**In Vitro UV Alate Choice Experiments**

**T = -18 hrs**

* Inoculate ~50 mL of PPM + glycerol with Pseud #220 and put in shaker

**T = 0 hrs**

* Prepare #220 supernatant
  + Measure OD of overnight culture, dilute to OD600 = 0.80 ± 0.05 using PPM + glycerol
  + Centrifuge diluted overnight culture to pellet cells
  + Pour supernatant into empty, sterile falcon tube and discard cell pellet
* Collect alates
  + Count 20-30 alates per plate from the alate cages
  + Keep them in petri dishes until ready to use
* Prepare plates
  + Label outer 4 wells of 6-well plates
  + Thaw aphid diet tubes in warm water
  + Add 5 mL of aphid diet to each of the 4 labelled wells per plate
  + Add 1 mL #220 supernatant to 2 of the 4 labelled wells on one side of each plate
  + Add 1 mL PPM + glycerol to the other 2 of the 4 labelled wells on the other side of each plate
  + Stretch out 2 rectangles of parafilm and cover the plates
* Add alates to a 1000 uL tip box cover and gently but quickly flip over the parafilm-covered plate to sit on top of the tip box cover
  + Use two half-strips of parafilm to seal the plate to the tip box cover
  + Tape the bottom edges where the parafilm meets the tip box cover
  + Repeat for each plate
* Put the plates in the growth chamber, plate side up (the bottom of the plate is facing upwards)
  + Cover half of the plates with the UV-blocking acrylic box
  + Cover the other half of the plates with the UV-transmitting acrylic box

**T = 4 hrs**

* Gently take each plate out of the growth chamber, photograph it, and record the number of alates/nymphs feeding in each well
* Return the plates to the growth chamber as before

**T = 24 hrs**

* Gently take each plate out of the growth chamber, photograph it, and record the number of alates/nymphs feeding in each well
* Freeze the plates to kill aphids for cleaning