

These procedures are used in the Shelton lab and were developed at Pioneer.

Standard Operating Method Insect Rearing

Title:

**LADYBIRD BEETLE (*Coleomegilla maculata*) REARING USING
DECAPSULATED BRINE SHRIMP EGGS**

Contact:

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1.0 Purpose

- 1.1 This standard operating method (SOM) outlines the procedure for rearing the ladybird beetle *Coleomegilla maculata* on decapsulated brine shrimp (*Artemia sp.*) eggs.

2.0 Definitions

- 2.1 Larva (pl. larvae) refers to the immature stage of an insect (between the egg and pupal stage of an insect with complete metamorphosis).
- 2.2 Neonate refers to a newly hatched insect larva
- 2.3 Pupation refers to the act of becoming a pupa. A pupa is a life stage between larva and adult in insects with complete metamorphosis. This stage is non-feeding and is usually an inactive period.
- 2.4 BS - Brine shrimp, *Artemia franciscana*
- 2.5 Decapsulated – removal of the outer protective layer of the brine shrimp egg
- 2.6 LBB – Ladybird beetle(*Coleomegilla maculata*)

3.0 Principle

- 3.1 This SOM is designed to reflect the maintenance of a small LBB colony that can be housed in an environmental chamber.

4.0 Apparatus and Ingredients

- Safety Glasses
- Heat Resistant Gloves
- 1.5mL snap cap Eppendorf microcentrifuge tubes (VWR product # 20901-437 or equivalent)
- Tube racks for 1.5ml tubes
- Large Petri dishes (150x15mm) (Falcon # 351058)
- Small Petri dishes (Lab-Tek 6cm x 2cm (or equivalent))
- Lids from 15ml centrifuge tubes
- Typing paper
- Scissors
- Microwave oven
- Graduated Cylinder
- 1000ml bottle with screw cap
- 1000ml beaker
- Agar
- 50ml Repeating Pipettor and tips
- Deionized water
- Balance
- Decapsulated Brine Shrimp eggs
(<http://www.brineshrimpdirect.com>)
- Refrigerator
- Freezer
- Plastic containers with lids
- Hot glue gun
- Organdy cloth
- Large rubber bands

5.0 Water Source Preparation

5.1 Setup 1.5ml tubes in racks in preparation for dispensing agar solution

5.2 Prepare a 0.5% molten agar solution

- Measure appropriate amount of water and add to the 1000ml bottle (Do not exceed 500mls of water in the bottle)
- Measure the appropriate amount of agar and add to the water in the bottle
- Secure the screw cap on the bottle and gently mix the agar and water
- Loosen the cap to allow venting of the bottle while heating
- Place the bottle in the microwave and heat until boiling
- Using heat resistant gloves, remove the bottle and molten agar solution
- Verify that all agar has gone into solution
- Pour molten agar solution into a 1000ml beaker
- Using a 50ml repeating pipettor, dispense 1.5ml of agar solution to each 1.5ml tube
- Allow agar tubes to cool
- Snap lids on agar tubes and store in the refrigerator

6.0 Food Source

6.1 Decapsulated Brine Shrimp (*Artemia sp.*) eggs

- BS eggs are purchased from <http://www.brineshrimpdirect.com> and held at approximately -20 C until needed.

7.0 Larval Care

7.1 Larval Containers

- 7.1.1 Plastic food containers (Ziploc, Gladware, or equivalent) with modified lids are used to house the LBB larvae
- 7.1.2 Cut a large hole in the lid of the container and hot-glue a section of organdy cloth around the hole
- 7.1.3 Allow the glue to cool and trim excess cloth
- 7.1.4 Fold a sheet of typing paper long-ways with alternating folds in an accordion-like fashion.
- 7.1.5 Cut several strips of the folded paper (approximately 1 cm in width) into the plastic container to provide surface area for the larvae.

7.2 Larval feeding

- 7.2.1 Decapsulated Brine shrimp (BS) eggs
 - Add BS eggs to the bottom of the container and add an agar tube
 - Refresh the BS eggs if needed (usually not required) at what intervals usually?
 - Refresh the agar tubes once a week until pupation occurs. Approximately 2 weeks at 28 C
 - Since the BS eggs are dry it is critical to refresh the agar tubes to provide fresh moisture and prevent cannibalization

7.3 Pupation

- 7.3.1 Allow LBB larvae to pupate in the larval containers
- 7.3.2 After emergence continue to provide agar tubes and BS eggs to the new adults

- 7.3.3 Allow the new adults at least 1-2 days to harden the exoskeleton before transferring them to the adult dishes

8.0 Adult Care

8.1 Adult beetles are kept in large (150 x 15mm) Petri dishes.

8.1.1 Dishes are clear and shallow for easy viewing of adults and egg masses, and maximize space in the environmental chamber

8.1.2 Adult dish setup

- Fold a sheet of typing paper long-ways with alternating folds in an accordion-like fashion.
- Cut approximately 1cm strips and place them on edge in the dish, approximately 4 strips per dish
- Add 1 tube of agar and 1 lid from a 15ml centrifuge tube (as a food dish) to each adult dish
- Add BS eggs to each food dish in each adult dish

8.1.3 Place 8-10 adult beetles into each dish and place lids on the dishes

8.1.4 Stack dishes 10 high and secure the stack with a rubber band

8.1.5 Place adult dishes in an environmental chamber set at 28 C, 16:8 light:dark, and 55% relative humidity

8.1.6 Check dishes twice daily for egg masses except for weekends. Adults will eat the eggs if they are not removed. *NOTE: For maximum egg collection, mated female beetles can be removed and set into individual dishes for egg laying and collection.*

8.1.7 Fresh agar tubes and BS eggs are added once a week

8.1.8 Beetles are transferred to new dishes every two weeks

8.1.9 Newly emerged adults require approximately two weeks to mature and mate before beginning to lay eggs

9.0 For Wild Collected Adults

9.1 Adults beetles are collected from field locations

- Sweep net samples from alfalfa fields
- Collections from overwintering sites

9.2 Field collected adults are brought into the laboratory

- Beetles are placed in adult dishes and maintained (See section 7.0)
- Field collected adults are kept isolated from the laboratory colony and observed for parasites and diseases before addition to the colony.

10.0 Egg Collection and Care

10.1 Adult beetles will lay eggs on almost any surface in the adult dish

10.2 Eggs are lighter in color immediately after being laid, they will turn darker orange as they mature

10.3 Eggs are typically laid in groups

10.4 Areas to check for eggs

- Paper strips
- Lids of the agar tubes
- Inside the agar tubes
- In the BS eggs
- On the Dish

10.5 Place collected eggs into small (6cm x 2cm) Petri dishes and incubate until hatch

- Eggs will hatch in ~3 days at 28 C

11.0 Approximate Time Tables

LBB Development at 28 C

Egg to hatch	Larval Development	Pupation to adult emergence	New adults to egg production
~3 Days	~2 weeks	~5-7 days	~1-2 weeks