Techniques for Rearing Plutella xylostella
at N.Y.S. Agricultural Experiment Station Geneva, New York
Shelton Lab

Diamondback moth on artificial diet (for 1 gallon or 50 styrofoam pint cups)
Dry ingredients from step 1 can be preweighed and stored at -20°C up to a month prior to use.
Agar should be weighed out separately and can be stored at rm temp.
Unless otherwise indicated, stocks of all ingredients should be stored at 4°C.
All bench top surfaces should be wiped with 10% clorox prior to and after making diet. Also, a razor blade can be used to scrape up diet that lands on the table.

1. Weigh out ingredients below. Place into a plastic bag or plastic container.

Vendor	Product No.	**Product**	**Amount**
Bioserv	G1659	**raw wheat germ* (finely ground)**	**175 g**
"	1100V	**casein**	**126 g**
"	3900	**sucrose**	**135 g**
"	F8680	**Wesson's salt mix**	**36 g**
"	6830	**potassium sorbate**	**4 g**
"	3425	**cellulose**	**25 g**
"	7685	**methyl paraben**	**5.4 g**
"	6265	**USDA vitamin premix***	**36 g**
"	7135	**aureomycin (14%)**	**4g**
"	6015	**ascorbic acid**	**14g**
ICN Biomed	102747	**propyl gallate**	**0.8 g**

***Wheat germ and vitamin stock should be stored at -20°C.

2. Weigh out agar. If preweighed, agar should be stored in its own plastic sandwich ziploc bag at room temperature.

(Vendor)	(Product No.)	**Product**	**Amount**
Bioserv	7060	**100 mesh agar**	**96 g**

3. Wet ingredients should be measured on the day that the diet is prepared. The oil, KOH, and formaldehyde can be measured separately and added to blender when you add the dry ingredients (see step 5).

(Vendor)	(Product No.)	**Product**	**Amount**
Bioserv	5680	**raw linseed oil**	**30 ml**
"	6795	**43.6% KOH***	**9 ml**
you choose	--	**38% formaldehyde (stock)**	**3 ml**

*52.4 g KOH plus 100 ml water for a total volume of 120 ml. Caustic, do NOT inhale KOH dust.

4. **To make diet.** Set up 50 styrofoam pint cups and measure out liquid ingredients from step 3 (linseed oil, KOH and formaldehyde). Combine agar (from step 2) with 3000 ml water in a steam kettle. Mix to dissolve lumps. Turn steam knob on about 1/2 turn. Stir every few seconds to ensure even heating of the agar solution. Agar will start to simmer at edges and will begin to thicken. Turn steam off when agar reaches 87°C. It should just begin to boil in center. Stir a little longer after heat is turned off. Empty bag of dry ingredients (from step 1) into bottom of blender container, then pour the hot agar into the container. (After steam kettle is emptied, fill with cold water to soak before you clean it.)

5. Add liquid ingredients to blender container. Mix well with a wooden spoon, making sure that unmixed ingredients do not accumulate on upper edges of blender. Secure cover and blend on low for 1 min. Everything should be well mixed. Diet temperature will be about 73°C at this point and will still pour easily.

6. Pour the diet into the pint cups to about 2 cm deep (there's a line in the cup that we fill to). Let diet cool 1-2 hours before infesting with eggs and putting on lids to avoid excess moisture in the cups, which promotes disease development.

7. Infest approximately 300 eggs per cup which will produce about 150 pupae per cup. These eggs should be fresh (i.e. 0-24 h old), sterilized, and air dried in a clean room for 1 h before they are infested on diet. To prevent inbreeding problems, make sure eggs are taken from all the mating cages set up. For example, if there were 3 mating cages, 1/3 of the infested eggs should come from each of the mating cages. Mark cups with eggs from each mating cage with a different color so that the next set of mating cages will contain equal proportions of each color. During larval development, cups should be kept on their side so that frass accumulates on the side of the cup as opposed to on the diet. Larvae are reared at 80°F (27°C), 35-50% RH, and 16:8h L:D photoperiod.

8. Keep records of number of batches set up, generation, % hatch, development times, and any other notes. For % hatch, cut out about 100 eggs from each egg sheet used and put into individual 1 oz. cups. After 1st instar larvae hatch and die, determine num eggs hatched/tot eggs.

Eggs to Adults

Cups are kept at 27°C, 16:8h L:D cycle, 35-50% RH. Eggs will hatch in 2-3 days, 2nd instar larvae will appear after 4-5 days, and after 10 days, larvae will pupate around upper portion of cup. At this point if your rearing operation is going well, you should see no disease, no cannibalism, and the pupae should have developed on schedule. Never let the adults emerge in the cup with the old diet. If you do, you will surely start to get disease in subsequent generations. With a razor blade we remove the upper 2.5 cm of the cup with lid and set up 10-12 lids per cage for adult eclosion and oviposition (about 1500-2000 adults per cage). Make sure that each oviposition cage mixes the entire gene pool. For example, if you always set up 2 oviposition cages A and B, then 1/2 of the diet cups should be infested with A eggs and the rest with B eggs. The next set of oviposition cages should have pupae from 7 A lids and from 7 B lids in each cage to mix the gene pool. Oviposition cages measure 39 x 39 x 39 cm, are screened on all sides and have a cloth sleeve for access on one of the side panels. 10% sugar water tinted yellow with food color is used to feed the adults. We fill two 50 ml erlenmeyer flasks with the sugar water and add a cotton dental wick that is held in place with parafilm or a lid with a hole cut through the center.

Our dental wick source: Absorbal.com

Egg Collection

Eggs are collected on cabbage-treated aluminum foil. Foil is cut to 11 cm x 30 cm (12" width foil) pieces then folded in half such that there is about a 1 cm overhang which is folded over again and the final piece measures about 11 cm x 14 cm. Each sheet is dipped into autoclaved cabbage juice (blend 65 gms cabbage in 500 ml. distilled H_20, autoclave, then strain), and stood on its long side to dry (leave for 24 h). After sheets are dry, they can be kept frozen until needed. The oviposition sheets are hung with wire hooks from the cage roof. Eggs are collected every 24 hrs for lab testing and can be stored at 8°C, 75% RH, 16:8h L:D photoperiod, up to 2 wks with little mortality. As described above, eggs infested on diet should be fresh, i.e. not stored at 8°C.

If egg hatch drops or development is slowed, then colony is showing signs of disease, malnutrition, or inbreeding depression. Investigate immediately because problems will just get worse. If egg production drops, double check your sugar water wicks to make sure that they are still moist.

Egg Sterilization

Eggs are sterilized 20 min in 3.8% formaldehyde (stock formaldehyde is sold as a 38% solution so just make a 10x dilution to obtain a 3.8% solution), then rinsed several times in distilled water, then set in 3 changes of distilled water, at 10 min each. Egg sheets are unfolded and allowed to air dry. Before infesting on diet, the eggs must be completely dry (it will take 1-2 hours). In a pinch, you can shorten the drying time by setting up a fan and taping the eggs down.

Dupont's method of egg sterizilation - Eggs could also be sterilized by washing in 5% clorox/distilled water solution for about 20 sec, then rinse in distilled water for about 1 min. Be careful, clorox can kill the eggs if concentration is too high or left in solution too long.

G88 DBM Rearing Schedule on Diet
(Chamber conditions: 27°C, 16:8h L:D photoperiod, 35-50% RH)

1. FRI - Collect 24 hr old eggs from oviposition cages on FRI. To prevent inbreeding depression, take a portion of the eggs from each oviposition cage. Sterilize eggs. Then make and infest diet. Color code cups so you know which diet was infested with eggs from which oviposition cage.
4. Set up pupae in oviposition cages on 2nd MON (10 days after infest date).
5. Add egg sheets for oviposition THURS (3 days after pupae set up). Use 2 egg sheets per cage; otherwise eggs will be too dense. Use Friday's eggs for the diet infest.
6. Freeze cages MON (7 days after pupae set up).

* Note: If development times start to get longer, then your insects are probably infected with Microsporidia. Many factors can trigger a Microsporidia infection including: egg sheets that are not completely dry at infest, overly high humidity, bad wheat germ, unsanitary conditions, letting the adults emerge in closed diet cups, etc.

Diamondback moth on broccoli plants

To maintain moth colonies on broccoli plants, infest approximately 500 eggs per plant by cutting sterilized eggsheets into pieces of about 100 eggs each, and pinning the pieces to several leaves on each plant.

For bioassays, we need several hundred 2nd instars. Since we are not rearing the larvae beyond this stage, we infest the plants at much higher rates, putting on about 1000 eggs per plant. After 4-5 days, we will have about 500-800 larvae per plant, from which we can easily get the number of 2nd instars we need for the bioassay. Leftover larvae are frozen if not needed.

Once the plants are infested with eggs, they are placed into 1 of our large cages. These cages measure 61 cm (24") high x 46 cm (18") wide x 61 cm (24") deep and are bound with cloth on at least 3 sides. In general, any cage will do as long as there is enough air circulation so that condensation does not form on the leaves or cage walls. If larvae run short of food, add clean broccoli plants to the cage. Once larvae have pupated, they are brought to a growth chamber where adults can be collected.

Adult collection and egg production

When pupae are formed, cages are brought to our walk-in growth chamber (27°C, 16:8h L:D photoperiod, 30-50% RH). Adults are collected on a daily basis by turning off room lights, shining a small lamp on the upper corner of the cage away from the door, and sucking adults up into a glass collection tube connected to a vacuum pump. Oviposition containers that we use for foliage reared insects (plexiglass cylinders, 13 cm diam x 16 cm tall) have lids with 2 screened openings and a center opening that is covered with a flat piece of rubber (cut up bicycle inner tube). A cross is cut into the rubber so that adults in the collection tube can be added to the cage. Masking tape is placed over the rubber door and a slit cut into it so that the oviposition sheet can be changed without letting adults escape. Our oviposition sheets measure about 2.5 cm wide by 11 cm long. Another piece of tape is then used to hold the egg sheet in place. We label all cages with the date the cage was started, the colony, the egg generation collected, the cage letter, and we keep a tally of the number of adults added to the cage on each day. This size cage cannot hold more than 250 adults so once we have that many, we start another cage. Just as we did when we changed the egg sheets of the diet reared colony, we label the egg sheets with the date the egg sheet was changed, the colony, the egg generation number, and the letter of the cage. Again, we use the letter to help us identify which egg sheet came from which cage so that we can be sure to include the entire gene pool every time we infest the eggs.