Measuring Leaves Protocol

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Note on nomenclature

This protocol can be usedboth on simple and compound leaves. If using on compound leaves, substitute "leaflet" for "leaf".

Note on monocots and gymnosperms

This protocol is not intended for use on monocots nor gymnosperms.

Installation of the program itself

Install Fiji.

https://imagej.net/Fiji/Downloads

Preparing to measure

Open image using Fiji (rgt click > open > other > fiji).

Assign numbers to mature, intact leaves (they must have their bases visible, visible and uneaten tips, and must not be folded in half). Do this by clicking on the paintbrush button and 'painting' the number next to the leaf. If there is only one 'good' leaf available, choose it.

Use a random number generator to choose a random leaf from among them. I used RandomNumberGenerator.c. Input your upper bound, desired number of numbers, and a seed and then run the program in CodeRunner or a similar environment.

Click on "Straight" button in ImageJ window.

Zoom in using UP arrow, center of zoom is the location of your mouse.

draw a line between two cm marks on the included scale bar.

Calibrate measurements (Analyze > Set Scale > "1" in 2nd box down).

Measuring the leaf

Blade base at point of petiole insertion. Enter value into column **BB**.

Measure the length of the blade ("cmd M", measurements are in separate window). When measuring this, if the leaf is curved measure from the base of the leaf to the apex of the tip, even if this means that the measuring line is not centered on the primary nerve. If the tip is missing (and there are no other suitable leaves) estimate where the tip would end based on other leaves in the picture. Enter value into column **LB**.

Draw the length of the blade ("cmd D", produces a white dashed line)

Width at half blade length. If a leaf has part of its blade eaten at the point where you are measuring and there are no suitable alternative leaves: (i) if the leaf is symmetrical, bring the halfway dot of the measuring

line to the primary nerve (ii) if the leaf is asymmetrical, observe the curvature of the leaf and estimate where the leaf edge would be and bring the far end of the measuring line there. This can be helped by observing other leaves in the photo. Enter value into column **W50**.

Blade apex width at about three-quarters length. Enter value into column **W75**.

Blade apex width at about one-quarter length. Enter value into column **W25**.

Petiole angle of insertion: press the "Angle" button on the toolbar and then click where you want one of the arms of the angle to begin. Draw to the vertex and click. Then drag the second arm to the proper place and click again. then hit "cmd M". Enter value (second cell from the right) into column **Angle**.

After measuring leaf

Save the altered image. (shift > "cmd S")

Click in measurements pane and delete data therein ("cmd A" > "cmd X").

Open next image (shift > "cmd O")