**Fecundity Experiments**

*Summer 2025*

Goal

Quantify the effect of epiphytic *P. pergaminensis* #220 on pea aphid (alate) reproduction.

Hypothesis

*P. pergaminensis* #220 negatively effects pea aphid (alate) reproduction.

Methods

**Day 0**

* Inoculate #220 overnights in KB media (~3 mL overnight per plant sprayed)

**Day 1**

* Prepare dilutions of #220 overnights at OD600 = 0.2 ± 0.02
* Spray labeled plants #1 with either #220 dilutions or fresh 10 mM MgCl2 buffer
  + Plate 100 uL 10 mM MgCl2 buffer on KB + Amp plates to check for contamination
  + Include one test plant each for #220 and buffer without any alates on them.
* Allow plants to dry
* While plants are drying collect alates (1/plant)
* Once plants are dry, add on alate per plant to base of plant #1 and cover with bread bag/rubber band
* Move plants to tent at 22 ± 2°C and 85 ± 10% relative humidity

**Day 2**

* Sample test plants and plate 100 uL leaf washes on KB + Amp + NYST plates [Should we sample test plants at 24 hours after spraying or 5/6 days after? We’ll have to wait 5/6 days to sample the second and third rounds of plants to avoid disturbing the aphids.]
  + Can do 10-1 and 10-2 dilutions for #220 plant and undiluted (UD) for MgCl2 plant
  + Leave plates on bench and check 2-3 days later

**Day 5/6**

* Count surviving alates/nymphs on each plant #1 and move surviving alates to labeled, unsprayed plants #2; cover and secure with bread bag/rubber band.
* Include one test plant without any alates on it as an epiphytic growth control.

**Day 11/12**

* Count surviving alates/nymphs on each plant #2 and move surviving alates to labeled, unsprayed plants #3; cover and secure with bread bag/rubber band.
* Include one test plant without any alates on it as an epiphytic growth control.
* Sample honeydew covered leaves from the plants (and one control plant) and plate 100 uL leaf washes on KB + Amp + NYST plates
  + Can do 10-1 and 10-2 dilutions for #220 plants and undiluted (UD) for MgCl2 plants/control plant
  + Leave plates on bench and check 2-3 days later

**Day 17/18**

* Count surviving alates/nymphs on each plant #2
* Sample any living alates for *Pseudomonas*
  + Plate 50 uL crushed alate on KB + Amp + NYST plates
  + Leave plates on bench and check 2-3 days later
* Sample honeydew covered leaves from the plants (and one control plant) and plate 100 uL leaf washes on KB + Amp + NYST plates
  + Can do 10-1 and 10-2 dilutions for #220 plants and undiluted (UD) for MgCl2 plants/control plant
  + Leave plates on bench and check 2-3 days later
    - ~3 weeks long
      * Full alate life cycle
    - 3 rounds of plant switching
    - Smallest pots for single plants (more reps in case alates die)
    - Griffin read fecundity comp. paper