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Rearing *Sarcophaga bullata* Fly Hosts for *Nasonia* (Parasitoid Wasp)

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INTRODUCTION

Nasonia vitripennis is a parasitoid of a number of calliphorid flies, such as *S. bullata. S. bullata* are relatively large, increasing the offspring yield that a single *N. vitripennis* female can produce. They are also easily reared in the lab if proper ventilation is available.

MATERIALS

REAGENTS

Beef liver, cut in 2″ cubes

Dry fly food (half dehydrated milk, half granulated sugar)

12mm glass test tubes and cotton plugs

Paper towels

Bleach

EQUIPMENT

Wire mesh fly cage

Ventilation hood

30C incubator

10×14″ plastic tubs with lids (cut large hole in lid)

16″×16″ cotton cloth squares (such as bed sheet)

8×8×3″ plastic containers

8×8×3″ plastic containers with 4mm drainage holes drilled in bottom

Steel colander

Plastic wash basin

Paper drinking cups with 1mm air holes poked in them

Rubber bands

METHOD

I. FLY CAGE

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1. Start and maintain a population of >50 flies (5 female: 1 male). Cover the bottom of the cage with a layer of paper towels. Change water and liver baits every day (see below).
2. Each day, change water: fill two 250mL Erlenmyer flasks with water and insert 5 rolled-up paper towels in each as a wick.
3. Each day, remove old liver baits and add 2 new pieces of liver (on Petri plates or similar).
4. Each day, add flies to the cage in a ratio of 5 females to 1 male (aim for 10:2).
5. Change fly food three times a week: two Petri plates of 50% sugar, 50% dehydrated milk.
6. Set up 32 fly pupae individually in 12mm glass vials with cotton plugs three times a week. Use the oldest hosts in the refrigerator, but crack a couple open to ensure they are good and will develop into flies. Healthy flies will have firm shells and clear eyes.
7. Clean the cage once a week. Remove paper toweling, sweep up debris, replace paper towels.

II. LARVAE REARING

A. LIVER

1. Remove liver from refrigerator. Place pieces onto paper towels to remove excess liquid.
2. Take necessary amount of liver (usually one 10lb bag) from freezer to defrost for later. Poke holes in bottom of bag and place bag in colander. Place this in the plastic wash basin. This allows drainage of blood from liver.
3. Always ensure that there is enough thawed liver for the next two days.

B. DAY 0 LARVAE

1. Add liver to cover the bottom of a 8×8×3 inch plastic container.
2. Add larvae from the fly cage liver baits.
3. Place into 10 × 14 inch larger holding tub with paper toweling in the bottom (about 5 sheets).
4. Place and seal lid and cloth cover over the large holding container. Make absolutely sure that the lid is completely closed.
5. Label with date. Place into ventilation hood.

C. DAY 1 LARVAE

1. Open container. Remove excess liquid with paper towels. Turn over liver if dry and sprinkle with water.
2. Close container, making sure to seal properly, and return to hood.

D. DAY 2 LARVAE

1. Open container.
2. Transfer liver and larvae into 8×8×3 inch plastic container with drainage holes. Stack in same size container without holes.
3. If larvae are crowded, split between two bins, adding additional fresh liver to each.
4. Close container and return to hood.

E. DAY 3 LARVAE

1. Open. Remove bottom container that catches the drippings.
2. Place drainage container on the paper towels in the larger tub. Prop up one edge of the container so it will drain.
3. Close container, making sure to seal properly, and return to hood.

F. DAY 4 LARVAE

1. Open. Living larvae should have congregated at the high end of the drainage container.
2. Scoop larvae over the side of the drainage container onto paper towels. Only move those larvae that are grouped away from the liver. Remaining larvae will follow.
3. Close container, making sure to seal properly, and return to hood. Larvae at this stage are most likely to crawl out of the bin and escape.

G. DAY 5 LARVAE: REMOVE FROM HOOD

1. Open. Remove smaller container with liver. Dispose of liver and any remaining larvae (put in plastic bag for freezing).
2. Remove old paper towels from tub, making sure to pick out larvae and pupae.
3. Distribute larvae evenly into two cleaned tubs, in between layers of paper towels. Cover with cloth and lid.
4. Place in 30C incubator for 3 days.

H. DAY 8 PUPAE

1. Check day 6 and day 7 pupae. If they are wet, change the paper towels.
2. Collect day 8 pupae into paper cups. Check pupa quality by cracking puparium with thumbnail in head region and examining quality of host. Firm puparia and clear eyes are good.
3. Write date on paper towel and cover cup using paper towel and rubber band.

4. Place cups into the refrigerator. Use a different refrigerator than that used to rear *Nasonia*, which can crawl through the air holes.

TROUBLESHOOTING

Problem: larvae appear dry, sticky, or covered with foam

Solution: wet hands and sift through larvae. Usually a small amount of water is sufficient for them to begin to clean themselves off. Use paper towel to mop up foam or standing liquid. Change paper towels under bin if they are wet.

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