**Experiment:**

Purpose: Test and identify the volatile profile emitted from microbes that affect the foraging behavior of SWD flies.

Hypothesis: The susceptibility of fruit (blueberries) to spotted winged drosophila is influenced by the microbiome of the fruit.

Test: record and compare the volatile profile of attractive and repellent bacteria in blueberry juice.

* Attractive; Bacillus subtilis, Bacillus stabeskii
* Repellent: Frigoribacterium faeni, Enterobacteriaceae sp.
* Neutral: Bacillus amloliquefacians
* Control: Blueberry juice

**Two-choice trap assay** *(Wong et al.)*

* Two-choice trap assays were set up as shown in Figure S1D. Flies were food deprived for 3h before being transferred to the test
* cages (350 3 260 3 150 mm). Each cage contained two traps (transparent cups, 34mm diameter) of 1ml MRS medium diluted in
* water, one seeded with the bacterium (AP or LP) and the other unseeded. Experiments were conducted at the same time of the
* day (between noon – 2pm) at room temperature, 12h:12h light-dark cycle. The number of flies in the test traps and outside the traps
* (no choice) was scored after 24 and 48h. Pilot tests were performed at different food deprivation length (3h, 6h and 18h) and we
* observed no difference in the proportion of flies entering traps 24h or 48h after (data not shown). Thus, the shortest length (3h)
* was chosen for the experiment. Experiments were conducted in biological replicates of arenas with multiple flies (mean number
* of flies per arena = 19.4; median = 17; Figure 3B), where n refers to the number of arenas (see STAR Methods).

Bacteria growth and inoculation protocol (*Wong et al.*):

* Obtain desired bacteria for testing either from glycerol stock or from an isolate on culture plate.
* Inoculate into a liquid medium at 3ml (LB/MRS/TSB, dependent upon which one it was isolated on originally).
  1. How much bacteria to inoculate: cell counter, volume, etc?
* Incubate the sample in the shaking incubator for a 24-hour period at 32℃.
* Pipette 1.5 ml of the liquid culture into an Eppendorf tube then centrifuge at 8,000 rpm for 10 minutes.
* Pipette off supernatant, add 1.5 ml of 1% saline water then homogenize the sample.
* In the A# Traps add the “3mL of bacteria” in 1.5mL saline solution
* Then insert 3 ml of blueberry juice into the A# traps.

Volatile collection: we will test superQ and SPME when cleaning reusable items DO NOT USE ACETONE

* SPME rotated through samples daily and SPME fibers not to exceed 100 runs before replacement.

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| --- | --- | --- | --- |
| Bacteria 1 | Day 0 | Day 1 | Day 2 |
| Rep 1 | SPME-1 | SPME-2 | SPME-3 |
| Rep 2 | SPME-2 | SPME-3 | SPME-1 |
| Rep 3 | SPME-3 | SPME-1 | SPME-2 |
| Rep 4 | SPME-1 | SPME-2 | SPME-3 |
| Rep 5 | SPME-2 | SPME-3 | SPME-1 |
| Control Juice | SPME-3 | SPME-1 | SPME-2 |

* Sample size n=5, Sample times: 2-hours, 24-hours, 48-hours
* Clean ball jars and scintillation vials after use
  + Ball lids: 80 Celsius
  + Ball jars, scintillation vials, Teflon: 100 Celsius
* SPME: no solvent delay needed and faster setup
  + Condition new SPME for 30m on Gladerial
    1. Method: SPME.CONDITION.M
  + Run 2 min blanks on SPME’s daily
    1. Datafile: JTB-E02-pg-line; lab book: project name-SPME name
    2. For the first SPME: Load method🡪insert SPME🡪start run🡪start a 5 minute timer🡪pull SPME and replace
    3. Remaining SPME’s: insert SPME🡪 set 2MINUTE timer🡪pull SPME and replace
  + Test blueberry juice background on each polymer type (mono-, bi-, and tri-)
  + Start low for P and E times (30s each)
  + Method:
    1. Lego: Legolas\_40\_4.M
    2. Gladerial: Gladerial\_DB1\_40\_4.M (this method will need to be created with the added solvent delay)
* SuperQ: solvent delay and flow can capture heavier volatiles
  + How to clean a SuperQ (setup in Shawn's)
    1. 600ul milli-q water
    2. 600ul MeOH
    3. 600ul DCM
  + Collect and run initial DCM rinse daily before use
  + Method:
    1. Leggo: Leggolas\_40\_4\_#
    2. Gladerial: Gladerial\_DB1\_40\_4\_# (this method will need to be created with the added solvent delay)

Data Mining: After polymer selection has been made and runs have been completed on both the DB-Wax and DB-1 columns complete the following steps

1. Opening data
   1. Load data file🡪Integrate (use default set integration parameters)🡪select Percent Report🡪select Spectrum then “Library Search Report” 🡪set library to “NIST” 🡪select ok, then select “Library Search Report” 🡪check screen report only🡪select correct integration parameter (the default one: ) and select “Apex” 🡪”ok”
   2. File🡪export to .csv file🡪”current file with current data” 🡪generate library search results🡪”ok” and take note of “appended” message
2. Building database
   1. Open file explorer and open the “Results” .csv file you just made
      1. Compare the retention times between the library search results and the integration results
      2. Copy and paste “RT”, “Area PCT”, and “Library” columns to the end of the integration results section and remove the remaining columns in lower results
      3. Same main database as a separate file, in .xlxs extension, and in a different location. As more data is collected it will be added to this main database.
3. Walking through peaks:
   1. Complete these steps on the DB-WAX and the DB-1
   2. Zoom into a region containing peaks
      1. Make a small “left click” box inside the peak to select the peak🡪make a small “left click” box at the baseline then navigate to the SUBTRACT option🡪double “right click” in the spectra area to obtain the identification result of the peak
      2. If the selected peak has a higher match rate than the match in the database file then replace the chemical name in the database with the newly found chemical name. Also add peak information for all peaks that were manually integrated and identified
4. Calculating RI’s: DO NOT SAVE OVER MACROS! EVER!
   1. Complete these steps on the DB-WAX and the DB-1
   2. Open “Huge list of VOCs” file🡪navigate to ”WAX” sheet🡪open main database🡪file🡪open🡪browse🡪navigate to DB-WAX macro, highlight it, and select open🡪”enable” it once the file opens as needed then minimize it
   3. Navigate to database🡪make an empty column next to chemical name column🡪select all the RT in question🡪press ctrl + g to calculate the RI values