This month we will be focusing on volatile extractions as you have been, as well as your new olfactometer tests.

We will be using diluted Methyl Salicylate (MeSA) dissolved into Dichloromethane to observe *A. swirskii’s* attraction to this plant volatile.

1. Make the following solution in the vent hood: Add of the 0.06 ml MeSA to 59.94 ml Dichloromethane in a graduated cylinder, transfer in a small glass container, swirl it around to ensure it is well mixed. This solution should be sealed with a lid and parafilm and kept in the vent hood during experiments and kept in the freezer when not in use to prevent evaporation

2. Put the two Erlenmeyer flasks in the metal rack and attach them to the olfactometer air flows, one labeled ‘control’ and the other ‘MeSA’

3. Triple-wash and cook a sectioning razor at 50 °C for a few minutes. The razors can be found in a small yellow container slightly above eye level in Dr. Funderburk’s lab, in the rightmost shelf on the back wall, text/ask Iris if you can’t find them. They can also be found in Dr. Martini lab near the microscope furniture closet.

4. Get out a sheet of clean aluminum foil to place the clean dental wick on, then use the sanitized razor to cut a clean dental wick in half (ask Dr. Martini if you can’t find them, they are in the closet in Dr. Martini’s lab)

5. Roll each dental wick piece separately into its own aluminum foil, mark half ‘MeSA’ and the other half ‘Control’ (just like we do for the volatile filters, this is so they cannot contact any contaminants or each other)

6. Do under the vent hood do the following:

7. Get the 100 ul glass pipette and the Drummond syringe used for volatile extractions

9. To the ‘Control’ wick, add 100 ul of Dichloromethane to it, then rewrap it in foil and put aside

10. To the ‘MeSA’ wick, add 100 ul of the MeSA solution onto it and store it in the same way

11. Let these solvents evaporate for 5 min before use

To begin a trial:

12. Take one wick labeled ‘MeSA’, unwrap it and add it to the glass beaker labeled ‘MeSA’

13. Take one ‘Control’ (Dichloromethane only) wick, unwrap it and add it to the other beaker labeled ‘Control’

14. Set the air flow to 0.3 as we have before

15. Grab a mite and record how long it takes to make a choice

16. Record time and choice in the lab notebook

We need to do 3 trials of 20 mites for 60 mites total, switching sides every five mites tested. Switch the metal Y after every ten mites are tested. After 20 mites, the MesA and solvent should be reapplied on the wick. The 20 mites should be run the same day. If you think you’ll not able to run a second set of 20 mites, postpone it for the next day. It is preferable to change the wicks every day.

Record all of your data in the lab notebook following the format in the datasheet labeled ‘James\_olfactometer\_assays\_spreadsheet’ in the shared drive.

I added an example of how to record a mite choice: write the treatment in each arm, and under ‘choice’ record the arm the mite goes towards, or ‘no choice’ if it doesn’t make a choice during the 5 minutes allowed.

Please upload your data at the end of each day so I can do some statistics to make sure that these concentrations are working.

Another thing: we don’t have to keep the small container of mites in the refrigerator during the week. Instead, sprinkle a small amount of Eddie’s bee pollen into the container, they should be fine feeding on that.