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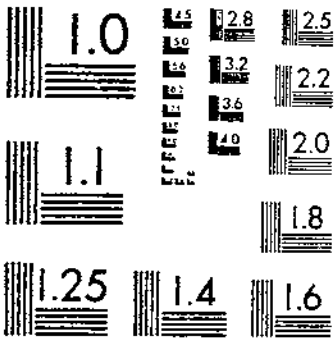


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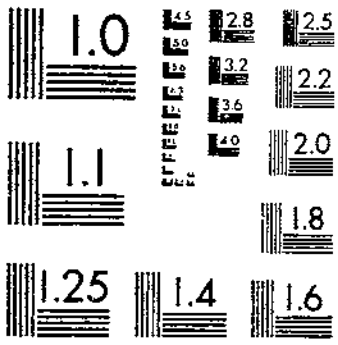


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# Mass Producing Eggs of the Greater Wax Moth, *Galleria mellonella* (L.)

Techn. l Bulletin No. 1510

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## Acknowledgments

We thank Don Hostetter for identifying the *Bacillus* associated with diseased larvae of *Galleria*, and Dr. E.W. Baker for identifying the mold mite infesting the diet of *Galleria*.

Washington, D.C.

Issued August 1975

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# Mass Producing Eggs of the Greater Wax Moth, *Galleria mellonella (L.)*[1]

By N. Marston, B. Campbell, and P. E. Boldt, *Biological Control of Insects Laboratory, North Central Region Agricultural Research Service, U.S. Department of Agriculture, Columbia, Mo.*

## Summary

Techniques for mass rearing larvae of *Galleria mellonella* and for collecting eggs from female moths are described and illustrated in this publication. It also gives cost estimates and presents a semiautomated scheme for production of eggs of *Galleria*.

Conditions for minimum cost of egg production are density of larvae in rearing pans—1,000 larvae/kg diet, density of moths in oviposition cages—898 to 1,345 pairs/m² of oviposition surface, and temperature for oviposition—30° C. Relative humidity had little effect on oviposition. Aureomycin can be added to the diet for disease control without adversely affecting egg production. Selection can increase the percent of body weight of females laid in eggs, decrease the number of days required for development, and decrease female body weight. Mean days to eclosion of eggs of *Galleria* vary from about 21.5 days at 20° to about 6.3 days at 35°. An increase in relative humidity from 20 to 90 percent decreased days to eclosion by about 1 day at all temperatures. No larvae emerged at 40°. Eggs that were 1, 2, or 3 days old and held at 30° were equally suitable for production of *Trichogramma pretiosum* Riley.

## Introduction

Eggs of Lepidoptera have been used extensively for rearing *Trichogramma* spp. and such predators as *Chrysopa* spp. for biological control programs. The Angoumois grain moth, *Sitotroga cerealella* (Olivier), has been chosen most often for producing eggs because it is easy to rear, and its diet of grain costs little. In this choice, egg size and quality have been given little attention. Marston and Ertle[2], however, demonstrated that eggs of the cabbage looper, *Trichoplusia ni* (Hübner) (13,872±266/g), produced more *Trichogramma minutum* Riley than eggs of *Sitotroga* (50,086±2,135/g). Also more of the parasites were females, and the females had greater fecundity, spent more time searching, and searched faster. They concluded that a *Trichoplusia* egg would provide females searching about 2.6 times more area than a *Sitotroga* egg. However, eggs of *T. ni* are expensive ($40.51/million, excluding labor) so a search was conducted for another species with eggs larger than those of *Sitotroga* to be considered as a host for rearing *Trichogramma* or predators.

Preliminary studies showed that *Galleria mellonella* (L.) has several advantages as a source of eggs for parasite production:

(1) Larvae can be reared on inexpensive artificial diet.
(2) No preoviposition period is present.
(3) Females require no food.
(4) Females have great fecundity.
(5) Most eggs are laid in a short time.
(6) Eggs have a tough chorion which resists breakage.
(7) Eggs are relatively large.
(8) The embryo requires a relatively long time to develop so that the eggs may be suitable for parasite development for several days.

Techniques were developed for mass producing eggs of *Galleria* so that it can be considered as an alternative to *Sitotroga* and other grain-infesting moths as a source of eggs for rearing *Trichogramma* or predators. Tests were conducted at the Biological Control of Insects Laboratory, Columbia, Mo., from December 1970 through January 1972.

[1] Lepidoptera: Pyralidae.

[2] MARSTON, N. and ERTLE, L.R. HOST INFLUENCE ON THE BIONOMICS OF "TRICHOGRAMMA MINUTUM" Ann. Ent. Soc. Amer. 66: 1155–1162. 1973.

## General Facilities

Two rooms, 3.05 by 2.44 by 2.55 m, were used for larval rearing and oviposition, and a work area, 3.05 by 4.57 m, outside the rooms was necessary for producing eggs of *Galleria* at the rate of about 1 million eggs per day. Each room had lights, a power source, and equipment for maintaining 65±5 percent relative humidity (RH) and 30±2° C. For larval rearing, there were three tiers of shelves, each 46 cm deep and 38 cm apart starting 38 cm from the floor on two sides of the room. Water, power, a floor drain, and at least 3.34 $m^2$ of bench space were needed in the work area. A refrigerated area for storing diet ingredients was also desirable.

For oviposition, boxes with shelving to hold pans of larvae and pupae were required along with the special oviposition cages containing plates from which egg collection was made at planned intervals.

## Larval Rearing

Larvae can be reared in any sort of pan or jar. It was most efficient to use 4.4-liter glass battery jars containing 2.2 liters of artificial diet (fig. 1) for the first 2 weeks of larval development because they required little space. Then, larvae were transferred to round, galvanized iron pans (11.5 liters) with more diet (fig. 2). Larvae required about 2 weeks to complete development, at 30° C, after transfer to the larger pans. Larvae constructed their cocoons in the diet around the edges of the pans. Larvae will construct their cocoons in a crumpled newspaper placed on the diet, but this may delay pupation of some larvae in cocoons for several months.[3]

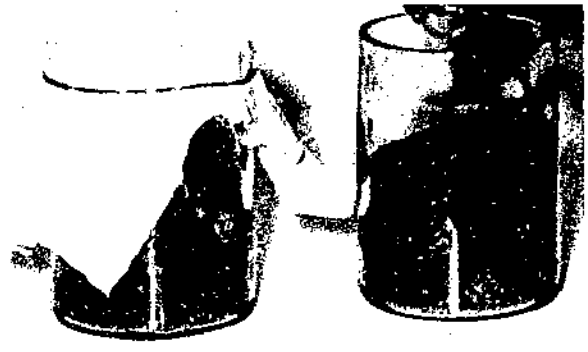

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Figure 1.—Application of eggs of *Galleria* to diet in the 4.4-liter glass battery jars used for the first 2 weeks of larval development.

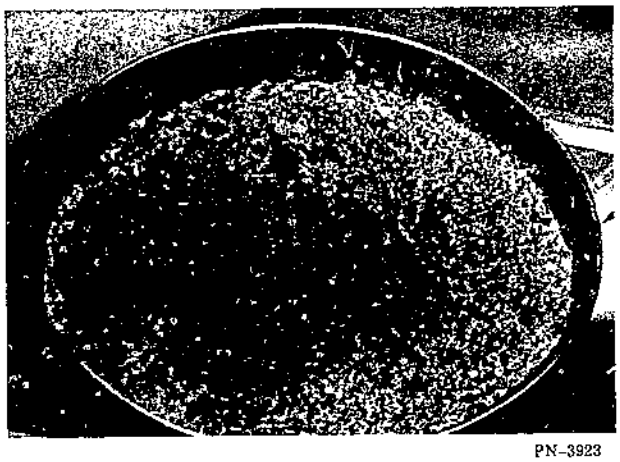

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Figure 2.—Cocoons of *Galleria* formed in diet in the galvanized iron pans used for the last 2 weeks of larval development.

The following diet is tentatively recommended for mass rearing *Galleria*: bran, 260 g; Wheast,[4] 65 g; wheat flour, 162 g; cornmeal, 162 g; glycerin, 193 g; and water, 158 g. Cost of the diet was $0.1866 per kilogram (bulk prices, September 1971). Tests to define the optimum proportion of each ingredient are in progress.

Liquid ingredients of the diet were mixed and added to the bran. Remaining dry ingredients that had been previously mixed were then added to provide even distribution of the cornmeal, flour, and Wheast over the bran. The diet may be mixed in any large container or barrel, but a 56.6 liter capacity cement mixer (fig. 3) greatly reduced labor.

The number of larvae necessary to infest the diet can vary widely without grossly affecting egg production. A decrease in larval density results in an increase in size of adult females and in higher egg production per female (app. 1). The optimum is about 1,100 eggs/kg (= 37.6 mg). If 89 percent of the eggs hatch (app. 5) and 79 percent

[3] MARSTON, N., and CAMPBELL, B. COMPARISON OF NINE DIETS FOR REARING "GALLERIA MELLONELLA." Ann. Ent. Soc. Amer. 66: 132–136. 1973.

[4] Registered trademark of a whey-grown yeast product.

of the first instars reach the cocoon stage, about 773 cocoons/kg would be produced. This is near the number produced with an initial infestation of 1,000 partly grown larvae/kg in the larval density experiment.

A daily production of nearly 984,000 eggs can be maintained if 3.5 kg of diet is infested with 1,100 eggs/kg (See Cost Estimates) daily. Diet was mixed and infested weekly for convenience without causing large cyclic fluctuations in egg production. About 0.5 kg of diet was placed in each of seven battery jars for the first 2 weeks of development. Each jar was infested with about 3,850 eggs (= 132 mg). After the 2-week initial incubation period, larvae in the battery jars were divided between two galvanized iron pans, each with an additional 1.5 kg diet. Seven battery jars and 14 pans were infested weekly, requiring 24.5 kg of diet.

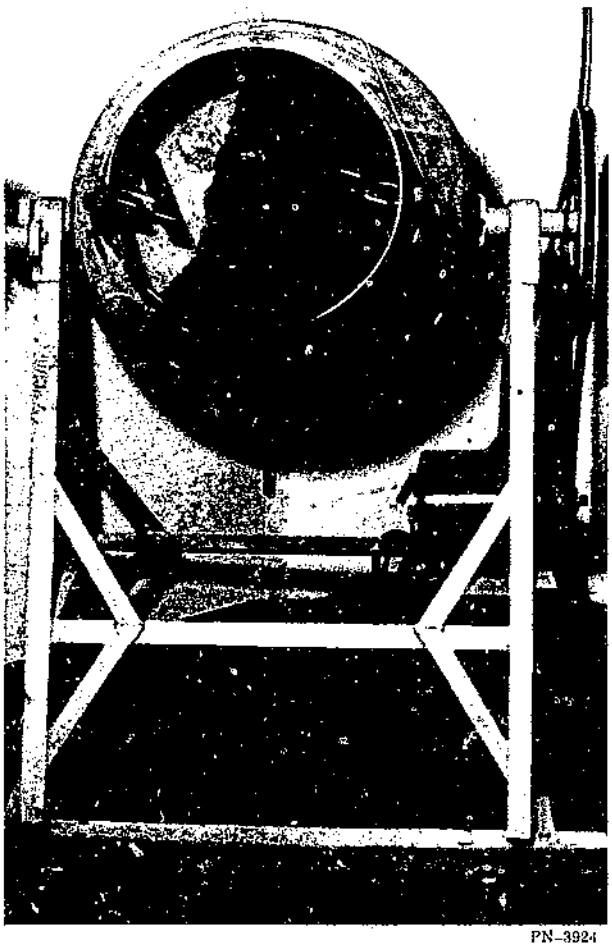

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Figure 3.—Mixing of diet for larvae of *Galleria* in a cement mixer with a 56.6-liter capacity.

Few pests were encountered in the tests. An infestation of a larval parasite, *Bracon hebetor* Say, was destructive at first. The parasite was controlled by placing screens over the rearing pans. The mold mite, *Tyrophagus putrescentiae* (Schrank), built up in large numbers on the diet of the wax moths and reduced the size and vigor of the adults. This mite was controlled by careful sanitation. Large populations of cockroaches built up on spilled food and scales. The straw itch mite, *Pyemotes ventricosus* (Newport), the most destructive pest of *Sitotroga*, was accidentally introduced into a container with *Galleria*, but died without reproducing. A few dead, black *Galleria* larvae, usually found in pans with high larval density, contained bacteria tentatively identified as *Bacillus thuringiensis*, var. *galleriae* Svecova. Aureomycin can safely be added to the diet for disease control, if needed (app. 7).

## Adult Emergence and Oviposition

Larval development was almost completed within 28 days. Then, pans with mature larvae and pupae were transferred to emergence boxes (fig. 4). The boxes were 121.9 cm high by 101.6 cm wide by 71.1 cm deep and had five shelves. Other sizes may utilize space more efficiently in an emergence room. The front of each box was closed with a plywood door attached by hooks at the sides. Cracks around the front of the box were sealed with masking tape to prevent escape of the moths. The number of boxes required at a given level of production depends upon the capacity of the boxes and the length of time required for moth emergence. Three boxes were necessary because emergence was almost complete in 3 weeks.

Emergent moths exited from the box through a plastic tube (2.54 cm diam) inserted into a hole near the upper end (fig. 5). A 25 W bulb on the floor attracted the moths through the translucent plastic tube into an oviposition cage. The sides and bottom of the oviposition cages were constructed of 32-mesh Saran screen over a frame 76.2 cm long by 61 cm high by 61 cm deep (fig. 6). Plywood tops, 1.27 cm by 45.7 cm (fig. 7), had 10 slits. The slits were lined with 1.9 cm weather stripping attached to the plywood with staples

PN-3925

Figure 4.—Pans containing cocoons of *Galleria* arranged in an emergence box.

(fig. 8). Duplicate cages were required so one could be cleaned while the other cage was in use.

Adults in these cages oviposited on plastic sheeting (45.7 by 61 cm), which had been sprayed with a fine mist of 10 percent sugar (sucrose) water, and coated (both sides) with granulated sugar (fig. 9). After the sugar dried, the plates were inserted through the slits in the top of the oviposition cage (fig. 10). Two bolts near the tops of the sheets prevented them from falling through the slits. Cages were alternated and cleaned at 1- to 2-week intervals. Because some moths in the first cage had not completed oviposition, half of a fresh set of plates were placed in the first cage and half in the second cage for 3 days after the cages were changed. Most moths in the first cage died in 7 to 10 days after the cages were changed. The cages were placed on blocks to permit circulation of air which inhibited molding of the dead moths. The oviposition room was cleaned weekly when the pans in the emergence boxes were changed. A small, portable vacuum cleaner with disposable bags removed dead moths from the emergence box and collected escaped moths from the walls.

## Egg Collection

Plates were changed at 3-day intervals since 1-, 2-, and 3-day-old eggs were equally suitable for production of *Trichogramma* (app. 10). The plates were placed in a washtub that had a hose at

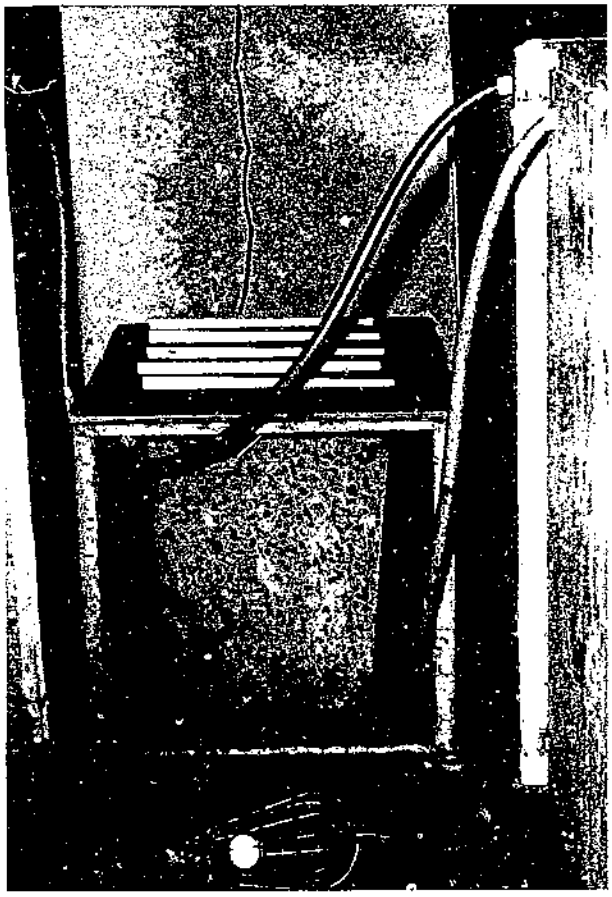

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Figure 5.—Plastic tubes for passage of *Galleria* from emergence boxes to an oviposition cage.

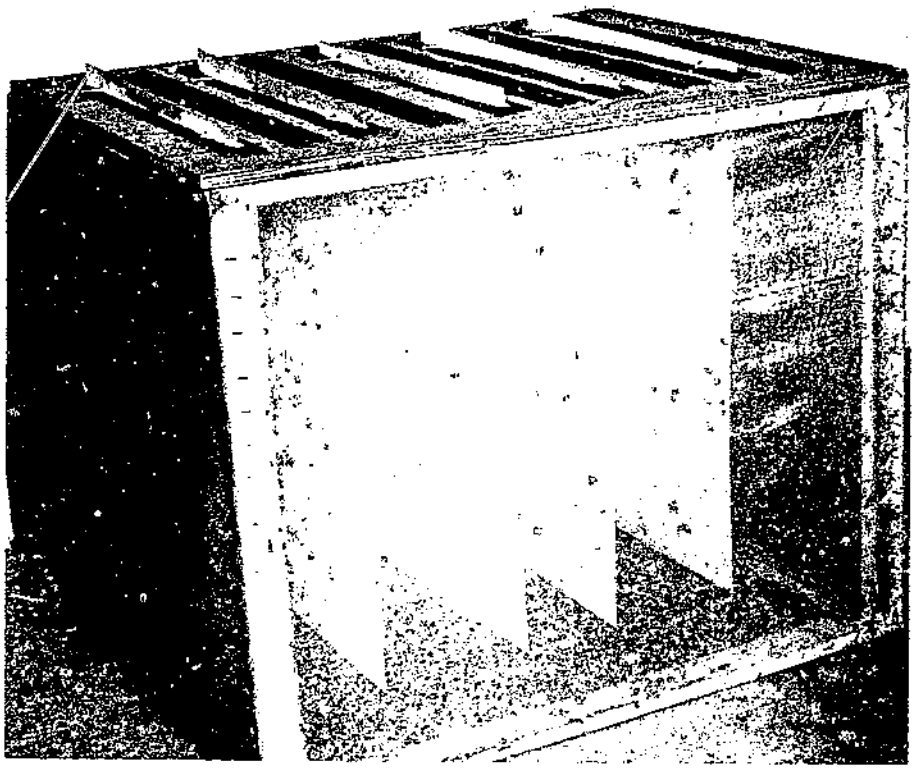

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Figure 6.—An oviposition cage for *Galleria* containing sugar-coated oviposition plates.

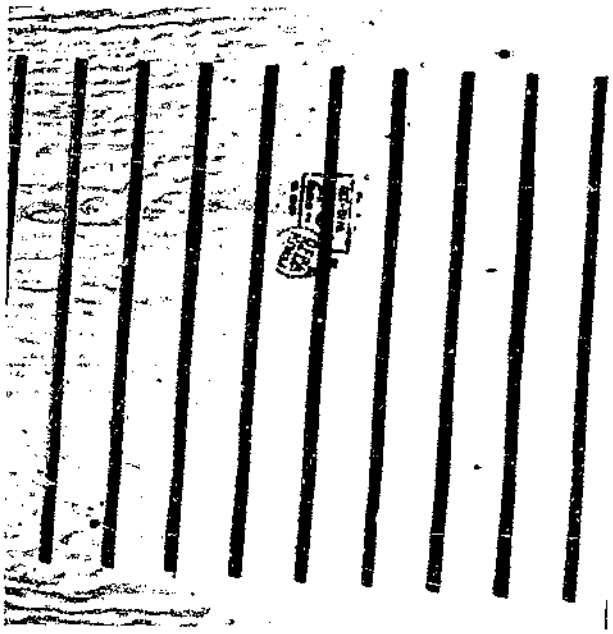

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Figure 7.—Underside of the lid of an oviposition cage for *Galleria* showing the slits through which the oviposition plates are inserted.

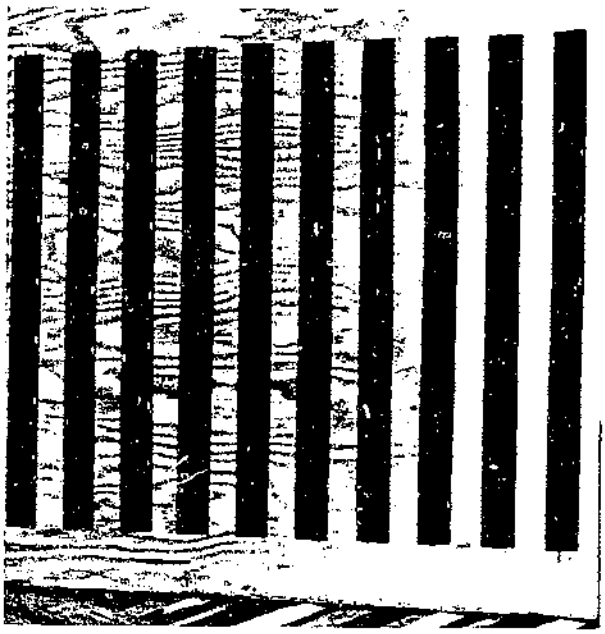

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Figure 8.—Upperside of the lid of an oviposition cage for *Galleria* showing the weather stripping that lines the slits through which the oviposition plates are inserted.

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Figure 9.—Application of granulated sugar to an oviposition plate which had been sprayed with a mist of sugar water.

the bottom. Then, the sugar was dissolved in water, and the eggs were dislodged with a sponge (fig. 11). The water and eggs passed through the hose to a 60-mesh sieve placed over a drain (fig. 12). Some scales were present along with the eggs, so about 20 ml of a dry biodegradable detergent was added to the water to induce the scales to pass through the sieve. (The detergent residues did not noticeably affect survival of *Trichogramma*.)

The eggs were then suspended in water, and poured onto a velvet cloth stapled to a wooden

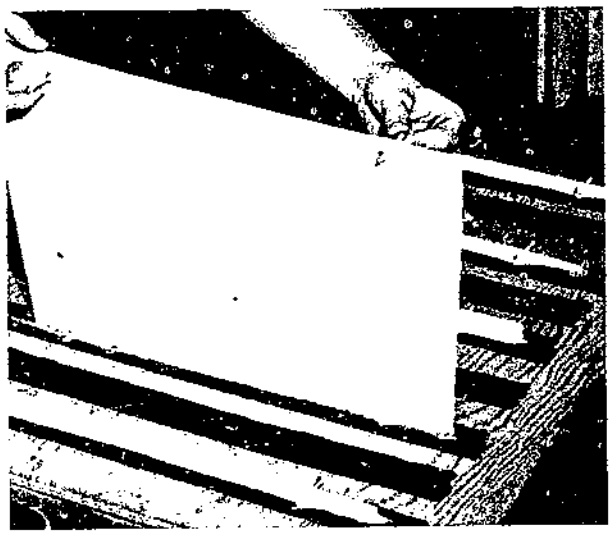

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Figure 10.—Insertion of an oviposition plate into an oviposition cage for *Galleria*.

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Figure 11.—Removal of eggs of *Galleria* from an oviposition plate.

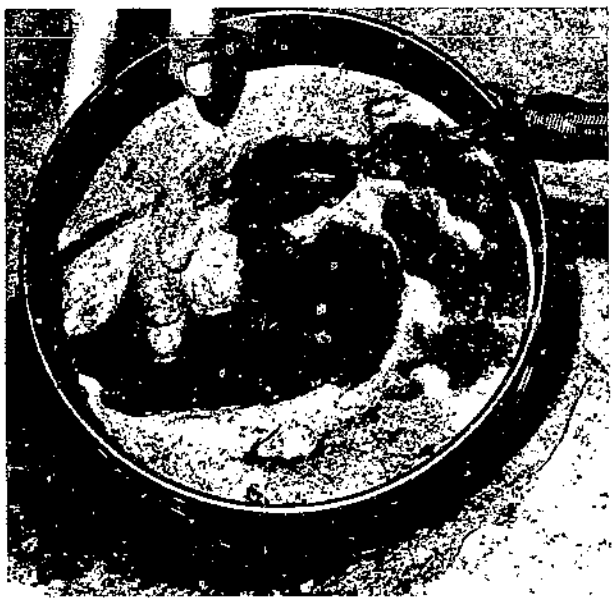

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Figure 12.—Passage of water containing eggs of *Galleria* through a sieve to concentrate the eggs.

frame (30.5 by 45.7 cm) (fig. 13). The cloth was wet thoroughly before the eggs were poured onto it so that the water would pass through. The eggs were separated singly or in small groups by brushing them across the individual fibers of the pile of the velvet. The cloth dried in about 15 min before a fan. The eggs were dislodged with a stiff-bristled brush into a wood and fiberboard trough (fig. 14) which funneled them into a wax-coated paper cup. Three cloth frames will dry about 1 million eggs.

PN-3934

Figure 13.—Distribution of eggs of *Galleria* over a velvet covered drying frame.

## Cost Estimates

Egg production from 24.5 kg/week infested at the rate of 1,100 eggs/kg can be estimated as follows:

24.5 kg × 1,100 eggs/kg = 26,950 eggs;

26,950 eggs × 89 percent hatch (app. 5) = 23,986 first instar larvae;

23,986 first instar larvae × 79 percent larval survival (app. 1) = 18,949 cocoons;

18,949 cocoons × 87 percent adult emergence from cocoons (app. 1, average for all treatments) = 16,486 adults;

16,486 adults × 53 percent females (app. 1, average for all treatments) = 8,738 female moths;

8,738 female moths × 27 mg eggs/female (app. 2, production at optimum density) = 235,926 mg eggs; and,

235,926 mg eggs × 29.21 eggs/mg (app. 6) = 6,891,398 eggs/week, or 984,485 eggs/day.

Survival of first instars would be lower than 79 percent since that figure estimated survival of 2-week-old larvae. However, lower larval survival would result in larger females which would then lay more eggs. Thus, quantity of eggs produced would be little affected. The expected production of eggs at optimum density ($\hat{y}$) was obtained from the formula: $\hat{y} = 35.38 - 0.15X$, where $X = 56$ females/445.74 cm$^2$ (app. 2).

The diet for rearing *Galleria* costs $0.1866/kg (September 1971). The cost would be $4.57 to produce about 6,900,000 eggs/week. About 4 h/week ($8 at $2/h) were required for mixing and infesting the diet, changing pans, and cleaning rooms and equipment.

Cost of collecting and drying the eggs depends upon the density of female moths on the oviposition plates and the number of times per week the eggs are collected. Number of females in the oviposition cage would be five times the number entering the cage daily if the females live an average of 5 days, for instance, 1,248 × 5 = 6,240 females. At the optimum density for egg collection (1,256 females/m$^2$; app. 2) 4.9 m$^2$ of sugar-coated surface area would be needed. About 1 h ($2) was required for collecting and drying the eggs from 10 plates covered with 2.9 kg of sugar on a total surface area of 5.6 m$^2$ ($0.70; app. 2).

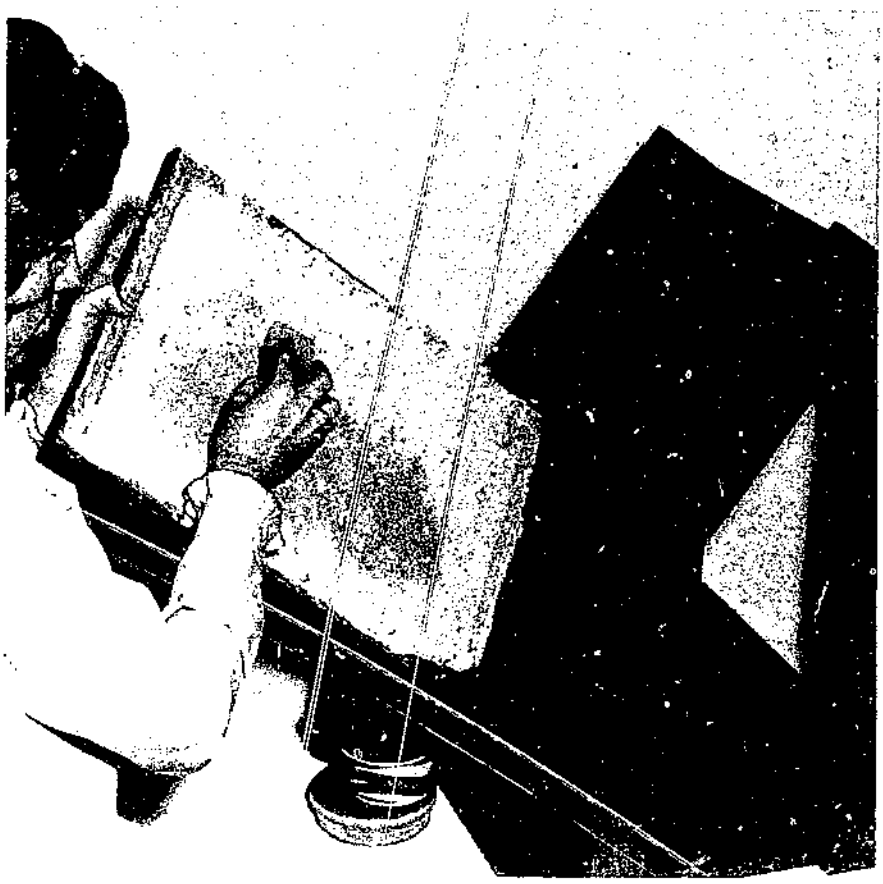

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Figure 14.—Removal of dried eggs of *Galleria* from a drying frame to a wooden trough and then into a waxed paper cup.

Total cost each time the eggs were collected would be ($2.00 + 0.70) × 4.9/5.6 = $2.36. Weekly cost would be $5.51 if the eggs were collected every 3 days (app. 10). Total weekly cost would be $18.08 ($4.57 + 8.00 + 5.51) for 6,900,000 eggs or $2.62 per million eggs. Total cost breakdown, excluding overhead would be: diet—$0.66; sugar—$0.21; and personnel—$1.75.

## Appendix

**1. Effect of Larval Density on the Bionomics of *Galleria mellonella*.**—Five larval densities, 50, 75, 100, 125, and 150 larvae/100 g diet, were evaluated to determine the best density for mass rearing moths for egg production. The diet consisted of 234 g bran, 351 g CSM,[5] 65 g Wheast, 175 g glycerin, and 175 g water/ kg. Larvae were reared in battery jars for 2 weeks, then transferred to 100 g diet in 1.1-liter polystyrene containers[6] and held in the dark at 30° ± 1° C and 60 ± 5 percent RH. Number and sex of emerging moths were recorded and females were weighed daily. Five females from each container were placed in oviposition cages[7] to determine their fecundity. Females tested for oviposition were those emerging 6 or more days after emergence of moths began, preventing bias from testing early emerging moths. Excess diet was removed from 2 to 14 days after emergence began to stop development of the mold mite. The mites had no apparent gross deleterious effects on the parameters measured. The tests, replicated six times, were ter-

[5]Registered trademark of product containing cornmeal, soybean flour, and dried milk.

[6]See reference in footnote 3, p. 2.

[7]See reference in footnote 2, p. 1.

TABLE 1.—Effect of larval density on the bionomics of *Galleria mellonella*[1]

| Larvae/ 100 g diet | Larval survival | Adult emergence | Females | Beginning of emergence | Span of emergence | Mean emergence | Body weight of females | Egg weight female | Body weight laid in eggs | Estimated egg potential/kg diet [2] |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Percent* | *Percent* | *Percent* | *Days* | *Days* | *Days* | *Milligrams* | *Milligrams* | *Percent* | *Grams* |
| 50 ..... | 87.20a | 90.18a | 51.01a | 17.20a | 13.20a | 23.19a | 143.01a | 48.57a | 34.79a | 10.77a |
| 75 ..... | 86.93ab | 83.81a | 54.86a | 18.00a | 15.00a | 24.04a | 130.56a | 48.11a | 37.29a | 14.19a |
| 100 ..... | 79.20ab | 88.11a | 47.52a | 19.40a | 18.20a | 25.27b | 108.99b | 40.80ab | 35.71a | 14.22a |
| 125 ..... | 65.12bc | 87.33a | 56.71a | 22.00a | 14.40a | 28.13c | 93.97bc | 36.84b | 35.52a | 12.35a |
| 150 ..... | 62.79c | 87.90a | 53.23a | 21.40a | 16.00a | 28.37c | 86.29c | 33.29b | 35.87a | 13.75a |
| Standard error | [3]3.93 | [3]3.06 | [3]3.83 | 3.36 | 1.67 | .66 | 5.01 | 2.78 | [3].62 | .21 |

[1]Means in the same column followed by the same letter are not significantly different at the 5-percent level.
[2]Total moths/100 g diet × 10 × percentage females × body weight of females × percentage of body weight laid in eggs.
[3]From arcsin transformations.

minated 6 weeks after the diet was infested, allowing 2 to 3 weeks for larval development and 3 to 4 weeks for adult emergence. Cocoons were counted when the tests were terminated to determine percentage larval survival, and dissected to determine percentage emergence.

Increasing larval density decreased larval survival (table 1) [b (sample regression coefficient) = -0.27 ± 0.16% larva] and increased mean days to emergence slightly (b = 0.06 ± 0.03 days/larva). The greatest effect was on female body weight which decreased by 0.59 ± 0.16 mg/larva. Because the percentage body weight laid in eggs was not significantly affected, egg weight decreased in proportion to body weight (b = -0.17 ± 0.07 mg/larva). Total egg potential per kilogram of diet was estimated by multiplying the total moths per 100 g diet by 10 by percentage females by mean body weight by mean percentage of body weight laid in eggs. The last two figures were necessary because the measure of actual egg production was for five females from each container, and their weight often varied greatly from the mean for all females. These estimates did not vary significantly between densities since the greater number of moths produced at high densities was smaller and therefore laid fewer eggs. Within the limits tested, larval density was not a critical factor in egg production of *Galleria*. A practical initial infestation was 1,000 2-week-old larvae/kg diet.

**2. Effect of Moth Density on Oviposition of *Galleria mellonella*.**—In the first of two experiments, moths were placed in a 1.1-liter polystyrene oviposition cage with and without a cardboard divider (374 cm² surface area). The sides and bottom of the cages were coated with granular sugar. One set of cages had 445.7 cm² covered with sugar and 547.8 cm² total surface area. The set with dividers had 445.7 cm² covered with sugar and 921.8 cm² total surface area.

Moths were collected from a mass production emergence box by inserting the end of the plastic tube into the cage through which the moths emerged into a container. The 0– to 1-day-old moths were anaesthetized with $CO_2$, females were weighed, and then 30 males and 30 females were placed in each oviposition cage. The containers were placed in darkness at 30° ± 2° C and 70 ± 10 percent RH for 3 days. Eggs were washed onto a cotton organdy screen, dried, and weighed. Larvae for the mass production unit were fed a diet containing 234 g bran, 351 g CSM, 65 g Wheast, 175 g glycerin, and 175 g water. There were 10 replications for each treatment tested in a completely random sequence.

The percentage of total female body weight laid in eggs was analyzed rather than total egg weight because the groups of females varied greatly in weight which is closely correlated to egg production. Females in the containers with dividers laid an average of 30.8 ± 3.7 percent of their body weight compared with 26.6 ± 4.6 percent for females in the containers without dividers. A *t*-test of arcsin transformations of the percentages indicated that the difference was nonsignificant ($0.10 < P < 0.20$), but the results are inconclusive because of the high standard error (1.6 arcsin units).

The second experiment was similar to the first except that number of pairs of moths in the containers was varied from 20 to 60 at intervals of 1. Dividers were used in all containers because egg production seemed to increase slightly when they were included. The mean weight of eggs per female was adjusted to be relative to the overall mean female weight (107.2 mg). This removed error because of the considerable differences in weight between the groups of females. The regression slope for the adjustment (b = 0.27 ± 0.07 mg eggs/mg body weight) was taken from another experiment with the same diet.

The regression formula for adjusted egg weight per female on pairs of moths per container was $\hat{y} = 35.38 - 0.15X$. Confidence interval for b $= -0.15 \pm 0.10$. Cost per million eggs depended upon (1) cost of sugar and labor in removing and drying the eggs, which decreased linearly as density increased, and (2) cost of moths, which increased linearly (within the limits tested) as density increased. Coating of the oviposition cages required 23.11 ± 0.53 g of sugar (= 518.46 ± 11.88 g/m²; $0.1254/m² at $0.242 kg). About 1 h was required to remove the eggs and replace the sugar on 10 plates, each with 5,575 cm² of surface area. At $2/h, cost would be $0.3583/m². Cost per container for sugar and labor was $0.0216 (= $0.4837/m²). Assuming diet cost of $0.1866/kg and labor of $0.3265/kg at $2/h (Cost Estimates section) and moth production as in table 1 from 100 larvae/100 g diet, cost of moths would be $0.0015/female.

Cost per million eggs (C) is given by the formula:

$$C = \frac{N}{\hat{y}}(M + \frac{S}{X})$$

Where, N = weight of 1 million eggs, 34,230 mg;
M = cost per female moth, $0.0015;
$\hat{y}$ = estimated milligrams eggs produced per female moth at the density, × (35.38 - 0.15X);
S = cost of sugar and labor for egg removal per container, $0.0216; and
X = density of moths per 445.74 cm².

It is difficult to extrapolate to mass production conditions in which moths would be continuously entering the cages and dying. Accurate estimates of effect of density would require estimates of longevity of females in the cages. If the density in mass production cages is assumed to be about five times as great as the number of moths entering daily (probably a realistic estimate because average longevity under dense conditions would be lower than the figures given in table 2), the expected egg production would be about 20 percent of the figures given in this experiment. For example, under mass production conditions egg production at 50 females/445.74 cm² would be 20 percent of the value obtained in this experiment because egg production would depend upon the number of females entering the cage daily, rather than total number of females in the cage. Cost of moths would also be 20 percent of the figure for a given density. Cost of sugar and labor for egg collection is based on the total number of moths in the cages, but it would be 33 percent of the figures used in this experiment if eggs were collected at 3-day intervals.

Given the above conditions, cost per million eggs would decline from $3.44 at a density of 20 pairs/445.74 cm² to $2.66 at a density of 56 pairs/445.74 cm² (= 1,256 pairs/m²). There would be little difference in cost between 40 pairs ($2.73) and 70 pairs ($2.70). A 4.9 m² sugared surface would be required for egg production at minimum cost with a weekly production of 8,738 females (Cost Estimates). Actual egg production would be somewhat greater than estimated because females in mass production cages would be ovipositing throughout their lifetimes rather than for 3 days.

Optimum density should be recalculated if sig-

TABLE 2.—Longevity and fecundity of females of *Galleria mellonella* at 5 temperatures[1]

| Temperature | Longevity of females | Total egg weight per female | Eggs laid in 3 days |
|---|---|---|---|
| °C | *Days* | *Milligrams* | *Percent* |
| 20 ..... | 19.61e | 47.58b | 49.37a |
| 25 ..... | 14.08d | 50.17b | 56.76a |
| 30 ..... | 9.74c | 67.56c | 77.06b |
| 35 ..... | 6.33b | 51.69b | 83.53b |
| 40 ..... | 3.79a | 14.81a | 93.32c |
| Standard error | [2,3].14 | [2]5.32 | [2,4]4.13 |

[1]Means in the same column followed by the same letter are not significantly different at the 5-percent level.
[2]Computed from average number of observations per treatment.
[3]From square root transformations.
[4]From arcsin transformations.

nificant changes occur in egg production per female (either through changes in moth size or percent body weight laid in eggs) or in cost of female moths or cost of egg collection (through automation or price increases). Information is not available on the effect of decreasing the proportion of surface area exposed to moths which is covered with sugar. Such decreases would greatly reduce cost of production, especially in a semiautomated system.

**3. Effect of Temperature on Oviposition of *Galleria mellonella*.**—To determine the optimum temperature for production of eggs of *Galleria*, 0- to 24-hour-old female moths reared from Beck's diet[8] were weighed, confined individually with a male in sugar-coated oviposition cages,[9] and placed in incubators at 20°, 25°, 30°, 35°, and 40° ± 0.5° C. Humidity was held at 70 ± 10 percent RH. Living moths were transferred to new cages after 3 days. Eggs were removed, air-dried, and weighed at this time and again when the females died. There were 14 replications, but because of accidents, the number of females tested at each temperature varied from 11 to 14. Female longevity (table 2) was transformed to its square root before analysis, because the variance was proportional to the mean, and percentage of eggs laid in 3 days was transformed to its arcsin. Differences between means were tested by Kramer's[10] modification for unequal sample sizes of Duncan's new multiple range test.

The optimum temperature tested for oviposition of *Galleria* was 30° C. At lower temperatures metabolism apparently slowed to such an extent that the females did not achieve their full potential, while at higher temperatures, especially 40° C, females died before reaching their potential. Percentage of eggs laid in 3 days increased as longevity decreased. Based on these results, the 3-day egg potential cited in the comparison of diets reported by Marston and Campbell[9] was about 75 percent of the true potential of the insects.

TABLE 3.—Oviposition of *Galleria mellonella* at 6 relative humidities[1]

| Relative humidity | Total egg weight per female | Body weight laid in eggs | Eggs laid in 3 days |
|---|---|---|---|
| *Percent* | *Milligrams* | *Percent* | *Percent* |
| 20 | 47.96a | 33.88a | 85.53a |
| 40 | 48.67a | 36.71a | 81.24a |
| 60 | 54.20a | 38.82a | 75.45a |
| 70 | 52.42a | 40.10a | 78.15a |
| 80 | 55.78a | 40.84a | 75.95a |
| 90 | 59.22a | 39.60a | 65.79a |
| Standard error | [2]4.88 | [2]1.83 | [2] [3]3.12 |

[1] Means in the same column followed by the same letters are not significantly different at the 5-percent level.

[2]Computed from average number of observations per treatment.

[3]In arcsin units.

**4. Effect of Relative Humidity on Oviposition of *Galleria mellonella*.**—Female moths were weighed and placed in oviposition cages as in the previous experiments to determine the optimum relative humidity (RH) for egg production. Cages were placed in incubators at 20, 40, 60, 70, 80, or 90 ± 10 percent RH and 30° ± 0.5° C. Eggs were weighed after 3 days and again after 6 days when the tests were terminated. Moths were reared from the CSM diet of Marston and Campbell.[11] Number of females tested varied from 6 to 7.

There was no difference in total eggs laid at the five humidities (table 3), but the data were highly variable. The percentage of body weight laid in eggs was computed to remove variability from differences in female weight which strongly affects fecundity.[11] Again, analysis of variance demonstrated no difference because of humidity, but there was a significant regression. Percentage of body weight laid in eggs increased by 0.9 ± 0.6 percent for each 10 percent increase in relative humidity. If humidity did affect fecundity, the effect was so small that expensive humidity control devices would be merited only in large-scale operations. Differences in percentage of total eggs laid in 3 days were nonsignificant by analysis of variance, but there was a significant regression, the percentages decreasing by 0.22 ±0.14 percent for each 10 percent increase in rel-

[8]BECK, S. D. GROWTH AND DEVELOPMENT OF THE GREATER WAX MOTH. Trans. Wis. Acad. Sci. Arts and Let. 49: 137–148. 1960.

[9]See reference in footnote 3, p. 2.

[10]KRAMER, C. Y. EXTENSION OF MULTIPLE RANGE TESTS TO GROUP MEANS WITH UNEQUAL NUMBERS OF REPLICATIONS. Biometrics 12: 307–310. 1956.

[11]See reference in footnote 3, p. 2.

ative humidity. This suggests that the equivocal depressing effect of low humidity on fecundity increased with time of exposure.

**5. Viability of Eggs of *Galleria mellonella*.**—Eighteen samples of about 50 eggs were taken from a mass production unit to test fertility of the eggs. The eggs were placed individually in cells and held at 23° ± 5° C and 51 ± 3 percent RH. Percentage fertility was recorded after larval emergence was complete. Fertility varied from 78.6 to 96.3 percent except for one group with 10.7 percent. A $\chi^2$ test showed that the 17 larger values did not vary significantly from their overall mean, 88.7 ± 2 percent ($0.25 < P < 0.50$). There is no ready explanation for the low percentage hatch from the one sample.

**6. Weight and Volume of Eggs of *Galleria mellonella*.**—The weights of 28 lots of 100 eggs were recorded to the nearest microgram to establish a confidence interval for number of eggs per gram. Eggs were laid by females reared on Balázs[12] diet. There were 29,214 ± 626 eggs/g or 34.23 ± 0.73 g/million eggs. There were 50,093 ± 2,135 eggs of *Sitotroga*/g or 19.96 ± 0.85 g/million eggs. Twenty 1-milliliter samples of *Galleria* eggs were weighed to the nearest microgram to establish a weight to volume ratio; 1 ml = 502 ± 19 mg.

**7. Effect of Aureomycin in a Diet for *Galleria mellonella*.**—A small number of larvae of *Galleria* regularly succumbed to an infection of *Bacillus thuringiensis*, var. *galleriae*, in dense populations. The disease did not become epidemic in the tests, but an experiment, with 10 replications, was initiated to determine the effect of Aureomycin (5.5% chlortetracycline hydrochloride) on the bionomics of *Galleria*, if it should be needed for disease control. Aureomycin was chosen because it was readily avalable as a constituent of the agar diet for rearing *T. ni* at this laboratory. Other antibiotics may prove superior for disease control. Techniques for testing the diets were described by Marston and Campbell.[13] The diets consisted of 25.9 g CSM, 17.3 g CSMA[14], 10.0 g Wheast, 20.5 g honey, 18.1 g glycerol and 8.2 g water with or without 0.25 g Aureomycin. Fifty first instar larvae were placed on 100 g of each diet and held in continual darkness.

The greatest effect noted was a decrease in survival of first instar larvae to the cocoon stage on the diet with Aureomycin as opposed to the check (28.6 as to 61%, respectively). Differences in days to beginning of emergence (36.7 as to 34.3) were nonsignificant, as were differences in mean days to emergence (40.3 as to 39.8), span of emergence (10.6 days as to 18.0 days), percent emergence from cocoons (89.4 as to 96.1%), percent females (50.7 as to 47.3%), female weight (141.6 as to 145.9 mg), and weight of eggs laid per female (57.4 as to 58.2 mg). However, inexplicably the percent of female body weight laid in eggs was significantly greater for the diet with Aureomycin, although the difference was small (41.4 as compared with 39%).

Because none of the parameters critical to egg production were adversely affected, Aureomycin can be added safely to the diet for protection from disease. The difference in percentage survival is of little importance since less than 0.5 percent of the egg production would be required for production of a succeeding generation. Number of eggs used to infest the diet should be increased by about 78 percent to compensate for the decrease in larval survival if Aureomycin is in the diet.

**8. Effect of Selection on Fecundity of *Galleria mellonella*.**—A test was initiated to determine if a strain of moths could be established which would more efficiently convert diet into eggs. Because weight of eggs depends on female weight,[15] selection for egg weight would lead to selection of large females which need not be more efficient in utilizing diet. Consequently, selection was made for high percent of body weight laid in eggs (PBW). The best five of 67 females were selected as the parents for the $F_1$. They came from another experiment, one that tested the effect of dried milk in the diet. The diet consisted of 254 g CSM, 170 g CSMA, 77 g Wheast, 194 g honey, 171 g glycerol, 50 g dried milk, and 77 g water/kg.

Fifty first instars were placed on 100 g diet for the $F_1$ and $F_2$ generations; for the $F_3$ generation the number of larvae was increased to 75 since larval survival decreased greatly during the preceding generation. The containers were held in

[12] BALÁZS, A. NUTRITIONAL AND NERVOUS FACTORS IN THE ADAPTATION OF "GALLERIA MELLONELLA" TO ARTIFICIAL DIET. Acta Biol. Acad. Sci. Hung. 9: 47–69. 1958.

[13] See reference in footnote 3, p. 2.

[14] Housefly medium.

continual darkness at 30° ± 1° C and 60 ± 5 percent RH during larval and pupal development. All emerging females were placed in individual oviposition cages.[15] Egg production was recorded after 3 days.

The five females with the best PBW were selected as parents for the succeeding generation. Even though no attempt was made to regulate mating, most females would have mated to siblings since they mate soon after emergence. When males were not present in the same container, they were transferred from another container. The test was terminated at the end of the $F_3$ generation when other work with *Galleria* was completed.

The increases in the PBW in the $F_1$ and $F_2$ generations (table 4) were highly significant ($P<0.001$) as was the decrease in the $F_3$ generation. Larval survival to the cocoon stage decreased from 42.0 ± 4.9 percent in the $F_0$ and 39.6 ± 6.1 percent in the $F_1$ to 17.6 ± 4.7 percent in the $F_2$. Most female moths emerged alone in the $F_2$ because of the decrease in larval survival. Because there were often no males from other containers to place with them, they were mated with males from the mass production unit. This may have negated the effect of the selection regime, resulting in the increase in the PBW in the $F_3$. Sixty of the 110 moths in the $F_3$ emerged from a container which had been infested with eggs from a female mated to a nonselected male.

The PBW was dependent in the $F_0$ upon days to emergence (b = -0.35 ± 0.23%/day) and female weight (b = -0.11 ± 0.5%/mg). Consequently, these parameters were also affected by selection for high PBW. Differences in female weight between the $F_0$ and $F_1$ and the $F_2$ and $F_3$ generations were highly significant ($P < 0.005$), but the difference between the $F_1$ and $F_2$ was nonsignificant ($0.20 < P < 0.40$). The decreases in days to emergence in the $F_1$ and in the $F_2$ were highly significant ($P< 0.001$), but the decrease in the $F_3$ was nonsignificant ($0.40 < P < 0.50$). Short developmental time is desirable in that less space would be required for housing larvae and less time would be required to make changes in level of production. Small female size would be desirable if the larvae require less food to complete development, and if there is less effect of moth density on oviposition of small females.

TABLE 4.—Effect of selection for maximum percentage of body weight laid in eggs on the bionomics of *Galleria mellonella*

| Generation[1] | Mean days to emergence | Weight of female | Egg weight/ female | Body weight laid in eggs |
|---|---|---|---|---|
| | | *Milligrams* | *Milligrams* | *Percent* |
| $F_0$ | 41.1 | 149.7 | 51.9 | 34.4 |
| $P_1$ | 35.8 | 135.8 | 61.6 | 45.3 |
| $F_1$ | 31.7 | 136.3 | 55.6 | 39.9 |
| $P_2$ | 32.4 | 136.6 | 64.4 | 47.2 |
| $F_2$ | 29.3 | 130.5 | 62.8 | 46.0 |
| $P_3$ | 30.2 | 128.6 | 62.8 | 48.8 |
| $F_3$ | 29.0 | 148.4 | 56.1 | 36.5 |

[1]The $F_0$ was the population from which the original parents were selected; $P_1$, $P_2$, and $P_3$ were the best 5 females from the preceding filial generation and were the parents of the succeeding generation.

Selection obviously can affect changes in the PBW, but the extent to which the PBW can be increased and the stability of the changes are still in doubt. Because inbreeding may lead to decreases in larval survival, it may be desirable in future studies to rear the larvae singly in 1-ounce plastic cups or to isolate the cocoons before emergence to prevent females from mating with siblings before they are weighed and placed in oviposition cages. Several strains may be established so that males from one strain can be placed with females from a second strain. This would allow rapid selection, but would reduce the extent of inbreeding. Males may be refrigerated to insure that they are available when needed. They were held near 5° C for several days without apparent detriment.

**9. Effect of Temperature and Humidity on Percent Survival and Rate of Development of Eggs of *Galleria mellonella*.**—One of the advantages of eggs of *Galleria* as a rearing host for *Trichogramma* is the relatively long period of development which would allow their use in parasite colonies for several days. To assess this advantage, tests were made on the effect of temperature and humidity on survival and development by observing eggs at five temperatures, 20°, 25°, 30°, 35°, and 40° ± 0.5° C, and five relative humidities, 20, 40, 60, 80, and 90 ± 3 percent, in a factorial design. Fifty eggs were scattered on 1.90 by 2.54 cm PRES-a-ply adhesive labels and

[15]See reference in footnote 3, p. 2.

TABLE 5.—Percent survival of eggs of *Galleria mellonella* at 4 temperatures and 5 humidities[1]

| Relative humidity (percent) | Survival at temperature (°C) | | | | |
|---|---|---|---|---|---|
| | 20 | 25 | 30 | 35 | Mean |
| | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* |
| 20 | 77 | 65 | 85 | 72 | 74.8 |
| 40 | 73 | 69 | 88 | 86 | 79.2 |
| 60 | 83 | 81 | 91 | 87 | 85.5 |
| 80 | 92 | 89 | 93 | 90 | 91.0 |
| 90 | 93 | 91 | 91 | 86 | 90.3 |
| Mean | 83.6 | 79.0 | 89.6 | 84.2 | |

[1]Standard error = about 5 percent; LSD = about 14.7 percent.

held in 1.1-liter polystyrene containers. Relative humidities were maintained by varying the specific gravity of glycerin-water solutions.[16] Number of eggs with eclosion holes were noted at 12-hour intervals from the beginning of eclosion until all eggs had hatched or collapsed. There were two replications. Data for individual eggs were analyzed to detect differences in development time while differences in percentage emerging were tested by analysis of variance of arcsin transformations of the percentage for each replication.

No eggs hatched at 40° C at any humidity. Analysis of variance of the percentage emerging for the remaining temperatures (table 5) detected no significant interaction, nor a difference because of temperature, but showed that differences because of humidity were highly significant. For all temperatures, percent survival increased by 2.4 ± 1.1 percent for each 10 percent increase in humidity. Such small differences would have little effect in a rearing program. Development time (table 6) varied greatly because of temperature and to a lesser extent because of humidity, but there was no interaction.

**10. Effect of Age of Eggs of *Galleria mellonella* on Parasitism by *Trichogramma pretiosum* Riley.**—Labor required for collecting eggs of *Galleria* can be reduced if the eggs are collected only every 2 or 3 days. Therefore, eggs 0–1, 1–2, and 2–3 days old (held at 30° ± 3° C and 65 ± 5% RH) were tested to see if they differed in suitability as hosts for *Trichogramma pretiosum* Riley. Eggs of each age were scattered on lids of separate 90 mm polystyrene petri dishes coated with a fine mist of water. After drying, about 10 *T. pretiosum* females were introduced into the dishes and eggs were marked with a grease pencil when parasitized. After about 50 eggs of each age were parasitized, the eggs were removed, placed singly in No. 1 gelatin capsules, and held at 23° ± 0.5° C and 51 ± 3 percent RH. Number of male and female parasites from each egg was recorded after emergence of the $F_1$. There were six replications. The strain of parasites used originated from parasitized eggs of the tobacco budworm, *Heliothis virescens* (F.), collected on cotton at Brownsville, Tex. It was maintained on eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier), until a colony was established on eggs of *Galleria* several generations before the tests were begun.

Differences in percentage of eggs producing parasites (table 7) were nonsignificant in an analysis of variance test, but regression analysis indicated a significant increase with increasing host age ($b = 6.6 \pm 1.7\%$/day; $P < 0.001$). Differences in percentage of female parasites were nonsignificant. Younger eggs produced significantly more parasites per egg than older eggs ($P < 0.005$). Number of parasites per host egg compensated for differences in percentage of eggs producing parasites, so differences in total female parasites per parasitized host egg were nonsignificant. Thus, eggs can be collected at 3-day intervals without loss of quality for production of *Trichogramma pretiosum*.

TABLE 6.—Duration of the egg stage of *Galleria mellonella* at 4 temperatures and 5 humidities[1]

| Relative humidity | Temperature (°C) | | | | |
|---|---|---|---|---|---|
| | 20 | 25 | 30 | 35 | Mean |
| *Days* | *Days* | *Days* | *Days* | *Days* | *Days* |
| 20 | 22.9 | 10.9 | 7.8 | 6.9 | 12.1 |
| 40 | 22.1 | 10.8 | 7.6 | 6.7 | 11.8 |
| 60 | 21.3 | 11.4 | 7.3 | 6.3 | 11.6 |
| 80 | 21.0 | 10.4 | 6.7 | 6.0 | 11.0 |
| 90 | 20.4 | 10.0 | 6.7 | 5.8 | 10.7 |
| Mean | 21.5 | 10.7 | 7.2 | 6.3 | |

[1]Standard error = 0.1 days; LSD = 0.3 days.

[16]BRAUN, J. V. AND BRAUN, J. D. A SIMPLIFIED METHOD OF PREPARING SOLUTIONS OF GLYCEROL AND WATER FOR HUMIDITY CONTROL. Corrosion 14: 17–18. 1958.

TABLE 7.—Suitability of 0-1-, 1-2-, and 2-3-day-old eggs of *Galleria mellonella* for production of *Trichogramma pretiosum*[1]

| Age of eggs | Host eggs producing parasites | Parasites per egg | Female parasites | Female parasites per parasitized egg |
|---|---|---|---|---|
| *Days* | *Percent* | | *Percent* | |
| 0–1 | 70.32a | 1.50a | 63.27a | 0.67a |
| 1–2 | 76.53a | 1.37b | 57.77a | .62a |
| 2–3 | 83.52a | 1.29c | 60.06a | .64a |
| Standard error | [2]2.55 | .03 | [2]1.92 | .04 |

[1]Means in the same column followed by the same letter are not significantly different at the 5-percent level.
[2]From arcsin transformations.

**11. Hypothetical Scheme for Semiautomatic Production of Eggs of *Galleria mellonella*.**—The proposed unit has four components (fig. 15): An adult emergence chamber (AEC), a mating oviposition cage (MOC), an egg removal and collection device (ERD), and a belt-coating device (BCD). The unit could be operated continuously in the absence of rearing pests.

Trays with cocoons would be left in the AEC for 4 weeks (until adult emergence is almost complete). Thus, one-fourth of the unit's capacity would be added weekly when a set of spent cocoons was discarded. The unit would not be used for rearing because emerging moths would oviposit in fresh diet, causing too great a larval density for optimum egg production.

Emerging adults would be attracted from AEC to a light source placed over the plexiglas top of the MOC. Adults would pass through a multicombed partition permitting free entry into the MOC but preventing return to the AEC. Females would deposit eggs on a belt covered with granulated sugar. Dead adults would collect in a removable tray at the bottom of the MOC. Eggs would be rinsed from the surface of the belt when it passed through water in the ERD. Eggs would be concentrated either through skimming or sedimentation, depending upon the specific gravity of the sugar solution. The wet belt would then pass through granulated sugar in the BCD. The sugar would be dried by a heating coil.

The belt could be geared to move through the MOC and ERD in 72-hour intervals if the eggs were for production of *Trichogramma*. It could be economically desirable to collect eggs at a younger age if they were used for other purposes, or if a device for recovering the dissolved sugar were attached to the unit.

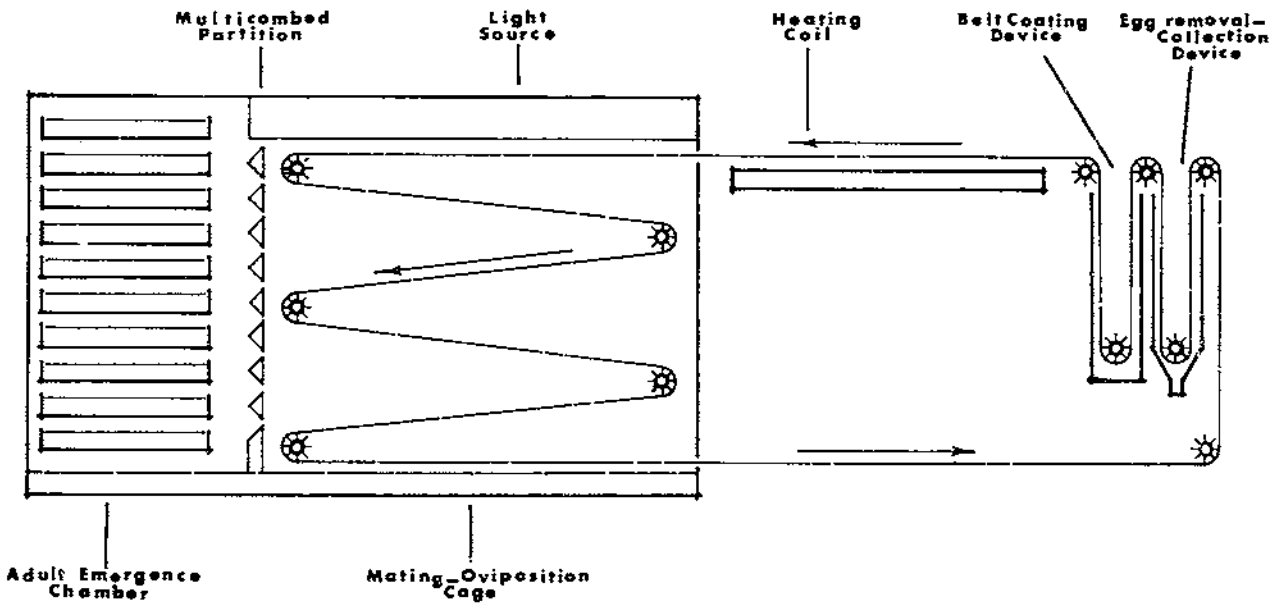


Figure 15.—Hypothetical scheme for semiautomatic production of eggs of *Galleria*.

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