through the media to crudely mimic the perforations. Using a less expensive vitamin mix decreased pupal yields by 12% (although differences were not statistically significant) and only decreased the cost of ingredients by 6%. Therefore, this mix may not be advantageous in mass rearing *G. molesta*.

In the preservative experiment, using increased amounts of MpH, sorbic acid, or benomyl caused decreases in pupal yields, the latter two significantly. Because of these deleterious effects, one would probably best maximize production by choosing a legume type that produces high yields along with a propensity for low mold growth (beans with unpigmented seed coats) rather than by altering the level of preservative.

Although fresh thinning apples provide the best diet for some fruit pests, several workers have reported a decline in the quality of *G. molesta* (Tzanakakis & Phillips 1969, Szöcs & Tóth 1982) and a related apple pest, the codling moth, *Cydia pomonella* (L.) (Dickson et al. 1952, Hathaway et al. 1973) when reared from apples over a period of time because the apples degenerate in quality. We reared the largest pupae from fresh, 0–2 mo old apples. The pupae reared from apples by Yokoya-

Table 3. *G. molesta* ($\bar{x}$ ± SD) reared in small plastic boxes on black bean diet containing differing amounts of preservatives

Modification of black bean diet[a]	No. pupae per 100 hatch	Male wt, mg	Female wt, mg	Sex ratio, ♀:♂	Egg hatch
Original formula[b]	37.3 ± 13.2a	11.1 ± 2.0a	14.0 ± 2.3a	1.15 ± 0.33a	92.1 ± 2.7a
3 g sorbic acid	6.2 ± 2.3bc	11.6 ± 1.5a	14.9 ± 2.2a	0.90 ± 0.04a	80.9 ± 11.8b
6 g sorbic acid	0.1 ± 0.4c	—	—	—	74.4 ± 9.7b
1.2 g MpH	36.3 ± 6.5a	11.2 ± 1.5a	14.4 ± 2.2a	0.78 ± 0.30a	92.1 ± 3.4a
2.4 g MpH	27.0 ± 5.3a	11.6 ± 1.8a	13.6 ± 2.9a	0.94 ± 0.60a	91.3 ± 4.9a
1 g 50% benomyl[c]	12.8 ± 4.9b	9.2 ± 1.8b	11.1 ± 2.8b	1.04 ± 0.60a	91.3 ± 4.1a
2 g 50% benomyl[c]	0.4 ± 0.5c	—	—	—	91.6 ± 4.0a

Values within a column that do not share the same letter are statistically different ($P < 0.05$) as determined by Tukey's studentized range test.

[a] Eight replicates of 100 eggs per replicate
[b] 0.6 g MpH, 1.5 g sorbic acid, and 0 g benomyl (Yokoyama et al. 1987).
[c] Benlate wettable powder, Dupont Co.

ma et al (1987) were significantly smaller (males, 7 8 mg; females, 9.6 mg) compared with pupae reared on lima beans (males, 11.4 mg; females, 15.3 mg). The apples used in their study were possibly old or otherwise suboptimal. Pupae reared on lima beans in their study were similar in weight to ours

The formulated diet serves an important role in rearing *G. molesta* in the interim period between successive annual supplies of apples. Although the lima bean diet may not produce as many pupae as fresh thinning apples, the pupae reared on diet are large and healthy. The diet provides for maintenance of a *G. molesta* colony throughout the year which cannot be accomplished on apples alone Infesting >2,000 eggs on 875 g of lima bean diet yielded >1,000 large, healthy pupae (R S V., unpublished data); thus, beans are an adequate apple substitute for mass rearing *G. molesta* when the quality of apples declines.

Finally, one concern about the use of bean diet in pheromone studies is that *G. molesta* males have been shown to sequester a variety of compounds from apples, which they use for close-range attraction of calling females; males reared on formulated diets lack trans-ethyl cinnamate (Baker et al. 1981, Nishida et al. 1982) Although this deficiency does not prevent the males reared on lima bean diet from mating with females, it may affect the frequency at which females accept or reject a specific male in courtship studies (Löfstedt et al. in press).

Acknowledgment

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