

Rhagoletis handling and processing

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### **Prepare tubs for holding fruits with maggot**

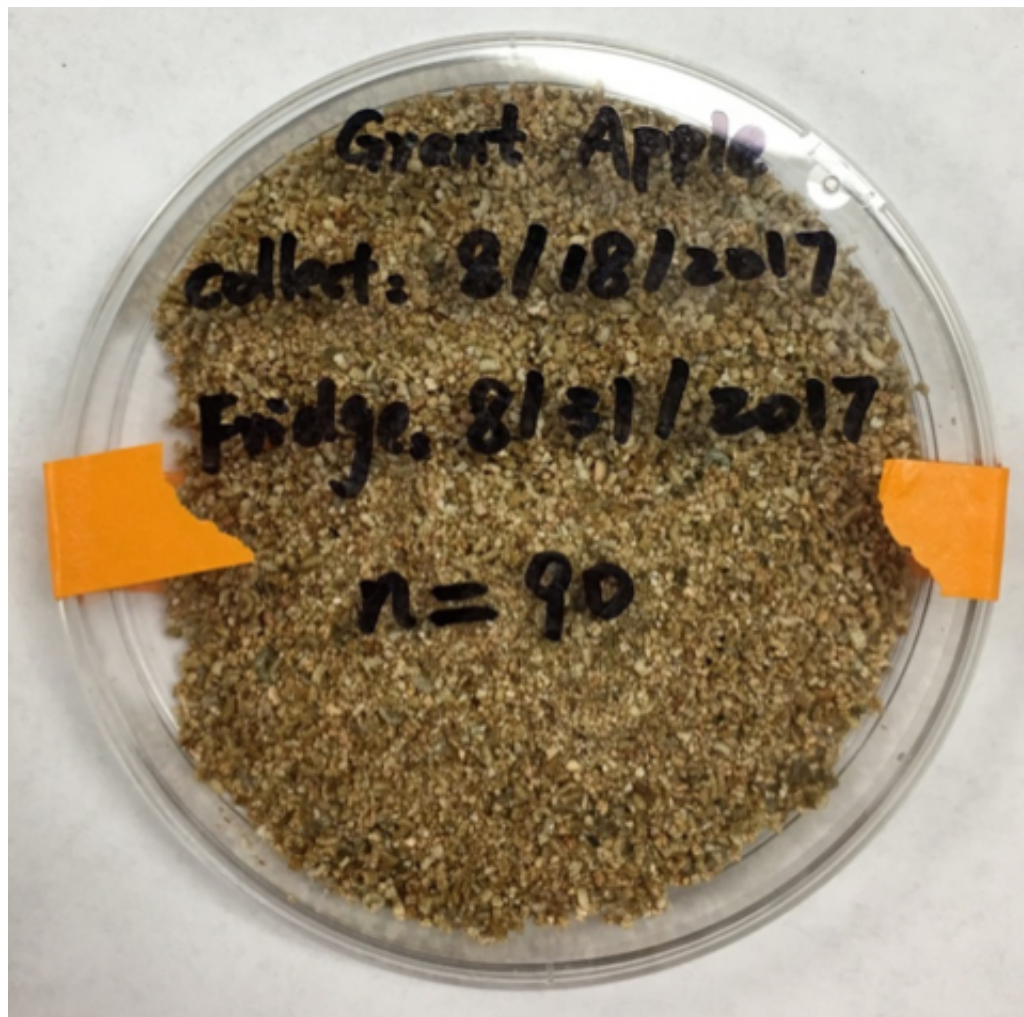
1. Put the metal mesh into the tubs, and pour the fruits onto the metal mesh
  - Make sure the apples are **1-2 layers thick**
  - As fruits keep rotting, there will be more juice at the bottom of the tub. Clean the juices up and place paper towels to absorb juice that will fall in the future.
2. Label all the tubs clearly especially by site and host fruit

### **Collect wandering larvae and pupae**

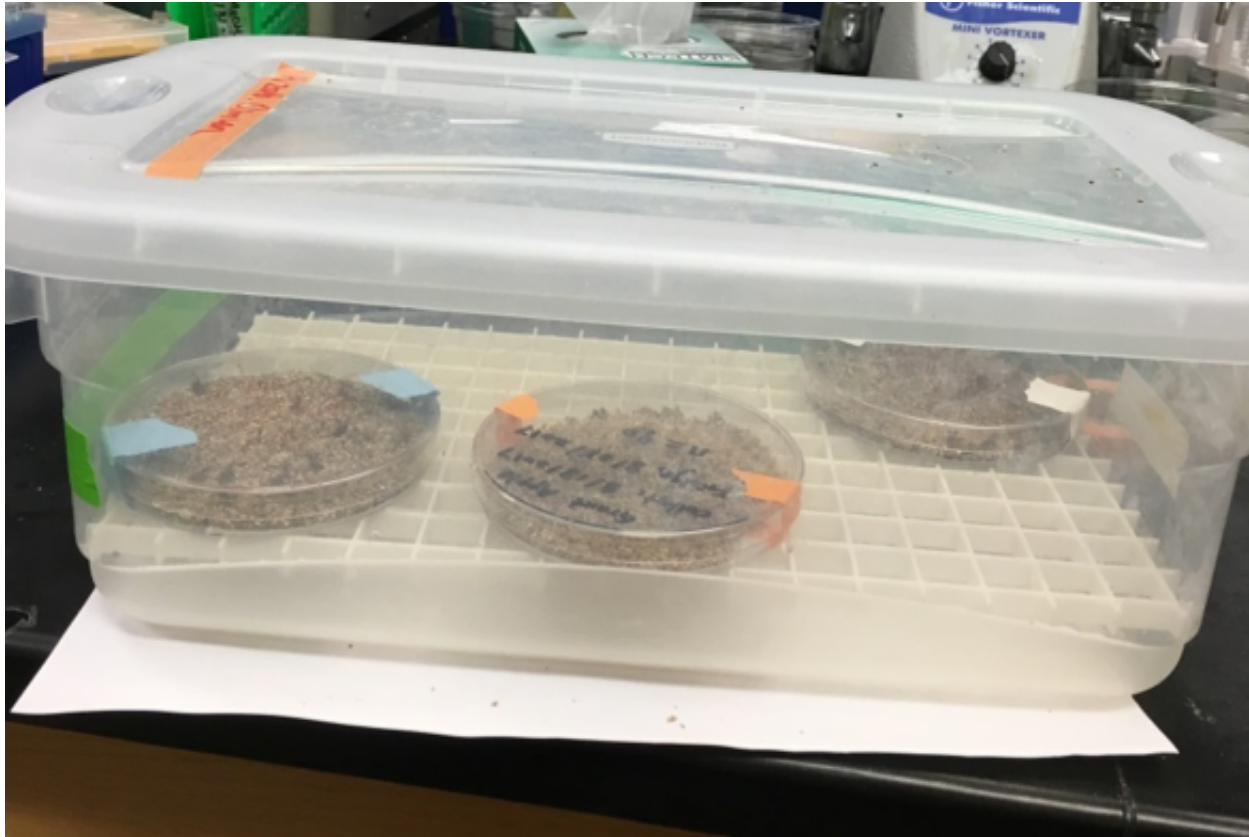
1. The wandering larvae will come out from the apple and drop into the tub. Take the metal mesh out so you can see the wandering larvae or pupae at the bottom of the tub.
2. Pick the larvae or pupae out from the tub very gently with your fingers and put them into one or more (> 100) petri dish
3. Clean the tub with paper towels and place paper towels at the bottom as apples become more rotten. Put the metal mesh with apples back.
4. Split the petri dish with all of your collections into several petri dishes containing wet vermiculite. Put no more than 100 pupae or larvae into each petri dish.
  - Prepare the petri dish by filling it with vermiculite and add water until all of it is wet, but make sure it isn't too wet. One way to assess wetness is to hold the petri sideways and squeeze the vermiculite. Water should not be dripping from dish.

5. Label the petri dishes by site, race, larvael/pupal collection date, fridge date, and number of individuals placed in dish.
  - The fridge date is 13 days from collection date.
6. Seal each petri dish with tape, and place the taped petri dishes into a container with saturated potassium chloride (KCl) solution to keep the humidity (85%).
  - KCl solution is made by mixing 1L water with 350g of KCl

**Example of petri dish set up with vermiculite and label**



Example of storing petri dish with KCl solution on the bottom



- Note: At the last few days of collection, the fruits will become very juicy so we sometimes clean the insects with water before moving into the vermiculite. Try to collect the insect at the same schedule every day

## **Respirometry:**

1. Take the pupae out from the vermiculite the day before respirometry. Normally we do purge in the afternoon and injection in the next morning. I can show you the details of respirometry later.