**In the shared folder:**

-Four subfolders where the images can be found. BAFOG, KAW and PARACOU are the three sites that vary in precipitation (BAOFG: 2500mm rain/year, PARACOU: 3000mm rain/year and KAW: 4000 mm rain/ year). FTH is part of the PARACOU site, but data was collected at a different time than the PARACOU folder.

- each folder has an excel spreadsheet with species code numbers and names, habitat and species type (generalist or specialist). In some it is not specified whether species are generalists or not, but this can be found also in the excel file called “species”.

- disregard the excel file called Scoffoni\_Pauldata. We won’t be using that one.

**In the excel file:**

* Pick 72 images you will be in charge of measuring and write your name under the column “student name”. Note that every single image will be measured by two different students, so there are two columns for each row with “student name” on it.
* To find the corresponding leaf, check the species name, and code number(s).
* If you picked the BAFOG site, go to the folder BAFOG. All the images will be there with code numbers and species name (first three letters of the genus and species) as the image title.
* If you picked the FTH site, go to the FTH folder. The image names represent the CodeFTH in the first column of the spreadsheet.
* If you picked the KAW site, go to the KAW folder. Images are arranged in subfolder by date of sampling. In each subfolder, image names represent the code number in the first column of the KAW spreadsheet.
* If you picked the PARACOUdata excel, go to PARACOU folder. Images are arranged in subfolder by species name. Image names represent the Plot\_Subplot\_FieldNumber\_SpeciesInitials\_CodeNumber

**Download FIJI to your computer**

<https://imagej.net/software/fiji/downloads>

**First, a few tips on how to use FIJI:**

1. *Zooming in and out*: zoom in on the scale by pressing the + button on your computer (you can zoom out using the -) OR in Image J by pressing the magnifying glass icon (10th from the left) where left clicking will permit you to zoom in and right clicking will make you zoom out.
2. Always try to have the image taking up as much of your screen as possible.
3. Going up and down/ left and right within the image: when you have zoomed in and enlarged your image to fit most of your screen, the full image might not appear. You will have to move right and left and up and down depending on what you want to measure. To do so press the space bar key and hold it down. Notice a little hand appeared instead of your normal cursor. While keeping the space button pressed down, click on the mouse and keep it clicked as you move up and down or left and right.
4. Set your measurement before you start: go to Analyze, Set measurements and click the area and perimeter boxes, unclick all the others.
5. Set scale to pixels: go to Analyze, Set Scale and clink “remove scale”
6. If your image is too dark: Go to IMAGE->ADJUST->BRIGHTNESS CONTRAST and adjust brightness and contrast until you see the veins well. Then click “Apply” and close the box
7. To increase line width: go to Edit-> Options -> Line Width. I put in 5 and it worked well for me if you want to try that width.
8. To change the color setting, double click on the icon to the right of the hand icon (12th icon from the left). A pallet of colors will appear, and you can double click on the color you want, then close the box.

* **Measuring leaf area and perimeter**

1. Zoom out of the scale, and zoom in at leaf lamina insertion, where the lamina starts to come off the petiole (the more you zoom in the more precise your measurement will be).
2. Enlarge your image so that it takes up most of your screen.
3. Select the polygon icon (3rd from the left) in Image j.
4. Double click where the lamina meets the petiole on one side of the leaf. You are going to be tracing around the leaf area. Follow the leaf margin with your cursor and click once to anchor a new point. The more points you anchor the more precise it will be. You can move up and down the image using as explained above the space bar key and mouse.
5. Once you have traced around the whole leaf, close off the area by double clicking where you started.
6. Press D and M to draw and measure
7. Copy the results from the results window that popped up. You want to report the area and perimeter to the excel file.

* **Measuring midrib diameter**

1. Zoom in at the middle of the midrib (at half the distance between the petiole/midrib connection and the leaf tip).
2. Select the segmented line by right clicking on the line icon and choosing segmented line.
3. Double click on one side of the midrib. You have anchored your point.
4. Now, draw to the other side of the midrib, keeping perpendicular to the midrib length and double click on the other end.
5. Your cursor is free from the line. Press D and M.
6. Your line is now colored. Check that it does follow well the diameter of the midrib, perpendicular to its length. Report value to your spreadsheet.

* **Measuring secondary vein diameter**

1. Pick a secondary vein at the middle of the leaf, on the side that is most clear to the eye. Zoom in at the middle of the secondary vein (at half the distance between the midrib and leaf margin).
2. Select the segmented line by right clicking on the line icon and choosing segmented line.
3. Double click on one side of the secondary vein. You have anchored your point.
4. Now, draw to the other side of the secondary vein, keeping perpendicular to the midrib length and double click on the other end.
5. Your cursor is free from the line. Press D and M.
6. Your line is now colored. Check that it does follow well the diameter of the secondary vein, perpendicular to its length. Report value to your spreadsheet.

* **Measuring other 1° vein length**

1. Zoom in on the bottom of the midrib, at the lamina insertion.
2. Select the segmented line by right clicking on the line icon and choosing segmented line.
3. Double click where the 1° vein begins (this would be at the “insertion point”, i.e., where lamina starts coming off the petiole). You have anchored your point.
4. Now, follow the 1° vein closely by clicking along it. You want to draw over the vein.
5. When the 1° vein ends, double click. Your cursor is free from the line. Press D and M.
6. Your line is now colored. Check that it does follow well the diameter of the secondary vein, perpendicular to its length. Report length value to your spreadsheet.

* **Measuring 2nd vein length**

1. Select the segmented tool. Double click on the first 2° vein, at the intersection of the midrib and 2° vein. Then click following that vein until the end. Double click once you have drawn over the whole vein. Press D and M.
2. The result box will show up with the length of that vein. Leave it open, and go to the next vein. Repeat step 4 until all the 2° veins of the category (ex: large) are measured.
3. Once all secondary veins are measured, select all the data in the result box (there should be one line per vein measured). You can right click and “select all”. Copy, and then paste it to a blank spreadsheet.
4. Enter and sum all the lengths together using the formula in excel: =SUM(data)

Copy the value, and 2nd vein lengths in your spreadsheet. Make sure that you use to “copy special” option in excel, and pick “values”. Otherwise you will just copy the formula you used, and the number will change.

* **Measuring third order vein lengths**

1. Partition the leaf into three parts: bottom third, middle and top third of the leaf. Select the Rectangular icon (the very first icon) and draw onto your leaf a rectangle making up half of the third of the whole leaf surface and on one side of the midrib, so as to avoid it (we have had it vary from 10 to 300mm², but depending on your leaf size, this could be smaller or larger than this range; make sure that the box spans at least two secondary veins). Draw your rectangle at the bottom of the leaf, avoiding if possible the midrib (although for very thin leaves that might be impossible), so that the rectangle fits in between the midrib and the leaf margin.
2. Press D and M, then drag your rectangle to the middle third of the leaf, place it like the first one between the midrib and margin and Press D.
3. Drag your rectangle toward the top third of the leaf; place it between the midrib and margin and Press D.

*Note: some leaves are really narrow toward the top, and the rectangle you drew for the bottom and middle might not fit at the top. In that case, draw a new rectangle of more or less the same size, but that would be longer than wider, so that it fits on your leaf lamina. Be sure to measure the area of that new rectangle.*

1. Record the area of your rectangle (or rectangles) in your spreadsheet.
2. Measure the total length of the secondary veins if present by tracing over them using the segmented line. Press M and D. Once all are measured, copy the results onto a spreadsheet and sum them all up. Copy the summed up value, paste special “value” and copy the result in your spreadsheet.
3. Proceed to measure 3° veins by using the segmented line and tracing over all tertiary veins. Then repeat step 5 for the 3°veins results.
4. Repeat steps 5 and 6 for all your rectangles. We suggest using a different color for different vein orders.

* Calculating vein densities for major veins

1. Vein densities for each order are calculated as the ratio of vein length/leaf area for 1o and 2o veins, and as 3o length / rectangle area for 3o veins.

However, because your 3 vein length per area measurement might be biased by the amount of secondary veins included in the boxed area, we recommend measuring 3 o VLA as

(1)

This calculation is for one box. To get the 3rd VLA of your leaf, you calculate the equation (1) for the other boxes and do the average of the three calculations.

1. **Finally, major VLA is calculated as the sum of the 1°, 2° and 3° vein densities.**

TIP: To undo measurements you can press ALT, keep it pressed as you move your cursor through the points you want to remove and then place your cursor back to where your point want to be. At that stage stop pressing ALT and continue your measurements.

Une image contenant vert, insecte

Description générée automatiquement

*Example of the measurements of Major VLA on a leaf from Chrysophyllum prieurii, following the protocol presented above. The petiole is drawn with a blue polygon. The midrib is drawn in black, the 2nd veins are drawn in red, the three boxes for the 3rd veins (colored in blue) are represented in yellow. The 2nd veins inside the boxes are in pink here.*