Measuring specific leaf area and water content

Etienne Laliberté

Abstract

Here we describe the standardised protocol used by the Canadian Airborne Biodiversity Observatory (CABO) to measure leaf water content and specific leaf area, using the WinFOLIA™ software (Régent Instruments). These leaf area and water measurements are done on a subset of leaves from the same bulk leaf sample used to measure leaf spectral reflectance and transmittance. Briefly, after removing their petioles, fresh leaves are weighed, rehydrated for 6 h, scanned for total leaf area and weighed again; they are then oven-dried at 65 ºC for 72 h, and weighed one last time. This allows us to measure leaf dry matter content and its complement, leaf water content, as well as leaf relative water content. Leaf area measurements are used to estimate specific leaf area, a key functional trait central to the leaf economics spectrum. Specific leaf area allows us to estimate equivalent water thickness and to convert concentrations of foliar biochemical constitutents from a leaf mass to a leaf area basis.

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Guidelines

Equipment

- Sartorius Secura 213-1S 1 mg (3 decimal places) balance or other similar balance
- Sartorius Secura 1102-1S 0.01 g (2 decimal places) balance or other similar balance
- Canon LiDE 220 portable scanner or other suitable flatbed scanner
- WinFOLIA™ leaf area software (Régent Instruments Inc.)
- Forced air drying oven
- Dessicator
- RezChecker color/resolution target (optional)

Consumables

- Paper towels
- Paper envelopes and/or bags
- Stapler and staples (for paper bags)
- Sealed plastic bags
- Weighing trays

Protocol

Leaf Sample Selection And Preparation

Step 1.

Select a sub-sample of leaves from the same bulk fresh leaf sample on which spectral reflectance and transmittance was measured.

NOTES

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The selected leaves do not have to be the same ones used for spectral measurements, but should be as similar as possible to those.

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Select enough leaves to entirely fill the scanner bed. Leaves larger than the scanner bed should be cut and scanned in multiple files.

Leaf Sample Selection And Preparation

Step 2.

Cut the petiole of each leaf that will be scanned.

NOTES

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For a compound leaf, the petiole is the extension of the rachis beyond which there are no leaflets. The rachis should remain on the leaf, since it is the functional analogue of the midrib vein for a simple leaf.

Record Leaf Fresh Mass

Step 3.

Immediately weigh the selected leaves (with petioles removed) and **record the leaf fresh mass** (g).

NOTES

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This fresh leaf mass should be done as close as possible to the spectral measurements; keep the fresh leaves in a sealed bag in which you have breathed into to prevent them from losing water.

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Use a 3-decimal place balance if possible. A 2-decimal place may be sufficient for very large leaves.

Leaf Rehydration

Step 4.

Store the selected leaves in a sealed plastic bag in which you have breathed into. Add a piece of damp paper towel (use deionised water).

NOTES

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Label the sealed plastic bag with the sample ID and/or a barcode label.

Leaf Rehydration

Step 5.

Store the sealed sample in the dark, in the fridge (never a freezer) or a chilled cooler for **12 h**.

Record Leaf Rehydrated Mass

Step 6.

Gently pat dry the rehydrated leaves to remove surface water.

Record Leaf Rehydrated Mass

Step 7.

Weigh the rehydrated leaves as a whole and **record rehydrated leaf mass** (g).

NOTES

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Use a 3-decimal place balance. A 2-decimal place balance can be sufficient for very large leaves.

Create Working Folder

Step 8.

Go the the shared 'leafscans' CABO Google Drive Folder for your project.

NOTES

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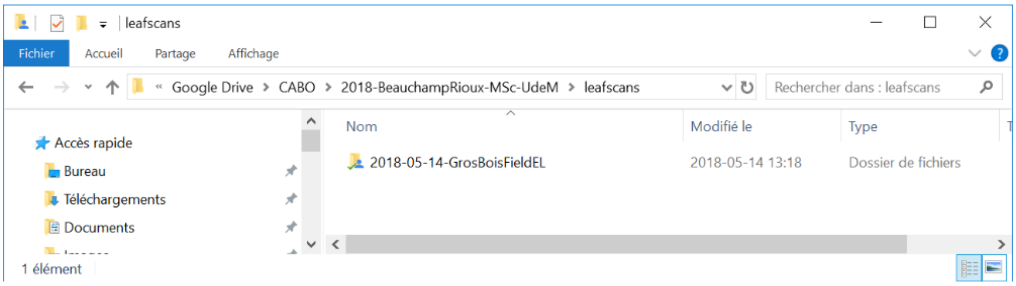
If you do not yet have a shared Google Drive folder for your project, contact the CABO data manager to create one (etienne.laliberte@umontreal.ca or jeremy.goimard@umontreal.ca).

Create Working Folder

Step 9.

Create a new folder named *YYYY-MM-DD-SiteID* (without spaces) within that 'leafscans' folder.



NOTES

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The 'Site ID' should be the same as the Site ID defined in the field for that site.

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This working folder is where the data file and all acquired images for that site on that day will be stored.

WinFOLIA Set-Up

Step 10.

Open WinFOLIA.

WinFOLIA Set-Up

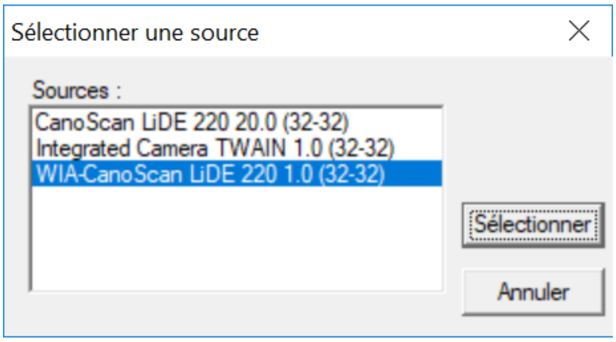
Step 11.

Choose the same settings as the last time WinFOLIA was run.

WinFOLIA Set-Up

Step 12.

Select the scanner to use; it should start with WIA...



NOTES

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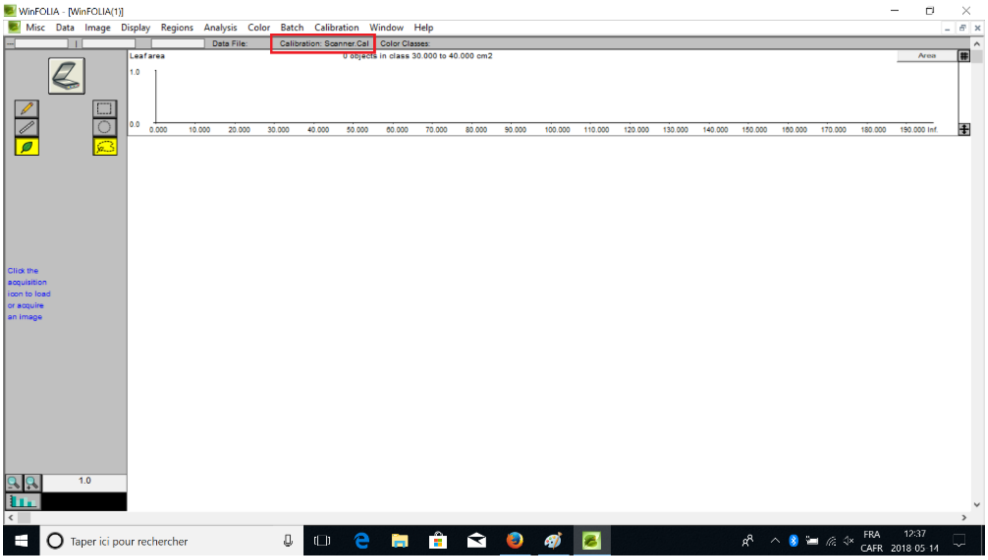
The scanner drivers must be installed first. Choose the scanned starting with WIA-...

WinFOLIA Set-Up

Step 13.

Ensure that the scanner calibration file is loaded.



NOTES

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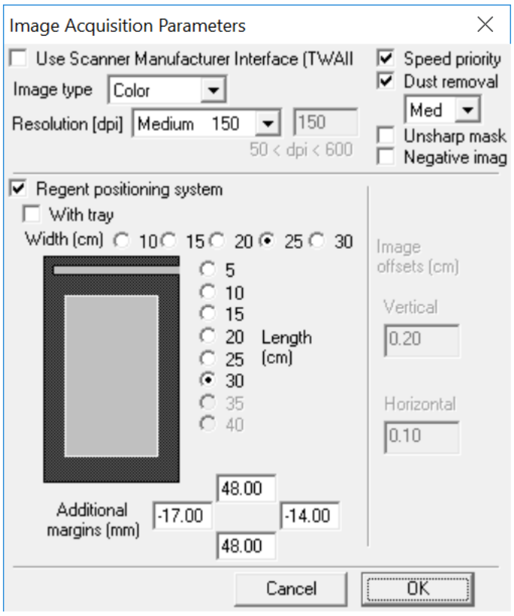
If the scanner calibration file is not loaded, refer to the WinFOLIA manual to load it.

WinFOLIA Set-Up

Step 14.

Ensure that the *Image > Acquisition Parameters...* are set as shown in the image below.



NOTES

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The parameters shown are for the Canon LiDE 220 portable scanner used in the field, and may differ on another scanner.

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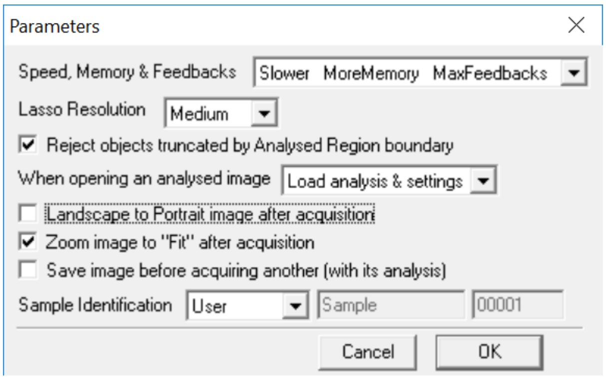
Although the leaf area analysis is done on greyscale images, **scan in color** at **150 dpi**.

For very small leaves, a higher resolution (e.g. 300 dpi) might be required.

WinFOLIA Set-Up

Step 15.

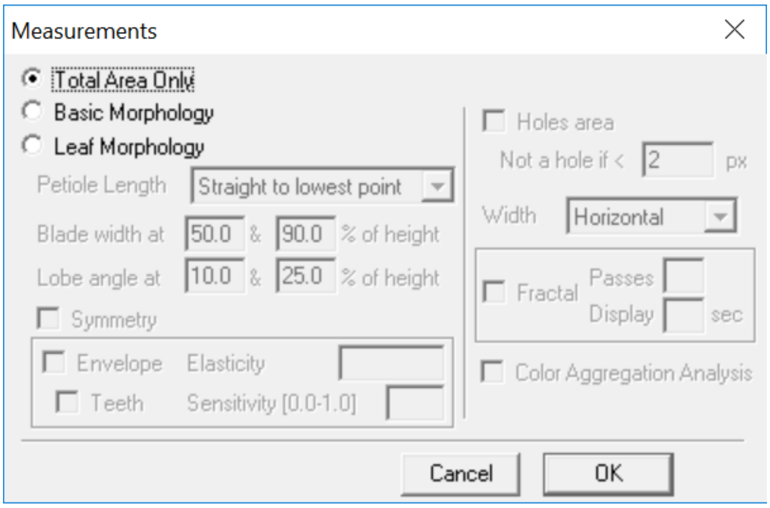
Ensure that the *Analysis > Parameters...* are set as shown in the image below.



WinFOLIA Set-Up

Step 16.

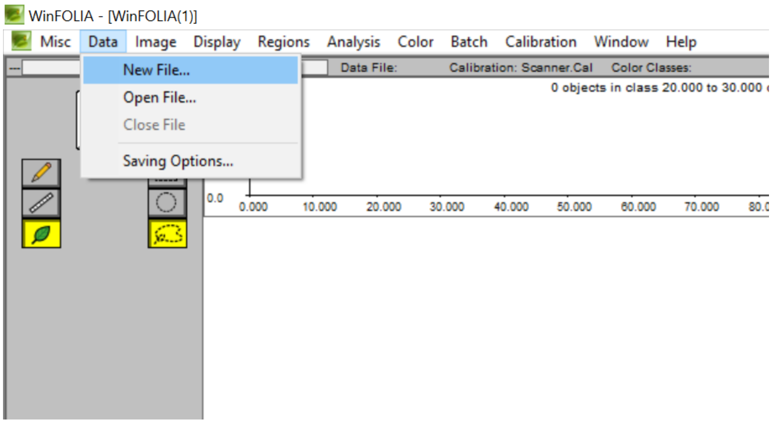
Ensure that the *Analysis > Measurements...* are set to *Total Are Only*.



Create New Data File

Step 17.

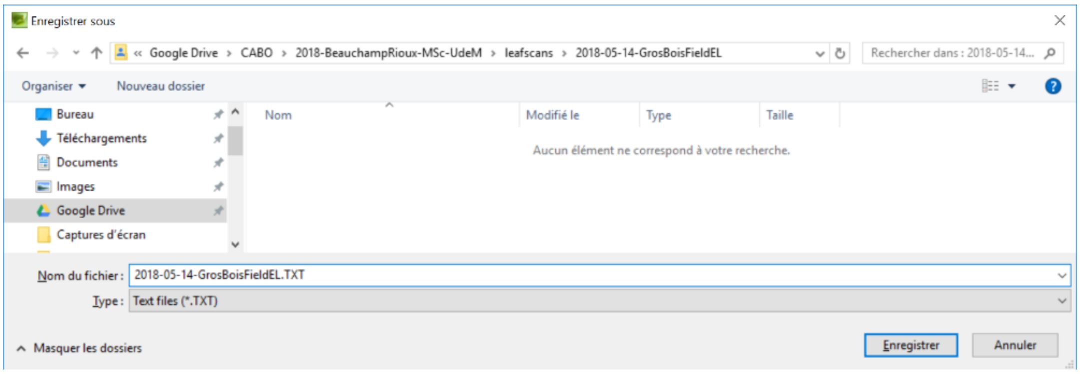
Create a new data file using *Data > New File...*



Create New Data File

Step 18.

Save the data file as *YYYY-MM-DD-SiteID.txt* in the 'leafscans' CABO shared Google Drive folder for your project.



Leaf Scan

Step 19.

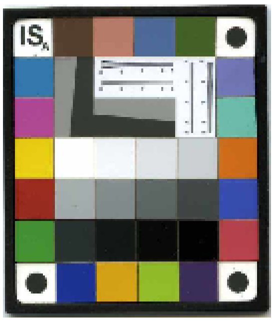
Position the leaves to fill the scanner bed:

- position leaf apex at the top of the image
- leave margins of scanned bed free
- ensure leaves are not folder
- ensure leaves do not overlap each other

Leaf Scan

Step 20.

Add the RezChecker target in the top left section of the image (optional; if target is available).



NOTES
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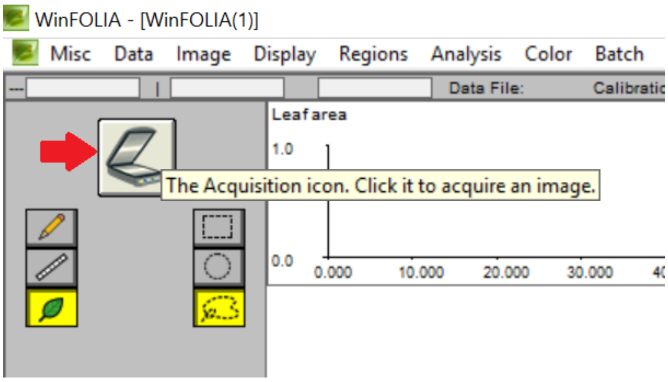
This target can be used to calibrate the color and/or resolution of the image later on.

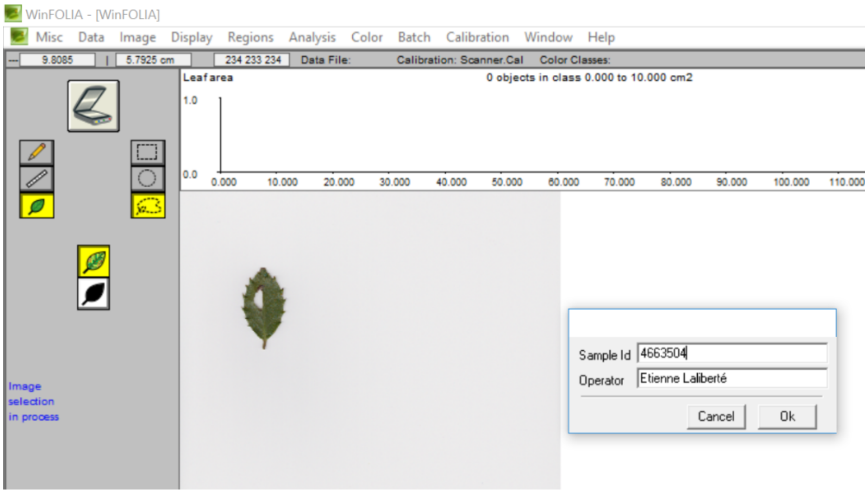
Leaf Scan
Step 21.

Acquire a scan.



Leaf Scan
Step 22.

Enter the Sample ID, and name of operator.



NOTES

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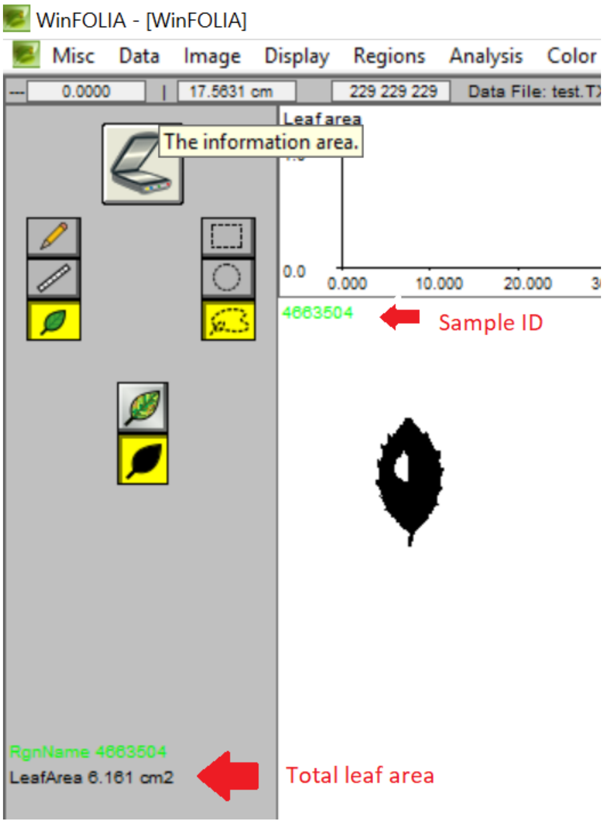
The 'Sample ID' should be **exactly** the same sample ID of the bulk fresh leaf sample, as defined in the field.

Leaf Scan

Step 23.

Click anywhere on the scanned image to start the analysis on the whole image, or select a particular region for analysis, and **record the total leaf area** (cm^2).



NOTES

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The total leaf area (cm2) for that image gets automatically saved in the data file.

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If the leaf sample requires multiple scans, repeat steps 15-17 as many times as required. In that case, add an identifier at the end of the Sample ID (e.g. 4663504-A, 4663504-B).

ANNOTATIONS

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The protocol should also explain what to do if the analysis is incorrect, and refer to the appropriate sections in WinFOLIA.

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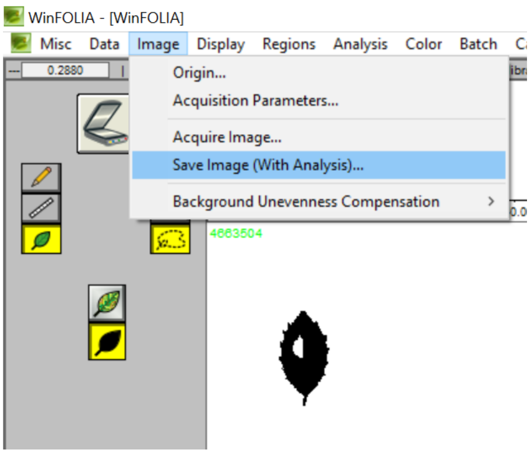
replace A by nothing, B by -2, etc.

Leaf Scan

Step 24.

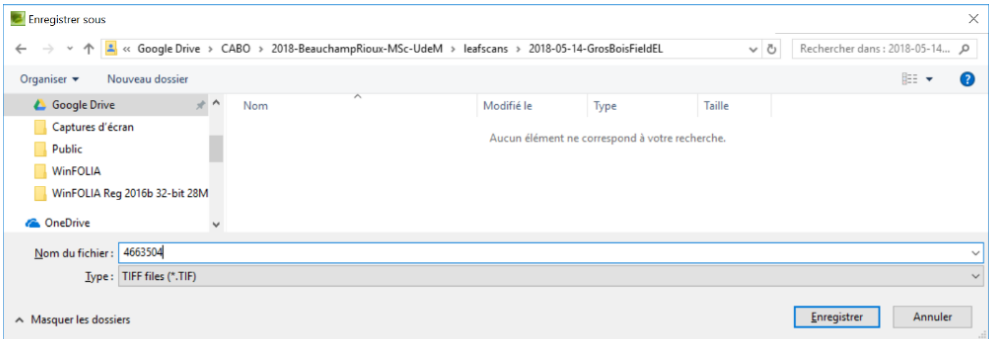
Save the analysed image(s) in the Google Drive working folder.



Leaf Scan

Step 25.

Name the image file using the sample ID.



Record Leaf Dry Mass

Step 26.

Transfer the scanned leaves in a labelled paper envelope or small paper bag.

NOTES

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Stape the envelope or paper bag to prevent leaves from falling out.

ANNOTATIONS

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Staple

Record Leaf Dry Mass

Step 27.

Dehydrate the leaves in forced air drying oven at **65 ºC for 72 h**.

Record Leaf Dry Mass

Step 28.

Ideally, cool the samples down to room temperature in a dessicator prior to weighing.

NOTES

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If no dessicator is available, lower the drying oven temperature to room temperature but with forced air still on while samples are being weighed.

Record Leaf Dry Mass

Step 29.

Weigh the dried leaves and **record leaf dry mass** (g).

NOTES

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Use a 3 or 4-decimal-place balance, depending on sample size. A 2-decimal place balance might be sufficient for very large leaves.

Close Data File

Step 30.

Once all leaf samples for that site/day are scanned and analysed for area using WinFOLIA™, close the data file using *Data > Close File*.

ANNOTATIONS

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Step should come before record dry mass

Calculating Specific Leaf Area (SLA) and Leaf Mass per Area (LMA)

Step 31.

Specific leaf area (SLA; $m^2\,kg^{-1}$) is calculated as:

SLA = (LA ÷ LDM) ÷ 10

where

LA is the total area of the leaf sample (cm^2)

LDM is the total leaf dry mass (g).

Leaf mass per area (LMA; $g\,m^{-2}$), the inverse of SLA, is calculated as:

LMA = LDM ÷ (LA ÷ 10 000)

Calculating Leaf Dry Matter Content (LDMC) and Leaf Water Content (LWC)

Step 32.

Leaf dy matter content (LDMC; $mg\,g^{-1}$) is calculated as:

LDMC = (LDM × 1000) ÷ RLM

where

LDM is the total leaf dry mass (g)

RLM is the rehydrated leaf mass (g)

Leaf water content (LWC; mg g^{-1}), the complement of LDMC, is calculated as:

LWC = 1000 - LDMC

Calculating Leaf Relative Water Content (RWC) and Equivalent Water Thickness (EWT)

Step 33.

The leaf relative water content (RWC; %) is expressed as:

RWC = [(LFM - LDM) ÷ (RLM - LDM)] × 100

where

LFM is the total leaf fresh mass (g),

LDM is the total leaf dry mass (g),

RLM is the rehydrated leaf mass (g).

Equivalent water thickness (EWT, g cm^{-2}, or cm^3 cm^{-2} = cm) is calculated as:

EWT = (LFM - LDM) ÷ LA

where

LA is the total leaf area (cm^2).

NOTES

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The relative water content expresses the actual water content of a given amount of leaf relative to the amount of water it contains in its fully hydrated state. It is one measure of leaf water stress at the time when spectral measurements were made.

Equivalent water content expresses the amount of water per leaf area, also at the time when spectral measurements were made.