Instructions for Botrytis Detached Leaf Assay - Image Analysis (R and ImageJ)

1. Run experiment, take images at 24, 48 and 72 hours post inoculation. Be sure to frame image to include all leaves. Include ruler for scaling. Check for focus and minimal shadows and glare.
2. Back up images DAILY from camera card.
3. Create a backup folder with a copy of all original images (01\_RawImages/)
4. Rename images (e.g. ExpName\_Timepoint\_Tray), rotate images, crop to fit only agar tray.
5. Create a backup folder with a copy of all renamed images (02\_ToMask/)
6. Copy images to a new folder (03\_Masked/)
7. Open R. Set working directory to 03\_Masked/ and run the script 01\_ImageAnalysis.R
8. Copy all images & masks to a new folder (04\_EditedMasks/)
9. Hand-edit masks of leaves and lesions in ImageJ
   1. See Analysis\_ImageJ.docx for this step
10. Copy all images & edited masks to a new folder (05\_FeatureCalc/)
11. Run 02\_FeatureCalc.R
12. Copy Results.xls to a new folder for editing
13. In Results.xls , add columns with experiment ID, object number, individual plant, growth flat, plant genotype and isolate genotype
14. Measure pixels per cm for each image in ImageJ
    1. Make a new spreadsheet (UnitConv.csv)
    2. In UnitConv.csv, add columns with experiment ID, image name, centimeters measured, and pixel count
    3. Start ImageJ
    4. Using ImageJ, File > Open > select your leaf image and leaf mask files
    5. Add number to pixels per cm column of UnitConv.csv
    6. Select the straight line segment tool
    7. Draw a line segment along 1 or more cm of the ruler
    8. Enter the number of cm measured into UnitConv.csv
    9. In ImageJ, go to Analyze > Measure (or Ctrl M)
    10. Copy the length measurement into UnitConv.csv