



User's guide for Ecofog's Ecophysiology Lab

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Contents

Contents	v
1 Introduction	1
2 Handbook architecture	3
2.1 Morpho-anatomy	3
2.2 Hydraulics	3
2.3 Fluorescence	3
2.4 Gaz fluxes and exchanges	3
2.5 Microbiology and metabarcoding	3
2.6 Greenhouse setups and tips	3
2.7 Device info	3
3 Morpho-anatomy	5
3.1 Root Morphology	5
Image Acquisition	5
Image processing	8
WinRhizo	8
3.2 Resources	11
4 Hydraulics	13
4.1 Leaf turgor loss point, π_{tlp}	13
4.2 Materials	13
4.3 Methods	14
Installing Vapro for measurements	14
Sampling on the field	14
Lab measurements	14
5 Leaf midday water potential \$	17
5.1 Pressure chamber method	17
5.2 Psychrometry method	17
6 Relative water content RWC (%)	19
6.1 Material	19
6.2 Method	19
6.3 g_{min}	20
6.4 gs	20

CONTENTS

7	Fluorescence	21
8	Gaz fluxes and exchanges	23
9	Microbiology and metabarcoding	25
10	Greenhouse setups and tips	27
11	Device info	29
11.1	Vapor pressure osmometer - Vapro 5520 cheatsheet	29
	List of Figures	31

Introduction

Plant functional traits are the features (morphological, physiological, phenological) that represent ecological strategies and determine *describe?* how plants respond to environmental factors, affect other trophic levels and influence ecosystem properties. Variation in plant functional traits, and trait syndromes, has proven useful for tackling many important ecological questions at a range of scales, giving rise to a demand for standardized ways to measure ecologically meaningful plant traits. The importance of these topics dictates the urgent need for more and better data, and increases the value of standardized protocols for quantifying trait variation of different species, in particular for traits with power to predict plant- and ecosystem-level processes, and for traits that can be measured relatively easily (Pérez-Harguindeguy et al., 2013)

This handbook presents the different protocols used in the ecophysio lab. We therefore suggest the methodological principles for a more open and transparent science. This handbook not only includes updated methods for the trait measurements, but also includes the excel worksheets for data collection and the associated R-scripts to upload/clean the raw data.

Handbook architecture

This handbook is written for operational ends. As such, it is not a review or scientific paper thoroughly presenting each traits but rather a list of protocols associated with routinely measured traits in this lab.

Each chapter of this book correspond to one trait and associated measurement process.

2.1 Morpho-anatomy

2.2 Hydraulics

2.3 Fluorescence

2.4 Gaz fluxes and exchanges

2.5 Microbiology and metabarcoding

2.6 Greenhouse setups and tips

2.7 Device info

Morpho-anatomy

3.1 Root Morphology

Root morphology analysis (length, diameter, etc.) are conducted using the Winrhizo software.

Winrhizo is a licenced software created by Regent Instrument Canada Inc. It exist 4 different version and we own the *Basic Version*. It allows root morphology analysis from scans.

Image Acquisition

Format

Supported image format are .TIFF, .JPEG and .BMP. .TIFFand .BMP are not compressed and are thus to be preferred. .TIFF images are compatible with all OS and should be privileged but you must be careful to save them *uncompressed* as WinRhizo won't be able to open *compressed* ones.

The higher the resolution, the more pixel you will have and the more precise will be your measurements. However, with resolution, scan time and image size increase. 800DPI is the standard in this lab but 400 is the winrhizo recommendation. This depend on the required level of precision as well as the size of the analyzed roots (the finer the higher must be the resolution to get more details).

Scanner

Any scanner can be used to acquire scans for Winrhizo software. However, be sure that the format is compatible and that all the images inside your project are saved in the same format and the same resolution. For coherence

3. MORPHO-ANATOMY

purposes we encourage you to use the same formats between studies at Ecofog's lab scale. EPSON's V800 scanners are the ones used as this document is being written. The scanner model isn't important but we recommend to use scanners with a transparent (double-lamp) option. This will allow cleaner root scans for complex root systems. And the scanning software is [ViewScan](#)

Scan process

Paste the wall-taped doc.

Flat scan You can decide to use basic scan options with light only coming from below. If you do so you need to have a white background installed under the scanner's roof (if black roots, if pale ones you'll need a black background).

Choosing this option will simplify your protocol and can suffice for simple and thin enough root systems.

EXAMPLE scan marion

Transparent If your root are too big 3.1, then self-shading can appear on flat scan and bias winrhizo's analysis. To avoid this shading you can remove the background from the scanner's roof to enable double-lamp scanning. The light coming from top and bottom as one, shading will be avoid and scans will be cleaner.



Figure 3.1: Roots too thick to be flat scanned

Another case where you can prefer **Transparent** option is for complex root systems (e.g. bromeliaceae, 3.2). For this type of roots, you can scan them in a thin coat of water to disentangle fine roots. Doing so you will have a better analysis of the root system morphology and structure but once again have shading issue. Supressing them requires the use of the **Transparent** mode.

BEWARE: The **Transparent** scan window is smaller than the normal mode scan. The actual scanned zone is showed 3.3 and you must make sure that your roots are well placed within this area.

3.1. Root Morphology



Figure 3.2: Complex bromeliad adventitious root system

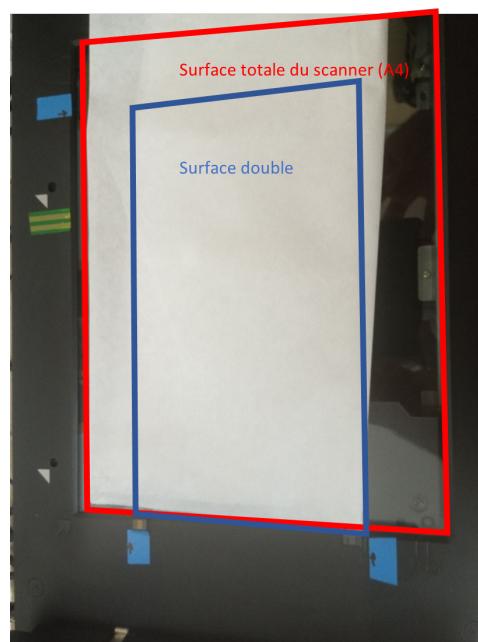


Figure 3.3: Flat (red) and Transparent (blue) scan zone of EPSON's V800 scanner

3. MORPHO-ANATOMY

Image processing

To analyze with winrhizo, you can either make it manually, one image at a time and by drawing rectangles around the roots you want to analyze. However, when having a lot of scans you might want to automatize the process using the **batch** option. If this is your choice, make sure that your images only contain roots!! Sometimes you will have to remove some parts of the scans to leave only roots in your images. For instance, this [3.4](#) is the scan from bromeliads roots. We can see the water-filled petri dishes border on the scan and this will be an issue for automatized Winrhizo analysis.



Figure 3.4: Scan of a bromeliad root system in water-filled petri dish

To re-crop these images we use the freeware **XnConvert**. The petri dish has always been placed in the same place using a stencil ?? on the scan window, enabling us to recrop all scans to the same size. Detailed **XnConvert** tutorial is available [HERE](#).

WinRhizo

Installation

The winrhizo software is contained on a CD (ask [Eliane Louisanna](#)). To be used you need to copy the software from the disk to your computer and install the protection key drivers (also on the CD). Once installed you don't need the CD to run the software but the protection key must be plugged. Unplugging it will prevent any use of the software.

Startup

First analysis

Once you have acquired your images and launched winrhizo you can start to analyze your scans. To display a single scan, click *Image -> Origin -> From File*. Then you can click the *acquisition* icon **PIC**. This will open a standard document opening window. Then you browse normally to find the wanted scan. Make sure that you are looking for the goor format, by

3.1. Root Morphology



Figure 3.5: Stencil used for inwater root scans



Figure 3.6: Stencil used for inwater root scans

3. MORPHO-ANATOMY

default, winrhizo display .TIFF. When you open it, winrhizo display the targeted image and you can then click on it (analyze whole image) or make a selection (only selected region) to start an analysis.

When an image or region is analyzed, winrhizo display the *sample identification* window which allows you to enter information about the sample. These informations will be saved with the measurements data. In this window click *OK* to do the analysis or *Cancel* to abort it.

After you clicked *OK*, winrhizo starts the analysis (can be stopped pressing *S*). When done, winrhizo is ready to save the data but a file must be opened or created first. Winrhizo display a window which asks whether to *open one*, *create one* or *save nothing*. Selecting *create one* will create a new .TXT file to store analysis data (more info about output [here](#)). Selecting *open one* will allow you to open a pre-existing file to add the new measurements at the end of this file. Clicking on *save nothing*, guess?

In the image, you can now see which roots have been analyzed. This have a skeleton line over them. The absence of this skeleton indicates that these roots have not been analyzed. This can be due to non-optimal pixel classification (see more about it [here](#)).

WELL DONE! You just analyze your first picture!

You can practice with scans available [here](#)

Calibration

If not calibrated (associated with a scale), winrhizo will display results in pixels. .TIFF files have an embedded scale, automatically detected by winrhizo. Check on your results if they are in px (pixels), in (inches) or cm (centimeters).

However, you can sometimes have uncalibrated files (mistakes or images from camera). Thus, you will need to “manually” calibrate your image. Winrhizo calibration files are saved as .CAL. In the *Calibration* menu you can load pre-existing calibration files. You will find the `calib_imge.TIFF` [here](#). To make your calibration at any DPI, you can print this image and scan it at the wanted DPI. The black square in the image delimit a white 1x1cm square. Loading this image in winrhizo, you can click on *calibration -> pixel size method -> object of known dimension -> 1 image -> width=1, height=1, border=0.35, units=cm -> Ok*

Then, winrhizo will propose you to save the calibration in a .CAL file that can be loaded later and used for all your images at a given resolution. **DO NOT NAME YOUR FILE Scanner.cal**. Please, when you create a .CAL at a previously not used resolution, store a copy of the calibration file [here](#) so that your work helps your successors!!

Batch

We saw how to analyze a **single picture or region** but you might have numerous scans to analyze and want to automatize this process. To do so you will give winrhizo a *batch* (i.e. a folder) containing any number of images you want.

Pixel classification

Output

3.2 Resources

Hydraulics

4.1 Leaf turgor loss point, π_{tlp}

We assessed the leaf turgor loss point, π_{tlp} in MPa, from a previously established relationship with the osmotic potential at full hydration, π_{osm} in MPa. π_{osm} is linked to the equilibrium solute concentration value C_0 (in mmol.kg⁻¹) directly measured with a vapor pressure osmometer (Vapro 5600, Wescor, Logan, UT). This is referred as the *osmometer method* (Bartlett et al. 2012a; Maréchaux et al. 2016).

4.2 Materials

- Vapor pressure osmometer (Vapro 5520, Wescor, Logan, UT)
- Vapro software (Vapro Lab Report)
- Fridge
- Liquid Nitrogen
- Ziplock bag
- Paper towel
- Distilled water
- Metal tea ball
- Tin foil
- Needle
- Liquid nitrogen gloves + goggles
- Liquid nitrogen contenant
- 2 Tweezers
- Cork borer

4.3 Methods

Installing Vapro for measurements

- Turn on Vapro the day before for the thermocouple's stability
- Test Water Quality *cf Vapro_cheatsheet*
- Clean
- Calibration *cf Vapro_cheatsheet*
- Control tests *cf Vapro_cheatsheet*
- Verify temperature
- Always have the black diamond at the center of the display

Used daily: * clean beforehand * select automatic mode (10 runs)

Sampling on the field

- Collect at least 3 healthy mature leaves on branch
- Place them in sample ziplock bag with:
 - wet paper towel
 - Exhale in bag to saturate in CO₂
 - Annotate bag with sample information
- Zip bag and stock in cooler

Lab measurements

Field day

- Recut branch under water
- Replace in ziplock bag with wet paper towel
- Put 24h in fridge to hydrate overnight

N+1 Field day

Vapro:

- check distilled water in vapro reservoir
- clean
- select automatic mode (10 runs)
- make sure vapro software is on

Sample measurement:

- Sample from a leaf a 5 mm disc with a cork borer: *avoid 1st and 2nd order veins to avoid apoplastic dilution that would lead to less negative osmometer values*
- Wrap disc in tin foil

- Immerse in liquid nitrogen for at least 2 min using metal tea ball
- Puncture 10-15 times with needle
- Place in vapro chamber

In total, disc are exposed to air for less than 40 seconds for all the steps.

- Record value C_0 when the difference between consecutive 2-min measurements fell below strictly 5 mmol.kg^{-1} after at least three runs.
- If error! or $\text{Nr_Run} > 10$:
 - try a 2nd cycle with same leaf
 - try a 3rd cycle with another leaf
 - otherwise record NA
- Beware of the stuck leaf inside the vapro! If so *cf Vapro_cheatsheet*

End measurements

Clean Vapro

For more information on the vapro machine, please refer to the *vapro cheatsheet* in the machine category.

Leaf midday water potential \$

5.1 Pressure chamber method

5.2 Psychrometry method

Relative water content RWC (%)

6.1 Material

Ziplock bag Paper towel *Distilled water* Fridge *Analytical balance* Sharpie
cooler envelop

6.2 Method

Before going on the field: * write individual code on ziplock bag * preweight ziplock bag

On the field: * collect leaf * clean leaf with clean paper towel * place leaf in preweighted corresponding ziplock bag * place bag in cooler for transport to the lab

In the lab: * weight the closed ziplock with the leaf (*fresh weight*) * delicately take the leaf out of the bag, wrap it in moist paper towel and place the wrap back in the ziplock bag * place ziplock bag in the fridge during 24 hours * 24 hours later, take out the leaf and wipe off water excess * weight re-hydrated leaf (*saturated weight*) * place leaf in envelop for oven-drying during at least 72h * weight dry leaf (*dry weight*)

Calculate RWC (%):

$$\text{RWC} = \frac{(\text{fresh weight} - \text{dry weight})}{(\text{saturated weight} - \text{dry weight})} \times 100$$

From Sapes et al 2020 and Barrs & Weatherley 1962.

6. RELATIVE WATER CONTENT RWC (%)

6.3 gmin

6.4 gs

C H A P T E R

7

Fluorescence

C H A P T E R

8

Gaz fluxes and exchanges

C H A P T E R

9

Microbiology and metabarcoding

C H A P T E R

10

Greenhouse setups and tips

Device info

11.1 Vapor pressure osmometer - Vapro 5520 cheatsheet

- π_{TLP} vapro cheatsheet

This template is based on *Bookdown* and the *Memoir* LaTeX class to allow writing a book, a report, a PhD thesis, etc. in *R Markdown*.

The main file is *index.Rmd* which contains the description of the book in its header. All other *.Rmd* files in the folder contain a chapter. The *references.bib* file contains the bibliography.

This file will have to be deleted, as well as *81-getting_started.Rmd* and *82-syntax.Rmd*: they have to be replaced by the content of the book.

To get started, create a new R project from this folder. Then open *index.Rmd* and click on the *Build Book* button in the *Build* window of Rstudio.

List of Figures

3.1	Roots too thick to be flat scanned	6
3.2	Complex bromeliad adventitious root system	7
3.3	Flat (red) and Transparent (blue) scan zone of EPSON's V800 scanner	7
3.4	Scan of a bromeliad root system in water-filled petri dish	8
3.5	Stencil used for inwater root scans	9
3.6	Stencil used for inwater root scans	9

Abstract English abstract, on the last page.

This is the user's guide of EcoFoG's ecophysiology lab

Keywords Keyword in English, As a list.

