**Pollen Tube Growth Treatment**

Make sure two plants from the same block, one being a northern plant and the other a southern plant, are flowering at the same time. Select a mature flower (petals completely open and straight across with a mature stigma) and cut at the pedicel.

Place five plates with pollen growth medium in a small circle. Hold the pedicel of the flower, while pointing the flower down toward one plate. Place the vibrating device against the stamen and turn on for a short burst (1-2 seconds). Repeat the same procedure for each plate and continue until pollen is distributed among the five plates. Repeat for the second flower with five separate plates.

Label the top of the plate with the plant’s unique identifier and one of five temperatures (10°, 20°, 25°, 30°, 40°). Place one plate from each flower at one of the five temperatures for 16 hours. After 16 hours, collect the plates and cover the agar with a thin film of ethanol. Store in the fridge at 4°.

*Imaging the Pollen Tubes*

Turn on microscope and computer. Navigate to Leica imaging software on the desktop. Place plate under microscope and focus using the course and fine adjustment. Once pollen grains and tubes are visible, click “Acquire.” Open file explorer and navigate to “Pictures.” Locate the image and rename file with genotype, ramet, temperature, and letter (ex. PI28B\_30\_A.jpg). Mark location where picture was taken on bottom of plate with a sharpie and then move to a new location on the plate. Repeat until four pictures have been acquired on the plate and labeled A, B, C, and D. After image “D” is acquired, place small section of ruler on plate and take an image of the ruler, for later length conversions.

Note:

* If there are only few pollen grains on the plate, more pictures may be necessary
* For ease in length measurements and counts, look for area where pollen grains are spaced out, rather than clumped together

**Measuring Pollen Tubes**

1. Starting with picture A, measure the lengths of all pollen tubes that are completely visible
   1. Begin with upper left quadrant and move counterclockwise
   2. Measure the length of at least 100 tubes that are fully visible
      1. Use the segmented line tool to create line
      2. Push analyze tab and then measure to record length
      3. Mark the tubes that were measured with the paintbrush tool
   3. Once completed, save spreadsheet as csv file
2. Count all pollen grains with tubes (even if they extend off the picture or extend beneath another tube) and all pollen grains without tubes
   1. Count all for the quadrants used for measuring the lengths
   2. Enter counts into the csv file with the lengths
3. Save image with as tiff with the same name as the original picture
4. Attain conversion from ruler image
   1. Enter length conversion into the spreadsheet

**Pollen Tube Growth Medium**

Fill a 1000 mL Erlenmeyer flask to 700 mL with deionized water and place with a stir bar on a hot plate. Add the following substances to the flask:

* 100 g sucrose
* 500 mg Calcium Nitrate (Ca(NO3)2 4H2O)
* 120 mg Magnesium Sulphate (MgSO4)
* 100 mg Potassium Nitrate (KNO3)
* 120 mg Boric Acid (H3BO3)
* 30 g Bacto-Agar

Fill to 1000 mL and continue heating and stirring solution until solutes are dissolved. Autoclave using the liquid setting for 15 minutes at 15psi and 121°C. \*Hold autoclave door until it catches, and pressure begins to build. Set out petri dishes. Fill the petri dishes halfway with the medium and allow to cool. After 10 minutes cover halfway with the lids. Once the medium completely cools, cover completely and store upside-down in the fridge at 4°C.

\*May be necessary to cut recipe in half if use is not consistent.