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LABORATORY REARING OF THE IMPORTED CABBAGEWORM

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The imported cabbageworm (ICW), Artogeia rapae (L.) is one of New York's most serious pests of cabbage; studies related to its control are an important component of our research program. For instance, we have experimented with the use of a granulosis virus for management of ICW, and our laboratory has an ongoing program screening cole crops for resistance to several lepidopteran species including ICW. Because these studies all require a constant supply of disease-free eggs and larvae, we continuously rear the insects in the laboratory. Fortunately, although the ICW is a crucifer specialist, it can be reared on a wheat germ-based diet. The major requirements for successful culture are: (1) supplemental lighting in the greenhouse to provide conditions suitable for mating and oviposition, and (2) strict attention to measures designed to prevent disease in the population, and to prevent microbial contamination of the diet.

Adults

Butterflies are kept in mating and oviposition cages (92 cm x 70 cm x 106 cm) in the greenhouse (Fig. 1), similar in size to those used by David and Gardiner (1952) who found that smaller cages caused problems with mating. We have had no problems with using smaller cages for ICW adults; large cages, however, are more efficient for the number of butterflies in the colony (as many as 2000 during the summer months).

The cages are constructed of wood and screening material (Lumite 25, 32 x 32 mesh, available from Chicopee, Gainesville, Georgia 30503); a muslin barrier confines the butterflies when the cage door is opened, and an inset sleeve allows access to the interior. Supplemental lighting is critical; the butterflies will not mate or lay eggs on cloudy days without it. During the winter, in particular, natural sunlight alone is not strong enough to allow normal activity. One thousand watt metal halide lamps J/vith 22-inch reflectors (Sylvania 1000 Metalarc bulbs which emit 3100-3600 lux when new) are placed one meter above the cages. These are replaced yearly.

Temperature in the greenhouse is not tightly controlled. In general, butterflies live from three to four weeks at daytime temperatures of 25-35°C, and nighttime temperatures of 18-25°C (Fig. 2). High temperatures are lethal (over 38-40°C). Before evaporative coolers are put into operation for the summer, the greenhouse must be watched carefully on warm spring days. If the greenhouse is overheating, butterflies tend to cease flying and remain near the bottom of the cage. One cage is kept in another greenhouse as a backup.

Cages are lined with fresh Kraft paper every week, and the floor and sides of the cages are vacuumed to help reduce the buildup of wing scales. Every few months cages are washed by taking them outside the greenhouse on a sunny day, scrubbing them with ammonia and water and rinsing thoroughly. Muslin sleeves are removed first, rinsed well in plain water to remove the uric acid excreted by the butterflies (which reacts with bleach), soaked in bleach and detergent, rinsed again, and then stapled back into place when dry.

Adults will not live long without an energy source. They are given a 10% sucrose solution (i. e., 100 g table sugar in a total volume of 1 liter of distilled water), which has yellow food color added (0.15%) to make it more attractive to the butterflies. Dental wicking (Absorbal®, available in 1370-cm rolls from Mohawk Dental Supply, Celi Drive, East Syracuse, NY, 13057), cut into ca. 24 cm lengths, is soaked in the sugar solution until saturated and placed in 250 ml Erlenmeyer flasks (three or four per flask) so that at least 5 cm of each wick extends beyond the opening of the flask (Fig. 3). Flasks are filled with 125 ml of sugar solution, the top and wicks are covered with aluminum foil, and the flasks are then autoclaved for 20 minutes. After the sugar solution cools, and just before the flasks are put in the oviposition cages, the foil is removed and a 5 cm by 2.5 cm piece of parafilm is stretched and wrapped around the tops to secure the wicks and to prevent excessive evaporation. Four or five flasks are generally sufficient for a cage containing 500 butterflies. However, time of year, temperature, and the age of the butterflies will change consumption rates. We provide enough for a week at a

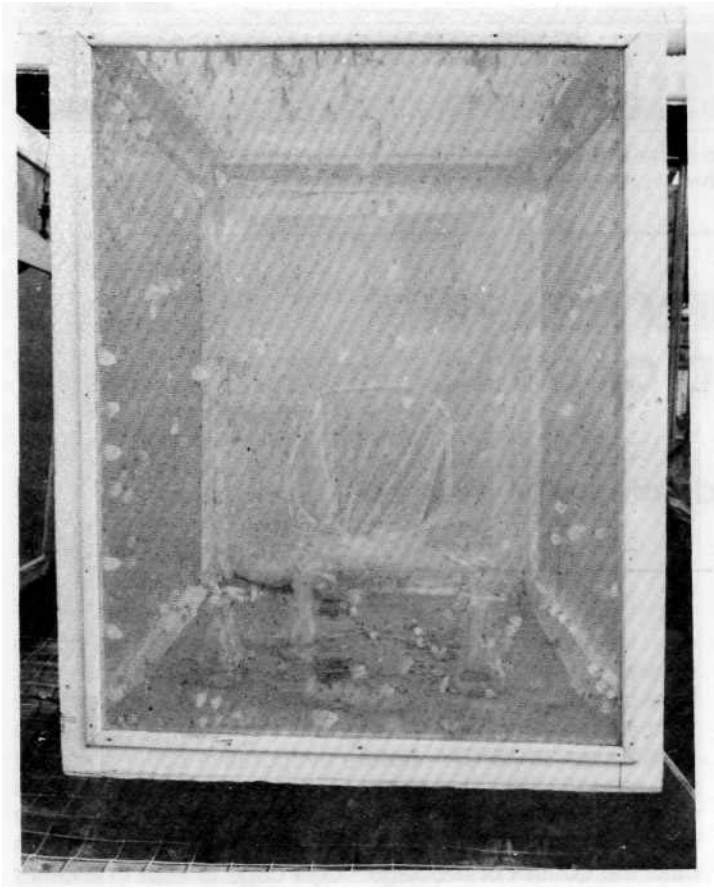

Fig. 1. Mating and oviposition cages for ICWin greenhouse.

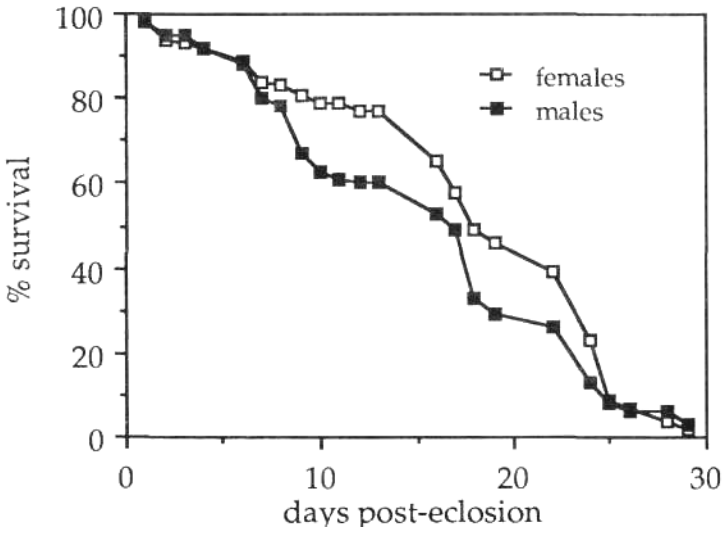

Fig. 2. Survival of ICW adults in greenhouse, June-July 1985. Percentages are means for three cages of ca. 200 butterflies each. Minimum temperature, 20°C; maximum, 37°C.

time, but not longer, since sugar water and wicks will become increasingly contaminated with mold and bacteria.

Eggs

Butterflies oviposit on parafilm-wrapped beakers that are covered with broccoli leaves (Figs. 4a and 4b). Tarsal contact with the leaf stimulates oviposition (Traynier 1979); the butterfly curls her abdomen as though ovipositing on the underside of a leaf, and deposits her egg on the parafilm. We use 100-ml beakers, filled one-third to one-half full with water and leaves that are large enough to cover the top of the beakers. The leaf is secured with a rubber band and a 5 cm by 20 cm piece of parafilm is carefully wrapped (but not stretched) around the beaker, the top edge of the parafilm flush with the top edge of the beaker. The parafilm is taped where it overlaps, and no leaf is left exposed below the lower edge of the parafilm (otherwise the butterflies will deposit eggs on the leaf instead of the parafilm).

If larvae synchronized in development are needed, oviposition beakers are left in cages for no more than an hour (butterflies are most active between 10 AM and 1 PM). For colony maintenance, collection is on a daily basis, as needed. Egg density can be controlled by the amount of time allowed for oviposition or by the number of beakers provided. If the density is too great, eggs are difficult to count accurately (500 per strip is about the optimal number for counting), and may not be effectively surface-sterilized. Larvae that hatch first will feed on nearby eggs, thus reducing apparent fertility. We have found that under summer conditions, with temperatures ranging from 2(37°C, butterflies will produce an average of 364 ± 30 eggs over their lifetimes (based on cage means for 123, 102, and 99 females), with peak oviposition approximately from days 3-10 (Fig. 5). Rainy weather will depress activity, however, even with supplemental lighting.

If eggs are to be used to infest plants in field plots, surface-sterilization is omitted, but periodically, a sample is allowed to hatch on broccoli leaves and reared to make sure that eggs are not contaminated with granulosis virus. Surface-sterilization of the eggs is essential, however, if larvae are to be reared on diet; the process reduces contamination of the diet by molds and bacteria in addition to eliminating viral contamination. The most serious contamination problem is bacterial, as judged by the surface of the diet becoming reddish-brown and/or slimy and foul-smelling. Only two species have been isolated from spoiled diet: a fluorescent pseudomonad (gram-negative motile rods, catalase positive, oxidase positive, producing fluorescent pigments in iron-deficient medium) that is ubiquitous in the environment and a possible Lactobacillus species. Because the antibiotic normally used, Aureomycin (chlortetracycline), is not effective in controlling the problem, and because routine use of antibiotics may encourage development of resistant strains of bacteria, many steps are taken to prevent contamination and spoilage from the time eggs are collected until the time they are placed in the diet cups

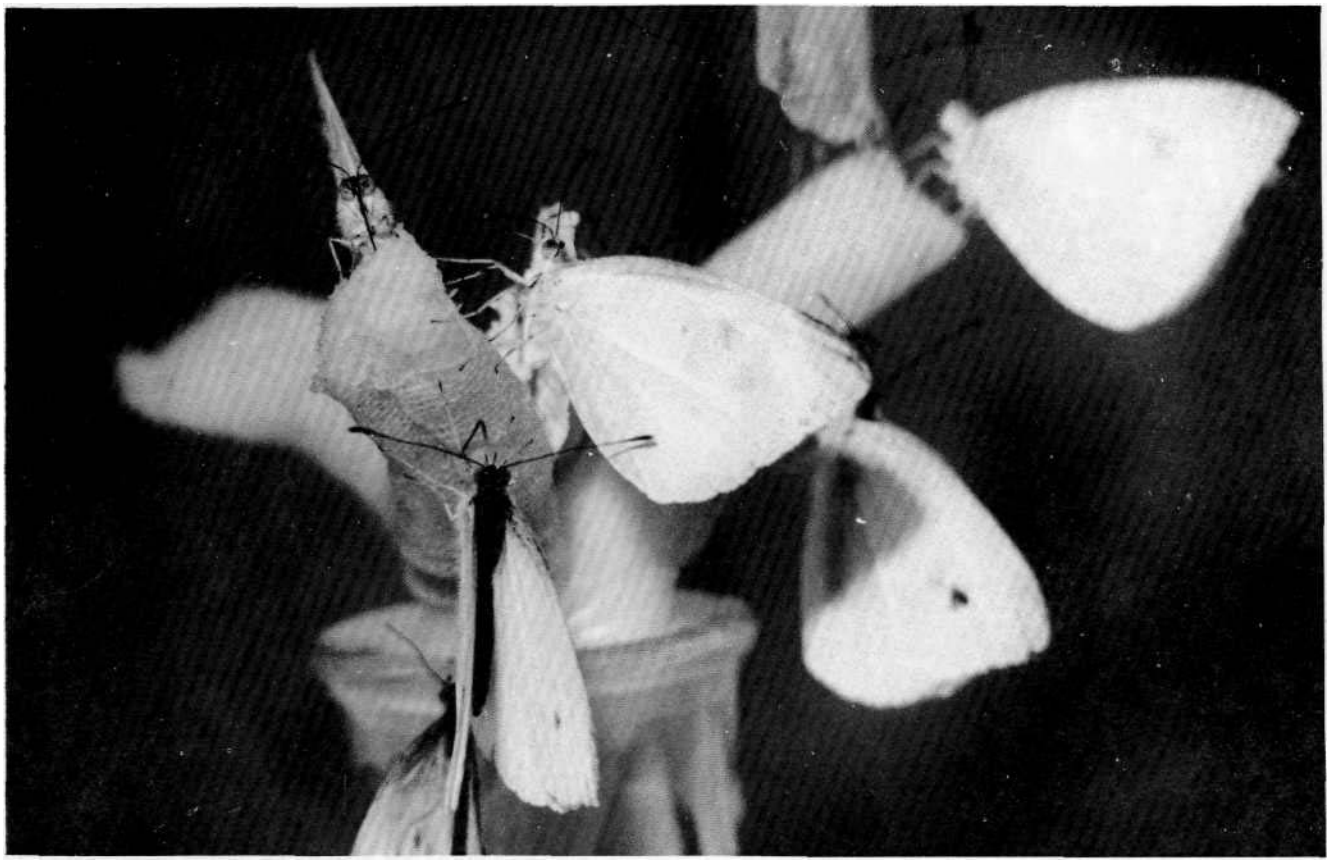

Fig. 3. Butterflies on cotton wicks saturated with 10% sucrose.

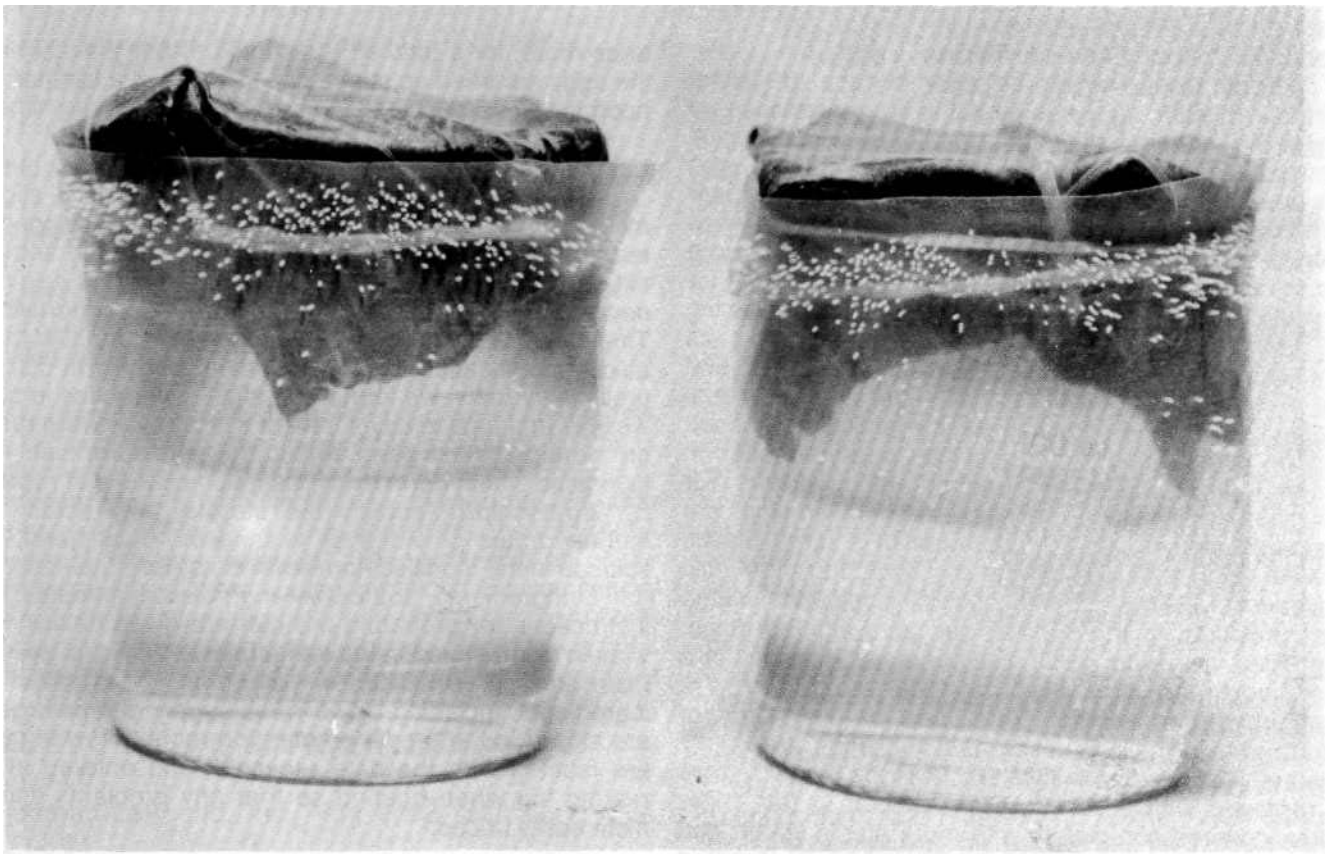

Fig. 4a. ICW eggs on parafilm-wrapped beakers. Fresh broccoli leaves cover the tops of the breakers.

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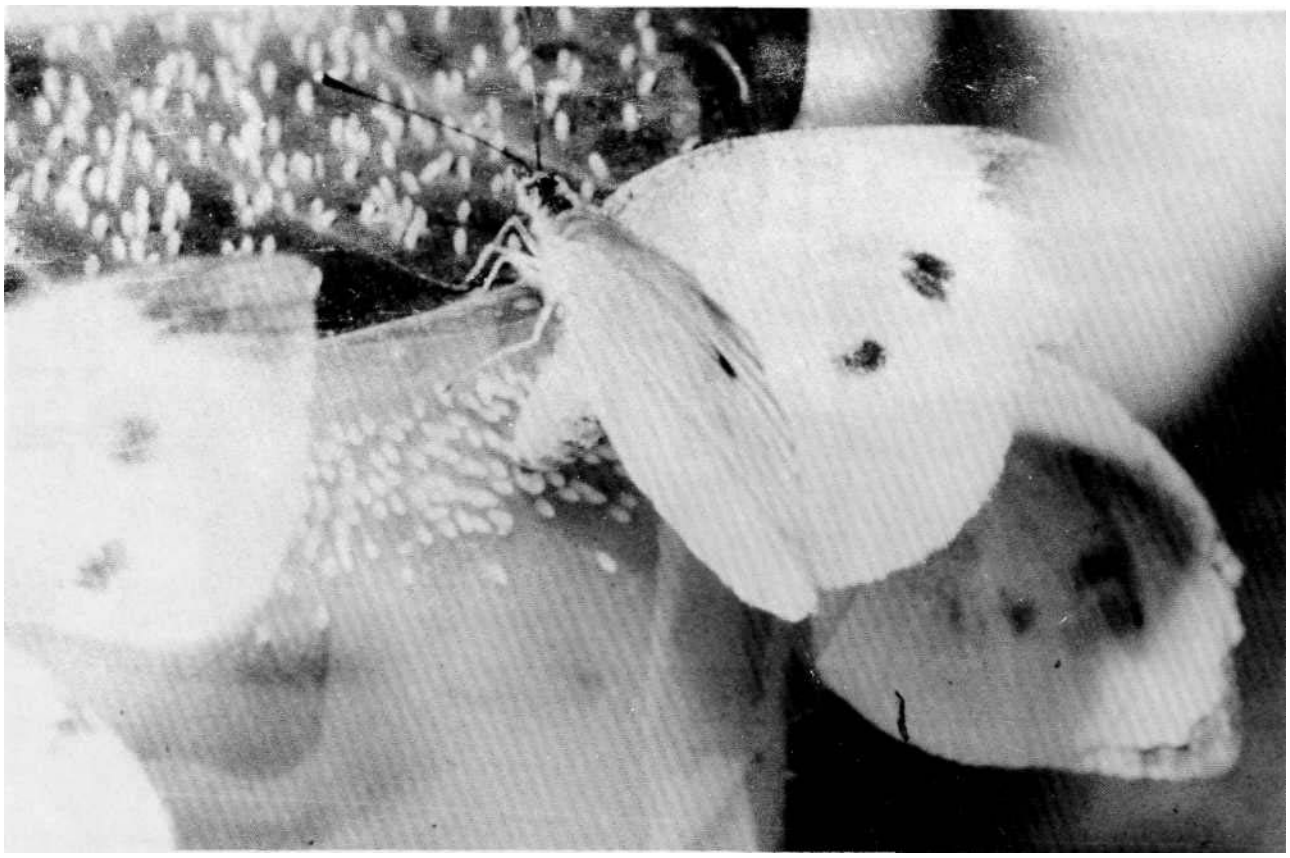

Fig. 4b. Butterfly ovipositing on beaker.

Procedure for surface-sterilizing eggs.

Eggs are never stored after surface-sterilizing. Before sterilization, eggs can be left in the greenhouse for a day or two, or kept between 5-10°C for up to two weeks. We have had problems with spoilage of the diet when eggs have been stored under warm, humid conditions (clear plastic box lined with damp paper towels, 28°C) even when the eggs were later surface-sterilized. The day before eggs are to be sterilized, the following are autoclaved for 30 minutes: two 3-liter Erlenmeyer flasks, each containing ca. 1.7 liters of distilled water, one 1-liter beaker, two pairs of forceps, one pair of scissors with metal handles, and three sheets of paper towels (not separated-individual sheets are too awkward to handle in the laminar flow hood). Scissors, forceps and towels are individually wrapped in foil, the tops of flasks and beaker are covered. For sterilization and rinsing of the eggs, the parafilm strips are iapea, egg side up, into the bottom of a large plastic container (26.5 x 33 x 10 cm,) using Scotch Brand Magic Transparent Tape® which will not come loose under water. Strips that float to the surface can be weighted down. Water-filled vials work well. The container is placed in a fume hood and one liter of 10% formalin (3.7% formaldehyde) is added. After one or two hours of soaking, the formalin is discarded and eggs are briefly immersed in sterile distilled water. Then the parafilm strips are removed with sterile forceps, placed in the 1-liter beaker, covered completely with more sterile water, and allowed to stand for 10 minutes. This rinse is repeated twice more, for a total of 30 minutes. After the last rinse, the eggs are dried on sterile paper towels in a laminar flow hood for 30-45 minutes.

Larvae

The artificial diet used to rear ICW is a slightly modified version of a high wheat germ diet developed for rearing gypsy moth (Bell et al. 1981), a simplifed and less expensive version of a wheat germ-casein diet originally developed by Yamamoto (1969) for tobacco hornworm. Troetschler et al. (1985) have reported a diet for ICW that is also a modification of the Yamamoto hornworm diet. In preliminary trials, we found only slight differences in pupal weights and time to development when larvae were reared on the high wheat germ versus the hornworm diet. The increased wheat germ (twice as much as the diet reported by Troetschler) supplies the sterols, fatty acids, and carbohydrates provided by linseed oil, cholesterol, and sucrose in the modified hornworm diets (Bell et al. 1981).

Ingredients, sources of ingredients, amounts, and instructions for preparing the diet are given below. The vitamin mix is stored in the refrigerator at all times, as is the main stock of stabilized wheat germ. The rest of the ingredients, including a two-week supply of wheat germ, are stored in the media preparation room. New supplies are labeled with the date received, and entered in the rearing log when opened so that any problems can be more easily traced.

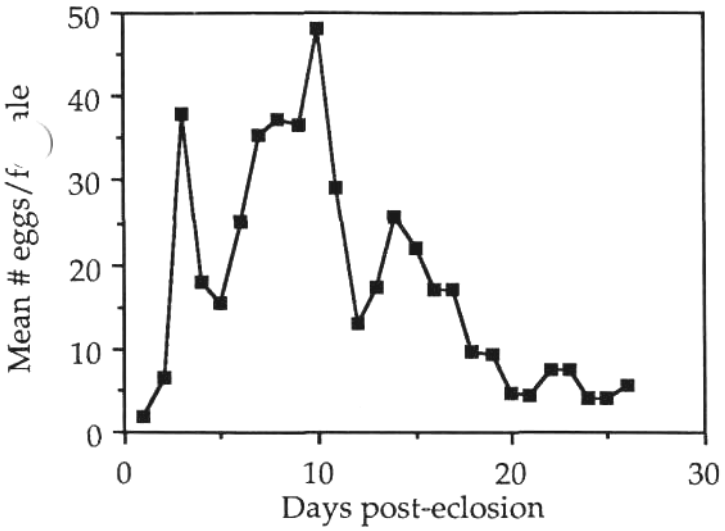

Fig. 5. Lifetime egg production for ICW in greenhouse, June-July 1985. Means are calculated from mean egg production per day per surviving female for three cages of butterflies (123, 102, 99 females).

High Wheat Germ Diet for Rearing ICW

(ca. 2 liters) raw or stabilized

wheat germ (Bio-Serv, Inc.)[1]	240 g
high nitrogen casein (Bio-Serv, Inc.)	68 g
Wesson salt mixture (Bio-Serv, Inc)	16 g
vitamin premix (#26862, Hoffman-LaRoche)[2]	20 g
sorbic acid (Bio-Serv, Inc.)	4g
methylparaben (Bio-Serv, Inc.)	2g
agar (Bio-Serv, Inc.)	30 g
distilled water	1750 ml

Approximately 2 liters of distilled water are brought to a boil in a steam kettle. While this is heating, 900 ml of distilled water and 30 g of agar are brought to a boil in a 2-qt. saucepan on a hotplate, stirring often to prevent agar from burning. While the agar is dissolving, the rest of the dry ingredients are weighed. Then, 800-850 ml of the boiling water (from steam kettle) are added to a commercial size Waring blender, and blended with the dry ingredients for ca. 30 seconds at medium speed. When the agar is thick and bubbling, it is added to the blender and mi/,ed at

[1] Bio-Serv, Inc., PO Box 450, Frenchtown, NJ 08825 [2]Hoffman-LaRoche, Inc., Box 2493, Salisbury, MD 21801

Figs. 6a. Styrofoam cup containing 5th instar ICW larvae on wheat germ diet. (Lid removed for picture.)

medium speed for another 30 seconds. The diet is poured immediately into the rearing containers (16-ounce [473 ml] squat styrofoam cups-Dart 16MJ32 with 32JL translucent, vented lids) to a depth of 1.5 cm, ca. 80-100 ml. The diet thickens quickly and becomes difficult to pour if it cools for even a few minutes.

The diet is covered with clean paper towels and allowed to cool for two hours. The cooled diet can be stored, covered and sealed in plastic bags, at 5-7°C, for at least a week, but is best used fresh. The egg strips, cut into pieces (using sterile scissors) bearing ca. 50 eggs, are placed in the containers of cooled diet in the media preparation room. The cups are then covered with vented lids, labeled with the date when eggs were added, and moved to a walk-in rearing chamber (21 °C, with a photoperiod of 16:8, L:D).

Once placed in the rearing room, the cups are turned on their sides (Figs. 6a and 6b), so that the frass will fall off the surface of the diet and will dry, making more of the diet available to the larvae. If the density of developing larvae is greater than 30-40 per cup, pupal weights are reduced (LaSota and Kok 1986), and bacterial infections can develop in larvae or pupae, either because of stress, or injury (overcrowded larvae will bite each other). If the larvae are overcrowded, some should be transferred to fresh diet when they have molted to the last (fifth) instar.

At 21°C with a photoperiod of 16:8 (L:D), the time from egg to adult is approximately one month. The larvae can be reared at higher temperatures to speed up development but the adults will be somewhat smaller than those reared at cooler temperatures, and will produce fewer eggs (Jones et al. 1982). The main reason, however, for rearing at 21 °C is to make it easier to collect second, third, and fourth instar larvae for bioassays. At higher temperatures, the second and third stadia may each

Fig. 6b. Interior of walk-in rearing room.

last less than 36 hours. Head capsule widths for each instar, based on a cohort of 100 larvae followed from egg hatch to pupation, are as follows (x + SD): 1st, 0.319 mm ± 0.01; 2nd, 0.575 mm ± 0.019; 3rd, 0.877 mm ± 0.036; 4th, 1.358 mm ± 0.033; 5th, 1.984 mm ± 0.046.

To lessen contamination problems, the benches in the rearing room are wiped down with 0.05% sodium hypochlorite (10% Clorox®) several times a week. Regular checks of a few cups from each batch of diet are made to detect spoilage. Any cups that show signs of fungal or bacterial contamination are discarded promptly, as are any cups containing virus-infected larvae (a rare occurrence). Contamination problems are noted in the rearing log, as are any deviations from normal procedures.

Once the larvae have pupated, chrysalids are removed with soft forceps and placed in the oviposition cages (chrysalids, if spread out in a single layer, can be stored for approximately one week at cool temperatures (5-10°C) to delay emergence). Larvae that develop very slowly or that are injured are destroyed by freezing, and discarded.

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