

Collecting *E. muscae*-infected fruit flies at your house

Materials:

- Container for your bait (bucket or dishpan)
 - This will get really gross and you will not want to use it for anything else afterwards, so keep that in mind!
- A protected outside location to place bait
- Organic watermelon
- Paper towels
- String, if you wish to hang your bucket up
- Mesh or netting, to cover bait from things bigger than a fruit fly (I used mesh cloth with 1/16" openings)
- Duct tape, to secure netting
- Aspirator
- Empty vials with breathable caps
- Vials with permissive food and breathable caps
- Newly-eclosed WT flies
- Paintbrush
- Small dish
- A freezer
- Patience
- Understanding housemates

Procedure:

E. muscae has been most commonly reported in the spring, summer and fall in temperate climates (Figure 1). The exact timing of *E. muscae*'s emergence will depend on your latitude and local climate, but you can expect that once fruit flies are plentiful *E. muscae* should be out circulating in hosts. The strategy that follows outlines how you can attract wild fruit flies to your backyard and learn if any are infected with *E. muscae*. Once you observe death by *E. muscae*, you can then act to isolate the fungus from wild flies. Of course, if you'd prefer to sample straight away and see what you get, that's up to you!



Figure 1. Reports of *E. muscae* sightings by month observed on iNaturalist.com. Green line indicates verified observations (“research grade”), gray line indicates suspected but unverified occurrences.

- 1) Identify a protected location for placing a bait that doesn’t get direct sunlight.
 - a) Also keep in mind that this bait will be attracting flies, so make sure you choose a location where this won’t bother you.
- 2) Prepare your bait
 - a) Quarter or halving an organic watermelon and placing it in either a clean bucket or a dishpan lined with paper towels. Use a bucket if you plan on hanging your bait (to make it harder for rodents and ants to get to); use a dishpan if this is not a huge concern.
 - b) The vessel should be washed with soap and water and dried before use. I would strongly advise against using cleaning products that are harsher than this.
- 3) Place your bait.
 - a) If you live in a cosmopolitan area where nocturnal rodents and other critters aren’t likely to go after your bait, you can place your bait on a stool and leave it open to the environment (e.g. Figure 2). This approach will attract all manner of creatures, including birds, ants etc, so you should be confident that the bait won’t be disturbed before you do this.



Figure 2. Example of open bait in dishpan. An organic watermelon was quartered and placed on paper towels in a clean dishpan. The dishpan was placed on a stool out of direct sunlight on a balcony in Berkeley, CA.

- b) If you live anywhere else, you will want to make sure that your bait is inaccessible to rats, birds, ants etc. You can achieve this by placing your bait on a stool or hanging the bait from the bucket's handle and topping with netting with holes small enough for fruit flies but not for other creatures. You want to secure the netting so that it can't be pushed away by hungry animals/wind/rain etc. Duct tape is a good option.



Figure 3. Example of closed bait in bucket. An organic watermelon was quartered and one quarter was placed on paper towels in a clean bucket. The bucket was hung off of a ceiling hook on the back balcony on the second floor residence in Somerville, MA.

- 4) Observe and wait.
 - a) Causing minimal disturbance to your flies, inspect your bait every day or so for cadavers. They may be adhered to the watermelon (check the undersides too!), the paper towel or the side of the container. If it's been a while between checks, they may have fallen to the bottom of the container. Hallmarks of infection are

upraised wings and remnants of fungal growth on their backs (especially poking up between their segments in a banded pattern). If you do not observe cadavers, don't despair - just keep waiting and observing.

Example of cadavers found stuck to watermelon:



Examples of old cadavers found on bottom of bait container:





- i) There are no guarantees that you will catch *E. muscae*, though if you live anywhere temperate it is a very safe bet that you will catch fruit flies! It will be tempting to poke around in your bait a bunch to see what's going on - try not to disturb your flies. Ideally, you want to create a safe, stable environment that flies will know to come to to safely get food. The more flies you can get to visit, the better, as high host density is favorable for *E. muscae*.

Once you have observed flies killed by *E. muscae* and confirmed its presence in the population of flies visiting your bait, then the fun begins.

- 5) Capture and monitor wild flies for death by fungus.
 - a) Prepare clear enclosures with preservative/anti-fungal free food which you can place wild flies in and monitor for death. This food can be chunks of organic banana, any fly diet omitting tegosept and propionic/phosphoric acids (see <https://bdsc.indiana.edu/information/recipes/index.html>), or sugar agar (recommended 5% sugar, 1.5% agar). The enclosure can be covered with a cloth, cotton or fly flug.
 - b) Fly visitation at your bait will be highest in the morning (in the hour or so following sunrise) and the evening (in the hour or so before sunset). Visit your bait at these times and collect flies using a mouth aspirator or net. Transfer these flies onto your prepared food and store at room temperature with access to ambient lighting. DO NOT LEAVE THESE OUTSIDE WHERE THEY CAN GET DIRECT SUN. Doing so will lead to baked flies.

- c) Check your captured flies daily ~2 hours after sunset for death by fungus. Look for the stereotyped death pose - fly stuck to elevated position in vial with wings upraised. Depending on when you check for death, the fungal growth may be more or less obvious (checking earlier - fungus is less grown, fungal outgrowth not apparent; checking later - fungus is much more obvious). If you observe flies killed by the fungus, you will need to remove them from the vial and place in a new vial with susceptible healthy flies to get new ones sick (see next step).
- 6) Collect fresh cadavers.
 - a) Place the container bearing your fungus-killed fly in your freezer for ~2 minutes to slow down the living flies. Orientation of the vial in the freezer is key - you want to position your vial such that when the living flies knock out, they will not fall in soupy food and never come back out. Depending on the consistency of your food, you can place your vial upside-down or on its side. While you're waiting for your flies to anesthetize, be ready with a paintbrush or aspirator and a container for your cadavers.
 - b) Once your flies have slowed, immediately removed from the freezer, uncap and remove cadavers using a paintbrush or aspirator. Recap closure and transfer cadavers to your prepared dish. **IT IS CRUCIAL THAT YOU DO NOT LEAVE YOUR FLIES IN THE FREEZER FOR MORE THAN A FEW MINUTES.** Too much cold will kill your flies.
- 7) Expose new flies to fresh cadavers.
 - a) Place cadavers (you can use as few or as many as you'd like, though I typically shoot for a ratio of 6 cadavers to 35-50 healthy flies) in a new vial of food.
 - b) Add ~35-50 young (ideally within first 5 days of eclosion, the younger the better!) to your vial. It is better to error on the side of too few flies than too many. If you use too many flies you may not have very many deaths.
 - i) If possible, confine the healthy flies within ~1-2 cm of the cadavers by pushing the cap down to cram the flies together. This will help increase the odds that the new flies get hit by spores. If you can't do this, don't worry - you can still infect new flies.
 - c) Place your vial in a cool (ideally ~68-70F), moist place overnight to let the fungus sporulate. Make sure the vial has access to ambient light.
- 8) Monitor your new vials for fresh cadavers.
 - a) Check your vials daily ~2 hours after sunset starting 3 days after exposure to the fungus for dead flies.
- 9) Repeat.
 - a) If you want to keep growing the fungus in flies, you need to keep providing more delicious flies as food. If you don't wish to culture the fungus, you can instead place cadavers in a container and freeze for genotyping by PCR or sequencing.