FORGENIUS

GCU Sampling Protocols

Version 2

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Author's contributions

Nicolas MARIOTTE was the field technical advisor. He made a major contribution to the protocol, particularly for the materials and methods developed in sections A to E and in the annexes. Joan PRUNERA-OLIVÉ was heavily involved in the field and lab measurements and contributed to the protocol especially for section F. Florence JEAN oversaw the logistics of the field and sampling collection, coordinated the drafting of the protocol. Nicolas MARTIN, FORGENIUS WP1 leader, supervised the entire protocol and was particularly involved in section F. Camilla AVANZI, Francesca BAGNOLI, Andrea PIOTTI and Giovanni VENDRAMIN contributed to section E. Nassim BELMOKHTAR, Delphine GRIVET (WP3 FORGENIUS leader), Frédéric LAGANE and Philippe ROZENBERG contributed to section D.7. Regis BURTLETT and Sylvain DELZON contributed to section F-VI. Hervé COCHARD and Jose-Manuel TORRES-RUIZ contributed to section F-II. Pierre-Jean DUMAS contributed to section B (PAI) and performed field and lab measurements. This protocol, particularly sections A to E, was largely inspired from the Gentree project protocol (project grant agreement No.676876) coordinated by Bruno FADY. Ana HERNANDEZ contributed to the protocol especially for section F and performed measurements in field and lab. Jordi MARTNIEZ-VILALTA contributed to the first version of the protocol particularly for section B.I. In addition to his role as project's coordinator, Ivan SCOTTI was especially involved in section A.1. Finally, Maurizio MENCUCCINI, FORGENIUS WP2 leader, contributed, coordinated and supervised the entire protocol including field and lab measurements. All authors approved the final version of the document.

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Contents

Introduction

In Europe, the broad network of Genetic Conservation Units (GCUs) represents a comprehensive collection of valuable forest genetic resources, as well as a network of sites for the monitoring of the status of, and trends in, forest diversity. Each GCU's stand-level characteristics and genetic properties are stored in the European Information System on Forest Genetic Resources (EUFGIS). In its previous version (until November 2025, when FORGENIUS released the current version), EUFGIS was conceived as a "directory" listing GCUs and providing general information about their location, size, and general environmental properties. However, considering the unprecedented speed and magnitude of the environmental changes we are witnessing, as well as the increasing scientific and societal demands for better characterisation of biodiversity (both for conservation and sustainable use), we needed a deeper description of the EUFORGEN conservation network, including not only genetic diversity (a baseline descriptor of a network of conservation of genetic resources), but also functional diversity and an assessment of resilience.

The FORGENIUS research project ("Improving access to FORest GENetic resources Information and services for end-Users" https://www.forgenius.eu/) adopted a multi-pronged and multidisciplinary approach that aimed at accurately assessing adaptive potential and resilience in GCUs. This project, funded by the EU Horizon 2020 programme, brought together nineteen partners in ten countries to characterise genetic and functional diversity in twenty-three European tree species. FORGENIUS aimed to provide a framework for the assessment of the potential impacts of climate change-related droughts and heat waves on natural genetic resources, to inform forest management and conservation. FORGENIUS was based on a nested sampling structure, with a complete coverage of all European GCUs using remote-sensing indices, and more limited but targeted coverage of a reduced number of GCUs at genotypic and phenotypic level. Modelling was included to connect between layers and enable generalization across European GCUs. Using this approach, FORGENIUS has provided novel genetic and phenotypic information of tree species present inside the European network of Forest Genetic Resources (Forest GenRes). FORGENIUS ultimate, end-user-oriented outcome is the new EUFGIS information system (eufgis.org); the protocols it established for functional trait-related field and laboratory operations have a general value, as they can be extended to the characterisation of any (wild, managed) forest stand in any ecosystem. We present them as a proposal for a unified, generalised strategy to describe the functional properties of forest stands, particularly from the point of view of their functional resilience to drought -and heat-related climate extremes.

This document describes the field and laboratory measurements that were performed in Genetic Conservation Units (GCUs) with the general goal of providing the phenotypic information necessary to inform forest management, particularly regarding the potential impacts of climate change-related droughts and heat waves. The specific objectives of this protocol can be summarised as follows:

· characterize the environmental conditions of the site, and the forest stand of the GCUs,

· describe the tree-level properties on a subsample of ten trees per GCU that constitute the Functional Subset (FS) of each GCU,

· collect biological materials for genetic analyses on 25 trees which constitute the Genomic Subset (GS) and on the 10 FS trees for which multi-trait phenotypic diversity is determined.

· determine a fundamental set of "soft traits" i.e., relatively easy-to-measure morphological, growth and physiological traits (of leaves and wood) that provide information on the performance of trees in the field.

· determine two sets of fundamental water relations and hydraulics traits designed to quantify water use and response of trees to drought stress.

To fulfil these objectives, this general protocol is structured into different independent parts with the aim to facilitate its use in the field (Chapters A to E) or in the laboratory (Chapter F):

- Field Sampling Strategy (See Chapter A)
- Environmental descriptors (See Chapter B I)
- Biotic descriptors (See Chapter B II)
- Tree ring & wood sampling (See Chapter C)
- Functional Subset (FS): Branch sampling protocol (See Chapter D)
- Genomic Subset (GS): Branch sampling protocol (See Chapter E)
- Pressure Volume Curves (See Chapter F I)
- Gmin and desiccation (See Chapter F II)
- Branch capacitance (See Chapter F III)
- Volumetric Wood Density (See Chapter F IV)
- Turgor loss point by osmometry (See Chapter F V)
- SLA, %N, d13C and Huber value (See Chapter F VI)
- Vulnerability curves measurements (See Chapter F VII)
- Soil texture analysis (See Chapter F VIII)

And additional documents in the appendices.

This protocol is based on the main following documents:

FORGENIUS Grant Agreement - number: 862221 - FORGENIUS - European Union's Horizon 2020 research, H2020-SFS-2018-2020 / H2020-SFS-2019-2 (I. Scotti).

Opgenoorth L., Dauphin B., Benavides R., Heer K., Alizoti P., Martínez-Sancho E., ..., Fady B., Myking T., Valladares F., Aravanopoulos F.A., Cavers S., 2021. The GenTree Platform: growth traits and tree-level environmental data in 12 European forest tree species. GigaScience 10(3), giab010 (https://doi.org10.1093/gigascience/giab010). https://hal.inrae.fr/hal-03282821v1.

This protocol must be supplemented by practical training with competent persons to implement these measures in the field and in the laboratory.

Keywords:

Phenotypic traits – functional traits - hydraulic traits - biotic descriptors - environmental descriptors – site descriptors - Forest Genetic Conservation Unit – materials - methods - protocol

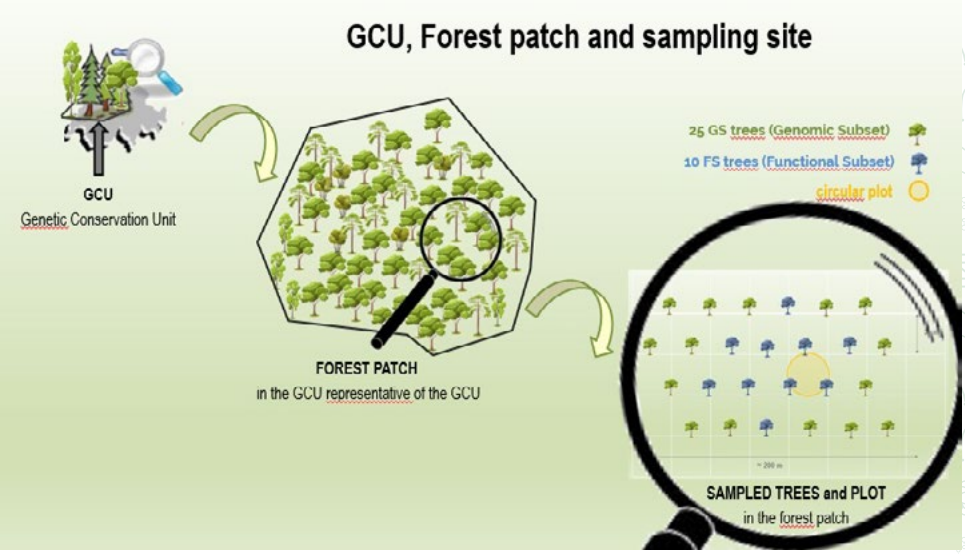
Abbreviations:

CAP: branch CAPacitance; Cbulk: leaf CAPacitance; cbh: Trunk circumference measured at breast height (1.3 m); CV: xylem vulnerability to embolism; DNA: DesoxyriboNucleic Acid; DTM: Digital Terrain Model; Eb: Mean leaf bulk elastic modulus; Eft: Maximum leaf bulk elastic modulus; EUFGIS: European Information System on Forest Genetic Resources; FORGENIUS: Improving access to FORest GENetic resources Information and services for end-Users; FS: Functional Subset; Fs: Fraction of leaf symplastic water; G/ha: Trunk cross-sectional area per hectare of a forest stand; GCU: Genetic Conservation Unit; Gmin: minimum leaf conductance; GPS: Global Positioning System ; Gres: residual leaf conductance (= Gmin); GS: Genomic Subset; Kmax: Maximum hydraulic conductivity; HV: Huber Value; LA: Leaf Area; LAI: Leaf Area Index; LMA: Leaf Mass per Area (1/SLA); LS: Leaf Succulence; NIRS: Near infraRed Spectroscopy; P50: xylem water potential value at which 50% loss of hydraulic conductance occurs; PAI: Plant Area Index; PV: Pressure Volume; RWC, leaf relative water content; SLA: Specific Leaf Area (1/LMA); Td: Time to full dessiccation; TLP: turgor loss point; WD: Wood Density; WP: Water Potential; π0: Osmotic potential at Full turgor.

Summary

The protocol characterizes 25 dominant or codominant trees inside a selected GCU. These 25 trees should be sampled for their DNA and correspond to what is called the Genomic Subset (GS) in the protocol. Ecophysiological measurements are taken on 10 of these 25 trees which correspond to what is called the Functional Subset (FS). These measurements are focused on traits related to drought resistance; water use efficiency and growth as a response to climate extremes.

Selection of forest patch inside the GCU



- **Identify the GCU area and its boundary in the field with the local partners.**
- **Check for a convenient location for field work inside the GCU, particularly the ease of access and the lack of thinning in the last 5 years in this forest stand.**
- **Select the forest patch, the potential area that should contain the sample trees:**
    - Choose an area representative of the GCU for the FS area i.e., retaining the dominant facies. *If there is a mosaic of facies, choose a homogeneous facies for the 10 trees of Functional Subset (FS) and complete with the other facies for the 15 additional trees of the Genomic Subset (GS)*
    - Choose a homogeneous environment with similar topography, aspect/slope for the FS area
- **Finally, check in this potential forest patch if you can find 25 dominant or codominant trees at least 30 m apart each: 10 trees for the Functional Subset (FS) + 15 additional trees to complete the Genomic Subset (GS).**

Selection of individual trees

Only adult, dominant or codominant trees should be sampled. It is important not to select senescent trees (with evident declining signs) or trees that have a defoliation level greater than 50%, unless these characteristics are present in most individuals in the forest patch.

These individual trees would ideally be selected along 4-5 parallel transects in order to keep a **minimum distance**

between trees ≥ 30 m. If it is not possible to follow this scheme, the number of transects should be reduced while increasing the number of trees per transect. FS trees should be selected first and GS trees afterwards.

Each selected tree must be individually tagged and georeferenced**, Geographical coordinates must be in decimal degrees with 5 decimal units (accuracy 1.1 m) and format must be in the WGS 84 reference system. Elevation should be measured in meters above sea level.

**** GCU code (EUFGIS code) + Tree number (001 to 025).**
Example: ESP00113_013

If trees are already labelled by local partners, no additional labels are added. The local reference must be recorded in the data file to allow linkage with the Field Tree Code.

The **EUFGIS Tree Code (GCUCode_Year_SpeciesCode_TreeNumber)** will be generated later for reporting purposes (e.g. ESP00113_2025_PINUS_SYL_013). The date (year) and species code (UPOV system) are recorded as separate variables in the data file and used to construct the EUFGIS Tree Code.

Important: Always keep the same Field Tree Code for the same individual tree and all associated samples throughout field and laboratory work.

The tree labelling is referred to all protocols as "GCUCode_TreeCode". **See Tree Labelling System section** for full details and rules.

Environmental descriptors assessment on a plot with soil samples collection

Traits measured	Brief explanation	Tools	Unit	Scale
Lat, long, elevation	Location of the centre of the plot (latitude, longitude, elevation)	GPS	decimal degrees / meters	Centre of the plot
soil_depth	Maximum soil depth	soil auger or soil pit	cm	Plot level
root_depth	Assessment of the deepest visible root	soil auger or soil pit	cm	Plot level
coarse_elements	Percentage of coarse elements and nature: • blocks: > 20 cm, • stones: 7 cm - 20 cm • pebbles: 2 cm - 7 cm • gravel: 2 mm - 2 cm	soil pit	nature %	Plot level
slope	Local slope	clinometer	degree	Plot level
aspect	Local aspect	compass	grade	Plot level
topography	Micro-topography assessed at the plot scale in 3 classes	naked eye	1 convex 2 neutral 3 concave	Plot level

Biotic descriptors assessment at tree level

Traits measured	Brief explanation	Tools	Unit	Scale
cbh	circumference at breast height	tape	cm	GS trees
ht	total height	vertex dendrometer	m	FS trees
hbc	basal crown height	vertex dendrometer	m	FS trees
dieback	Index for the level of decline in 4 classes corresponding to a defoliation percentage assessment.	visually	0 [0-25%] 1 [25-50%] 2 [50-75%] 3 [75-99%] dead.	FS trees
crown_area	Tree crown projected area assessed by 4 lengths projected in the 4 directions: north, east, south and west.	telemeter	m	FS trees
n_living_ha n_dead_ha g_living_ha g_dead_ha	Stand density and stand basal area corresponding to the sum of basal areas at breast height of all living trees and all dead trees	Bitterlich relascope or Bitterlich relascope APP for Android	N/ha m2/ha	surrounding each FS trees
Dead trees	Note for the presence or not of dead trees around FS tree for the target species.	visually	0 absence 1 presence	FS trees
core_lg	Total length of the core without bark measured on the tree ring core after collection	a graduated ruler (mm resolution)	cm	FS trees
sap_lg	Sapwood length measured on the tree ring core after collection.	a graduated ruler (mm resolution)	cm	FS trees
bark_lg	Bark length measured on the tree ring core after collection.	a graduated ruler (mm resolution)	cm	FS trees

The CBH measurement on 15 additional GS trees is carried out at the same time as the collection of leaves or needles for the Genomic Subset sampling (see chapter E).

Biotic descriptors assessment at forest stand level

Traits measured	Brief explanation	Tools	Unit	Scale
CBH	Circumference at breast height (> 23,6 cm) of all the trees inside a circular plot representative of the GCU.	tape	cm	Circular plot level
Structure	Forest stand structure of the GCU	visually	1 single layer 2 multi-layered 3 sparse forest	Circular plot level
Composition	Composition of the forest stand assessed visually inside the circular plot representative of the GCU	visually	1 single species 2 mixed forest	Circular plot level
Regeneration	The presence and abundance of natural regeneration in 4 classes for the target species.	visually	0 Absence 1 Scattered 2 Groups 3 Abundant	surrounding each FS trees
PAI	The projected area of green leaves, needles and some branches or trunk per unit horizontal ground surface area.	Digital camera	m2/m2	in the forest patch area surrounding FS trees.

Sampling strategy

The strategy aims at collecting material for both genotyping and phenotyping individual trees to assess their resistance and adaptability to drought.

Collection of leaves for DNA analysis

From each of the 25 trees (GS) of a GCU, a few healthy, green leaves or needles (5-6 leaves for broadleaved species, 15-20 needles for conifers with long needles, and 40-50 for those with short needles) are collected from the lower branches by hand or with a pole pruner. They should be stored in dry conditions in a paper bag or tea bag. Aerate in the evening. Once back in the lab additional oven drying (50°) is strongly recommended to perfectly dry the material (see chapter E). This collection of genomic samples is carried out at the same time as the tree selection (the 10 FS trees and the 15 additional GS trees).

Branches for ecophysiology

On each of the 10 FS trees, 2 to 3 long, sun-exposed branches (1-2 m long and 3 cm in diameter at the base) are collected either with a pole-pruner or by climbing. Each branch is subsampled to collect shoots for the assessments of the functional traits (Chapter D). Among all the branches collected on one tree, start by choosing the CV samples as it is difficult to find the straightness and diameter criteria and then the CAP ones.

Tree ring core and wood sampling

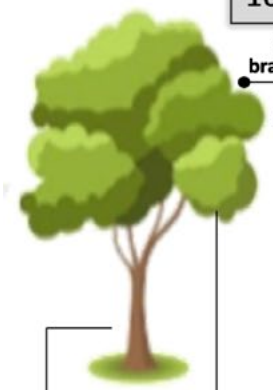
On each of the 10 trees of FS, 2 long cores (from the bark to the pith) are collected (1) for micro-density, radial increments and age (2) for isotope measurements. On the same trees, one short core or dust drill is collected for NIRS analysis (chapter C).

Soil samples

Soil samples will be collected by soil horizon every 20 cm down to the maximum depth (with a maximum of 100 cm) along the soil pith or with the soil auger according to the method used (see chapter B.I). Put the soil samples from these soil horizons in a bag with the GCUCode_TreeCode the soil depth [0-20] or]20-40] or]40-60] or]60-80] or]80-100] on it. Double-check for the code in putting a plastic label with the code into the bag.

Sampling strategy per tree at a glance

SAMPLING STRATEGY PER TREE



	CAPacitance Huber Value	Cavitation Vulnerability (CV) / NIRS	Gmin *(Drought box)*	Pressure Volume (PV)
Number of samples	3 shoots (1 for CAP + 1 back-up + 1 for HV)	2 stem samples 1 segment at the base of CV stem for NIRS (for WP3)	3 shoots (1 + 1 back-up for Gmin + 1 for SLA) *On 8 trees/GCU for all GCUs*	3 small branchlets: 1 for PV + 1 back-up 1 for SLA *Same trees as Gmin samples*
Length – Diameter / Methods	 **CAP**: 50/60 cm length, well leafed with 20 leaf shoots. Paper towel at the bottom of the 2 capacitance branches. Cut the base of shoots under water. **HV**: 55 cm for HV sample. Collect all the leaves excepted the first 5cm from the base of the branch. Keep the base of the stem (15 cm length). No paper towel.	**CV**: L = 40 cm - 0,5< D_{base} < 1 cm As straight at possible – Avoiding big ramification- Removed ramification/needles or leaves. Cut everything under water. **NIRS** sample : stem 10 cm lenght at the base of the CV sample	35-40 cm for conifers 50-60 cm for angiosperms Cut the base of shoots under water.	10-15 cm. Cut the base of shoots under water.
Tags on label	*GCUCode_TreeCode*_CAP_1 / CAP_2/HV	*GCUCode_TreeCode* _CV_1/CV_2/NIRS	*GCUCode_TreeCode*_$Gmin_1$ / $Gmin_2$/ $Gmin_3$	*GCUCode_TreeCode* _PV e.g.: ESP00129_010_PV
Storage	**CAP**: Put wet paper towel at the base of the 2 CAP branches. Put these samples in one 100 l black bag. Keep in fresh conditions (or in the shadow in the field). **HV**: store the base of the stem (15cm) and leaves in a ziplock.	Wrapped with wet paper and cellophane. All CV samples are stored inside one very large black double-bag in fresh conditions until the sample preparation for shipment the day after or 2-3 days after.	Put wet paper towel at the base of the 3 branches. Put it in a 60L garbage bag. Put this individual bag in 1 large bag with 5-6 wet paper balls with another Gmin samples. Store in fresh conditions.	Put all the branchlets in a ziplock with 1 ball of wet paper. Store in a cool place.

5-6 leaves for broadleaves

15-20 needles for conifers with long needles

40-50 needles for conifers with short needles

Storage in dry condition in tea bag

- 2 long cores (to the pith): 1 core for the Functional Subset (FS) + 1 core for microdensity
- 1 short core or dust drill (according to the species) for NIRS analysis

Logistics

A dedicated mobile team composed of 2-3 members carried out the sampling across all Genetic Conservation Units (GCUs) in collaboration with local partners. This approach ensured methodological consistency across sites and minimized potential human errors during field sampling. For more information, please see the checklist in Appendix 2.

In general, the fieldwork takes one week per GCU with 2 people from the mobile team (2 climbers if tree climbing is needed for sampling collection):

- The 1st and 5th days for travelling.
- The 2nd day for the choice of the trees, and the measurements (biotic and environmental descriptors)
- The 3rd (and 4th days if necessary) for the sampling collection.

One usually needs the help of local partners for 2 days (particularly the 1st day on field for the visit of the GCU and the choice of the area).

Materials List for FORGENIUS GCU Sampling Protocol

(*not allowed in case of traveling by plane)

Site, Plot and Tree Selection

- Handheld GPS or DGPS (Differential Global Positioning System)
- Android tablet
- Laser distance measuring system (e.g., telemeter or Vertex), better and easier than tape measure
- Compass for direction
- Durable metal or plastic tree labels to identify and mark the tree + Dymo
- Staples and stapler
- Spray of recyclable forest paint* or construction tape
- Construction tape or milestones for delimiting virtual plot*
- Construction tape for selecting tree*
- Camera
- Pruning knife / hand saw

Environmental Descriptors

- Clinometer
- Compass
- Soil auger*
- Pickaxe*
- Folding shovel*
- Rigid tape measure*
- Knife
- Screwdriver
- Pruning shears (for cutting roots)
- Basin (to collect soil)
- Photo camera
- Plastic bags (to carry soil sample)
- Record sheet + pencil
- Munsell soil colour chart
- Hydrochloric acid

Biotic Descriptors

- Measuring tape for cbh
- Dendrometer Vertex or tape measure to check radius
- Bitterlich relascope
- Field sheet and pencil
- Chalks (to mark inventoried trees)

PAI Equipment

- Digital camera
- Digital single-lens reflex camera (≥12.3 MP, no compact camera)
- Fish-eye lens compatible with camera
- Field of view: 180°
- 4.5mm lens for APS-C cameras, 8mm for full-frame cameras
- Tripod or telescopic monopod with levelling head
- Measuring tape or stick (1.3m height)
- 3D bubble level or self-levelling mount
- Compass (for camera alignment to true North)
- Inclinometer (for slope measurement)
- Material for calibration of lens and camera

Sample Collection – Tree Cores

- Manual Increment borer
- Electric drill for increment borer *
- Battery for electric drill for increment borer*

- PVC cylinder tube and core holders (e.g., paper straws)
- Paper bags and polycarbonate multiwall panels to store
- Waterproof pencil
- Masking tape
- Graduated ruler
- Field-sampling sheets
- Borer cleaning and sharpening set

Sample Collection – Branches

- Climbing equipment and materials
- Pruning shears (2 or 3) for collecting samples
- Long pole pruner (~15-18m)
- Hand saw

Sample Storage

- Bucket*
- Plastic box (60cm × 40cm × 20cm)*
- Water
- Manual shears*
- Plastic labels
- Paper labels
- Indelible felt pen or pencil
- Zip lock bags
- Paper towel
- Packaging film
- Plastic tray
- Pen
- Note sheet (for data management)

Laboratory Protocols

- Falcon tubes (for rehydrating small samples for 12-24 hours)
- Bucket (for rehydrating large samples for 12-24 hours)
- Paper towel
- Shears
- Zip lock bags
- Alcohol
- Analytical balance (0.001g)
- Gas cylinder (N2 gas)
- Temperature and humidity sensor (e.g., Hobo ProV2)
- Fan
- Perforated bags (for dehydrating samples)
- Oven
- Razor blades
- Paper envelopes
- Desktop scanner (for LMA)
- Pencil
- Notebook (for data entry)
- Pressure chamber (for CAP + PV)
- Osmometre (for TLP)
- Leaf grinder (for Isotope analysis)
- Empty serum bags + tubes (for HV)

Safety Equipment

- Toolbox
- First aid kit

Check list of traits to measure

Activities / measurements	Field lab	Scale activities
Selection of sampling site, trees and plot see § A	F	
• Selection of forest patch • Selection of trees: GPS coordinates • Selection of plot: GPS coordinates on the centre of the plot • Photo of plot		- GCU - FS/ GS trees - plot - plot
Environmental descriptors see § B.I	**F**	
Bioclimatic environmental descriptors		
• Micro-topography		- plot
• Slope and aspect		- plot
Soil descriptors		
• Soil auger survey or soil pit description and soil sample collection		- plot
Biotic descriptors see § B.II	**F**	
• Stand forest composition and structure and forestry inventory (CBH > 23,6 cm)		- plot
• Stand density with Bitterlich relascope		- surrounding each FS trees + in the centre of the plot
• PAI		-in the FS patch area surrounding FS trees + in the centre of the plot
• Tree characters		
CBH		GS trees
Height (total/crown basal)		FS trees
Crown size		FS trees
Regenerative traits with seeding density assesment		surrounding each FS tree
Dieback index observations		FS trees
• Tree ring core, see § C.t		FS trees
Branch sampling for phenotypic traits, see § D	**F**	**FS tree**
Sampling for NIRS and micro-density, see § C	**F**	**FS tree**
Sampling for the Genomic Subset, see § E	**F**	**GS tree**

A. Field Sampling Strategy

I. Selection of sampling sites, trees and plot

1. Selection of GCU

The GCUs should be selected based on bioclimatic representativeness with an over-representation of areas in climatically marginal areas.

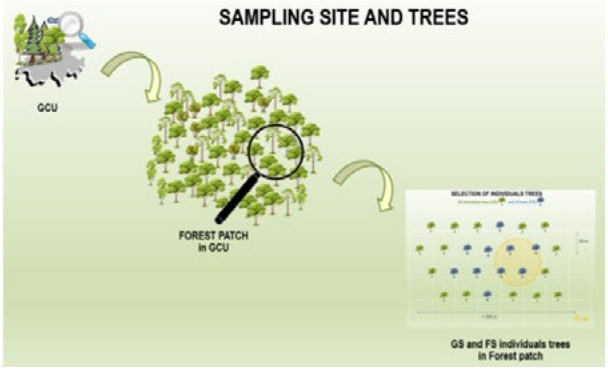
2. Sampling site

2.1. Selection of forest patch

If there is a dedicated mobile team to carry out sampling, it is essential to organize fieldwork in collaboration with local partners. The local partners help organise the campaign measurements for each country: planning, contacting the GCU forest managers, requesting permits from local authorities, etc. The local partners accompanies the mobile team in the field because they have a good overview of the GCU. It would be helpful to choose the suitable **forest patch** together.

Once the general GCU population is identified in the field, the following concepts regarding the selection of the specific forest patch in which individual trees are selected must be considered and optimized:

- Check for **representativity** of GCU local ambient conditions
- Check for an **homogeneous environement** with similar topography, aspect/slope.
- Check for a convenient location for field work inside GCU: easiness of access as much as possible.



To be representative of the GCU : retaining the dominant facies. If there is a mosaic of facies, choose an homogeneous facies for the 10 trees of Functional Subset (FS) and complete with the other facies for the 15 remaing trees of the Genomic Subset (GS).

Finally, check in this potential forest patch if we can find 25 dominant or codominant trees at least 30 m apart on this surface area. Delimite visually this forest patch.

2.2. Metadata at forest patch level

For each forest patch, it's necessary to take general information such as :

1. Location name, GCU-code (EUFGIS code)

2. GPS coordinates and altitude (a reference point corresponding to the centre of the circular plot representative of the GCU is recorded (see the § I.4. below)).

3. Observations about accessibility when necessary

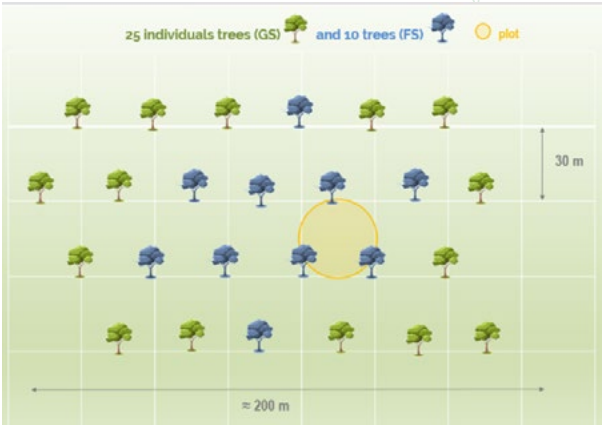
4. Date and name of the persons collecting the data

3. Selection of individual trees

3.1. Size of sampling

Once the forest patch is visually delimited, select 25 individual trees :

- 25 trees/site for genomic samples = **GS : Genomic Subset**
- Including 10 trees/site for phenotypic samples = **FS : Functional Subset**



3.2. Sampling plan

To ensure the environmental homogeneity while getting a compact area patch, the recommended sampling plan for tree selection is systematic sampling along randomly positioned transects.

This scheme involves selecting trees positioned along several virtual parallel linear transects across the selected forest patch. Selected trees must be at least 30 m from one another (the distance between sampled trees being a block). Typically, 3-4 virtual transects over a few hectares (2-3 ha) provide enough trees. Otherwise, the number of transects could be reduced while increasing the number of trees per transect (case of Populus nigra riverine woodlands). For each sampling point within each transect, sample the closest tree randomly, meeting the criteria above. The overall sampling area is around 3 ha.

The main direction of each transect should follow:

- In the case of sloping ground, the same contour line, i.e. perpendicular to the direction of the slope.
- Otherwise, according to the source of the heterogeneity of the environment, i.e perpendicular to the variation gradient.

3.3. Criteria

For the purposes of this sampling protocol, an individual tree is characterised by a stem that, at its base, is not connected to other stems.

- It is very important to select **only adult, dominant or codominant** trees considering the social status inside the forest stand.

Trees need to be at least 30 m apart to avoid inbreeding as much as possible.

Discarding trees

A tree at the given distance in the given bearing should be discarded if it has one or more of the following characteristics:

- Declining tree due to age (senescent trees) or excessive defoliation (more than 50%), except in cases in which the (high) level of decline or biotic/ abiotic damage is representative of the whole stand. Please see § II. 3.2. for defoliation assessement.
- Affected by fungi, pests, bark damage (animal activities, fire, mechanical damage, but see exception above).
- Affected by parasitic plants (e.g *Viscum spp.*), except if the entire forest has high mean levels of infestation a tree with parasitic plants.

3.4. Selection of individuals trees

First, select the 10 trees of the FS. For this, choose the most homogenous area inside the dominant forest facies of the forest patch (see § I.2.1). Begin by choosing the first tree starting in the first block of 30m length of the first virtual parallel linear transect. If the tree meets the selection criteria, mark it with a metal or plastic label attached to the bark at the bottom of the tree (§ 3.5). For each sampling point within each transect, sample the closest tree randomly, meeting the criteria (§ 3.3). Once a tree has been sampled, move to the second block, at least 30m ahead and repeat the operation.

Continue, until finding the 10 FS trees in 3 or 4 parallel transects. Then, complete the selection by choosing 15 additional trees for the GS around this FS area.

A consideration: for linear or quasi-linear populations (e.g., riparian *Populus*) simply select individual trees (according to the agreed criteria) each at a minimum of 25-30m of each other along the population.

3.5. Tree labelling

To balance field practicality with EUFGIS standardisation, a dual tree identification system is applied:

3.5.1. Field Tree Code (Primary identifier in the field)

The Field Tree Code is a simplified identifier used during sampling to minimise errors and facilitate rapid data collection.

- If trees are already tagged by local partners, these tags are not replaced.
    - The local identifier must be recorded in the data file (e.g. in the comments column).
    - A clear link must be established between the local tag and the Field Tree Code.
- If trees are not pre-labelled, assign sequential numbers:
    - Format: GCUCode_TreeNumber (3 digits)
    - Example: ESP00113_013
- Rules:
    - Tree numbers range from 001–025 per site.
    - The same Field Tree Code must be used consistently for all samples from the same tree (field and lab).

3.5.2. EUFGIS Tree Code (Official identifier in EUFGIS)

The EUFGIS Tree Code is the complete, standardised identifier used for data reporting and integration into EUFGIS.

- Format: **GCUCode_Year_SpeciesCode_TreeNumber**
- Components:
- GCU code (as in EUFGIS)
    1. Sampling year, corresponding to the year the sample was collected
    2. Species code (UPOV standard), following the coding system of the International Union for the Protection of New Varieties of Plants (UPOV) – see https://www.upov.int/genie/species.xhtml
    3. Tree number (3 digits)
- Example: *Tree number 13 of Pinus sylvestris GCU "ESP00113" in Spain sampled in 2025 gets the label: ESP00113_2025_PINUS_SYL_013*

Linking Between Codes

The Field Tree Code and EUFGIS Tree Code refer to the same individual tree and must be explicitly linked in the dataset.

- The GCU code and tree number (last 3 digits) is identical in both codes.
- Additional variables (year, species) are recorded separately in field files and used to construct the EUFGIS Tree Code.

The tree labelling is referred to all protocols as "GCUCode_TreeCode"

GPS measurements of individual trees

A simple handheld GPS is used to record the coordinates of all 25 trees. Geographical coordinates must be in decimal degrees with 4 decimal units (accuracy 1.1 m) and format must be in the WGS 84 reference system

4. Selection of a representative plot

Delimit one circular plot per site to assess the biotic and environmental descriptors as forest stand structure and composition, local topography, aspect, slope and the soil characteristics. This plot should be homogeneous and representative of the GCU forest patch. Place the plot around the FS tree having the reading from the Bitterlich relascope closest to the mean (see § methods/stand density). The radius of the circular plot was set at 15 m (around 7 ares) but should be increased to 20 m (around 12 ares) to get a large enough plot which contains at least 10 dominant trees.

4.1. GPS measurement of the centre of the plot

Get that reading for georeferencing the center of the plot with normal handheld GPS or with DGPS (differential global positioning system) if possible. Use coordinates in decimal degrees with 4 decimal units (and accuracy) in the WGS 84 reference system. Please position milestones at the 4 cardinal points 15 m from the centre to virtually divide the plot into 4 sectors for subsequent photos and soil survey.

4.2. Photo of plot

Take one photo per plot quarter from the centre of the plot to have an overview of the forest stand. Try to start with the Northeast quarter and turn clockwise. One person in the photo can be used as a reference scale. Name the photo with the GCU code and the sequential number of the camera.

5. Materials

- A metal or plastic (durable) label to mark and identify the tree
- Staples and stapler
- Spray of recyclable forest paint or works ribbon
- Milestones for delimiting temporarily the FS plot (with telemeter)
- Construction tape for selecting tree

II. Field sampling plan and team organisation

1. Team organisation

Within the H2020 FORGENIUS project (2020–2025), a dedicated mobile team composed of 2-3 members carried out the sampling across all Genetic Conservation Units (GCUs), in collaboration with local partners. This approach ensured methodological consistency across sites and minimized potential human errors during field sampling. For a guidance on how to get prepared in advance of the field sampling, please see the checklist provided in Appendix 2.

2. Planning

In general, it takes one week per GCU with 2 to 3 people from the mobile team (2 climbers if tree climbing is needed for sampling collection) and 1 local partner at least:

- The 1st and 5th days for travelling.
- The 2nd day for the choice of the trees, and the measurements of biotic and environmental descriptors.
- The 3rd (and 4th day if necessary) for the biological sampling collection.

The support of local partners is essential the 1st day on field for the visit of the GCU and the choice of the forest patch.

3. Precautions

- Phenology is limiting for the choice of FS GCUs. For some species, depending on distribution, it may matter whether the sampling is done during the spring, early summer (prior to summer drought) or late summer (post summer drought). And of course, leaves of deciduous species should not be sampled in the autumn. For conifers, it may in fact be preferable to sample needles/shoots in late autumn, early winter, but this needs to be thought through carefully i.e., consulting with partners on this.
- Samples from the same year for a given species: the team travels as many times as necessary and does not collect all species at once to avoid having same-species samples from multiple years.
- For each country and site, it is necessary to contact local partners in order to organize the campaign and to check to have the different permits (immigration requirements and local transportation, specific admission to parks, compliance to the Nagoya protocol …) in time, particularly for sampling in non-partner countries.
- Coordinate with other labs for different samples and different dates of data collection.

B. Environmental site description

I.Abiotic environmental descriptors

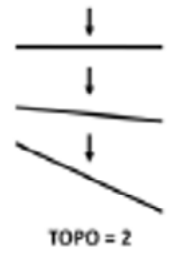
Check if GCUs are not already described by local partners: soil map, forest stand composition, age …etc.

1. Bioclimatic descriptors

Except for general topography and shade created by opposite mountain sides, assessed by Digital Terrain Model, all bioclimatic factors are measured at the plot level (I.4).

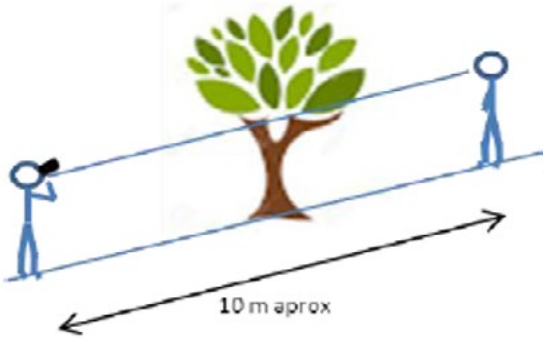
1.1. Micro-topography

Micro-topography affects the flow of water at the site. Topographical indications characterize the local conditions of the water balance between the arrival and departure of water. Micro-topography is assessed at the scale of the plot according to an index ranging from 1 to 3. The index allows us to determine whether there is more in- or outflow of water.

	Level 1 is equivalent to a negative balance: there is more outflowing than inflowing water. This is the case in convex situations, slope increases, high slope situations with breaking downstream slope ... etc.
	Level 2 is equivalent to a zero balance: water in- and outflow are balanced. This is particularly true in homogenous terrain, independent of slopes angle.
	Level 3 is equivalent to a positive balance: the flow of water slows down at the plot level: there is thus a water-surplus. This is the case in situations such concave hollows, ledges on the slopes, and where the lower slopes gradually decrease.

1.2. Slope

At the level plot, one member of the field team walks 5 meters down, starting at the plot center, and another member walks 5 meters upslope (see figure on the left). Using a clinometer and looking at the other´s eyes, one person writes down the value (the two members need to have similar height). Other methods are possible.



1.3. Aspect

Local aspect at the site of the plot is measured by compass (5 degree steps) in the direction of the steepest slope. The absolute value has to be noted on the record sheet (0 for North).

2. Soil descriptors

Determine the soil descriptors either by excavating a pit or by extracting a soil core with a soil auger to the depth at which rocks are found or the extraction of further soil was impossible. The auger sampling method provides an overview of the heterogeneity of the soil within the plot. However, it does not allow to assess the coarse material content. Moreover, it is difficult to apply on overly rocky soils. As a result, the preferred method of soil description should be pedological pit sampling. However, the description of the two methods is indicated below.

Soil samples were collected horizon per horizon for every sum of 20 cm for the subsequent soil texture analyses in the laboratory. These descriptors will be used to calculate the available soil water capacity of the soil that can be used by the trees.

2.1. Soils that are not rocky or only minimally rocky: soil auger survey

To evaluate the soil depth with the soil auger survey, dig 5 sampling points with a soil auger inside the plot (see the scheme opposite): (1) on the centre of the plot (2) on the first quarter of the plot at the north (3) on the second quarter of the plot at the east (4) on the third quarter of the plot at the south (5) on the 4th quarter of the plot at the west. At each of the five points, take soil samples every 20 cm down to the maximum depth of the auger. Pool the soil samples from these 5 points by soil horizon in a bag with the EUFGIS code_ soil depth [0-20], [20-40] …

Note the maximum soil depth (in cm) when it is impossible to go deeper at each point.

2.2. With a rocky soil: soil pit

Describe the soil horizon by horizon: [0-20], [20-40] … following the chronology below:

a) Selecting the location and excavation of the soil pit

Dig the soil pit inside the circular plot representative of the GCU

b) Opening the pit

For the digging of the pit use a shovel.

c) Photo of the pit

Take a picture after positioning the tape measure in the pit. The camera should be positioned in a plane parallel to the wall of the pit. The top of the photo must match the top of the litter and bottom of the picture at the bottom of the pit.

d) Description of dominant coarse elements

The coarse components are the individual mineral constituents, of size greater than 2 mm. Depending on their size, there are:

- blocks: > 20 cm,
- stones: 7 cm - 20 cm
- pebbles: 2 cm - 7 cm
- gravel: 2 mm - 2 cm

Estimate the content of coarse material in % of the soil volume horizon by horizon. This percentage is estimated by type of coarse elements: block, stone, pebble and gravel. The percentage of remaining volume corresponds to that occupied by fine soil and voids. This percentage is estimated visually in horizons of 20 cm and then averaged across all horizons. The assessment must be based on the chart in Appendix 6 and should be completed by taking a soil subsample to separate the coarse elements from the fine elements of the soil (see picture on the left).

e) Determination of the maximum soil depth

The soil depth corresponds to the vertical distance from soil surface to the bottom of the excavated pit or of the soil auger core. Measure the maximum soil depth with a tape in cm. As far as possible, the maximum depth to reach is 100 cm.

f) Estimation of the rooting depth

Note the rooting depth i.e. the depth beyond which there are no roots inside the auger or inside the pit.

g) Soil sample

Collect soil samples by soil horizon every 20 cm down to the maximum depth. Put the soil samples in a bag with the EUFGIS code_the soil horizon [0-20] or]20-40] or]40-60] or]60-80] or]80-100] on it. Double-check for the code in putting a plastic label with the code into the bag.

3. Materials

- Clinometer,
- Compass,
- A Soil auger,
- A pickaxe,
- A folding shovel,
- A rigid tape measure,
- A knife,
- A screwdriver,
- A pruning shears (for cutting roots),
- A basin (to collect the soil),
- A photo camera,
- Plastic bags (to carry soil sample),
- Record sheet + pencil
- A pruning shears (for cutting roots)
- A basin (to collect the soil)
- A photo camera
- Plastic bags (to carry soil sample)
- Record sheet + pencil

4. Data management

4.1. Record data in field

In the field, record these environmental descriptors on the specific spreadsheets with Trimble Ranger or digital tablet. See the spreadsheets called Template_PLOT_SOIL_FIELD_DATA.xlsx which contains:

a. Some general descriptors as the GCU Code, the date of observation, the operators or comments.

b. The environmental descriptors described at plot level: bioclimatic and soil descriptors.

c. Some forest stand's biotic descriptors at the forest patch level surrounding the 10 FS trees, such as structure, composition and the PAI picture number (see chapter below).

Once the fieldwork is completed, transfer this raw data file to the dedicated folder in the official FORGENIUS repository (/ DATA / Raw_data). Don't forget a backup on your PC.

This file is then formatted (harmonisation of remarks, etc.) and data curated (verification of units, consistency of data, possible graphic verification, etc.) and saved in the file provide for this purpose: / DATA / Curated_data. Please change the version number accordingly and don't forget a backup on your PC.

4.2. Related document

A report (including photos and list of team members) should be provided by the referent of each GCU to ensure traceability of activities within the GCU.

II. Biotic descriptors

1. Stand structure, composition and density at forest stand level

1.1. Forest stand features: structure and composition

These factors are described at forest patch level.

- Specify the stand structure: note the structure using an index ranging from 1 to 3: (1) tree crowns inside a single horizontal stratum as regular woodland (2) tree crowns contained inside multiple horizontal strata as uneven-aged high stand (3) stand composed of scattered tree crowns.

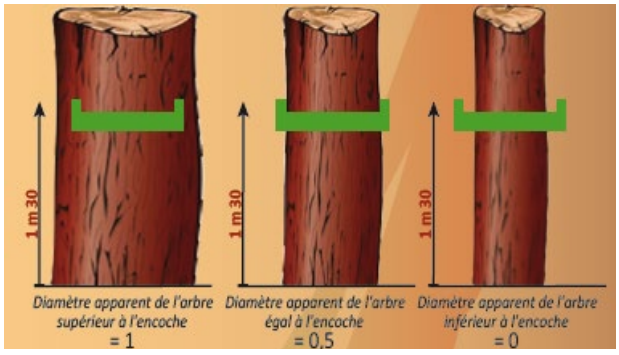
- Specify the composition of the forest stand. Note the composition with an index ranging from 1 to 2: (1) single species (2) mixed forest with several species in the same strata.

1.2. Forest stand features: density and basal area

The stand density assessment is required to estimate the competition level of the site which can partly explain the variation of the functional traits surrounding each FS tree. To capture spatial heterogeneity, it is necessary to combine a basal area assessment over the larger sampling area around each FS tree and a stand forest inventory inside a circular plot representative of the GCU.

Around FS tree level. Estimate the trunk cross-sectional area in m2/ha, all tree species merged, over the larger sampling area around each FS tree using the Bitterlich relascope or Bitterlich relascope APK for Android (see Appendix 4). Choose the band (1, 2, 3 or 4) to have at least 15 trees on the counter. Don't consider the trees < 23.6 of cbh (already assessed with the regeneration presence/abundance class). Systematically move away from each FS tree around 1,5 m so that it does not block the overview. Count separately the number of living trees and dead trees around with the relascope and according to the chosen band.

During the overview, trees whose trunks extend beyond the band, count as 1. If the trunk of the tree corresponds to the band, we only count as 1/2. And finally, if the trees are entirely visible inside the band, they should not be counted. Note on a paper or directly on the excel spreadsheet.



During the overview, trees whose trunks extend beyond the band, count as 1. If the trunk of the tree corresponds to the band, we only count as 1/2. And finally, if the trees are entirely visible inside the band, they should not be counted. Note on a paper or directly on the excel spreadsheet.

Band	Equivalence between basal area per ha (G/ha) and the number of trees counted (N)	Code entered on note sheet to specify the band used
1	G/ha = N	1
2	G/ha = 2N	2
1/4	G/ha = N/16	1/16
2/4	G/ha = N/4	1/4
1+ 4/4	G/ha = 4N	4

At plot level. The forestry inventory inside the circular plot provides an assessment of trunk cross-sectional area per hectare (10000 m2), i.e, the stand basal area of all living and dead trees per species at breast height (1.3 m) noted G/ha. For that,

Measure the trunk circumference at 1.30 m, i.e. approximately at an adult's breast height using a circular tape (see Appendix 3). The unit is in mm. Species must be specified by Latin name.

All trees (dead and alive) in the circular plot with a circumference at breast height (CBH) larger than 23.6 cm must be measured given that the regeneration density is estimated using trees with a CBH < 23.6 cm (see § below).

1.3. Materials

- A measuring tape for CBH
- Dendrometer vertex or decameter for checking distance of radius
- Construction tape or milestones for delimiting virtual plot
- Field sheet, pencil
- Chalks if needed to mark trees already inventoried
- Bitterlich relascop APP for Android.

2. Leaf Area Index

Leaf Area Index (LAI) is defined as the projected area of green leaves or needles per unit horizontal ground surface area (Stenberg, 2006). It corresponds to the main surface exchange of carbon and water fluxes between the forest canopy and the atmosphere. It is also a key variable driving ecosystem functioning, as many processes in a

forest depend on LAI, including light and rain interception (Gash et al., 1995), gross productivity (Davi et al., 2006), transpiration (Granier et al., 2000), seedlings growth and soil respiration (Davidson et al., 2002).

In the FORGENIUS project, measuring Leaf Area index serves three purposes:

- The first is to help to qualify surfaces for transpiration and for photosynthesis.
- The second is to parameterize the models, since LAI is one of the main stand-specific parameters. It can be estimated via allometric relationships, but LAI measurements are more accurate.
- The third is the scaling up of our estimation of GCU resilience from the studied plot to the whole GCU by linking in situ measured LAI and remote sensing data.

To fit these three objectives, we want to measure maximal LAI as much as possible. For deciduous species, this corresponds to the period after budburst and full leaf expansion and before leaf fall in autumn. For conifers, the situation is more complex and species-dependent, making fieldwork timing more difficult.

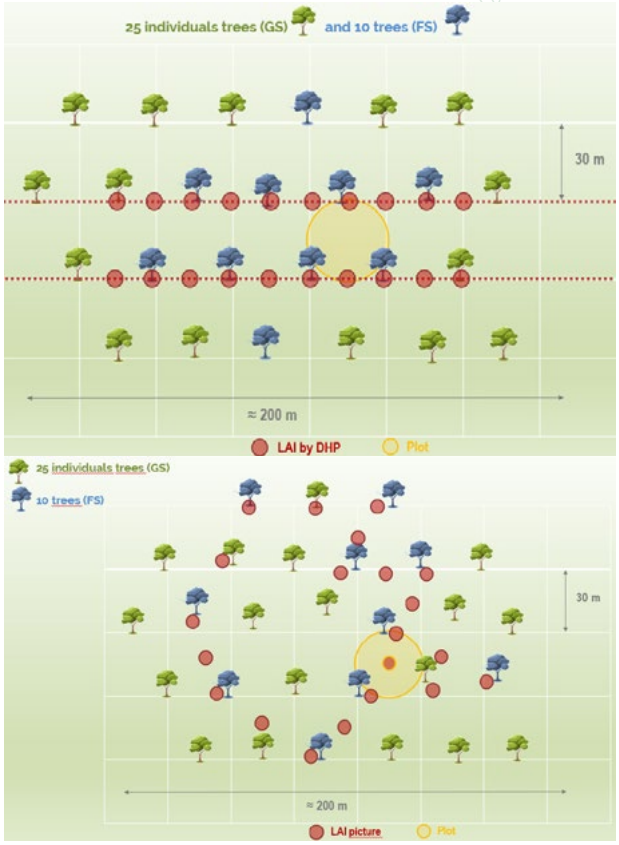
Note that the term LAI is usually used but in fact the measurement corresponds to the PAI (Plant Area Index) which includes non-green elements. Throughout the rest of the document, we will focus on the materials and methods used for taking hemispherical pictures and estimating the PAI (see analysis section).

2.1. Sensors and material section

- Digital camera
- Digital Single lens reflex camera: at least 12,3 Mpixel (no compact camera)
- Fish-eye lens, compatible with the camera
  - Field of view: 180°
  - 4.5mm for APS-C cameras and 8mm for full frame cameras (case of CANON 5D)
- Tripod or telescopic monopod with levelling head
- Measuring tape or stick of 1.3m to set tripod height
- 3D Bubble level or self-levelling mount
- Compass to align the camera to the true North
- Inclinometer to determine the slope of the terrain in case of complex terrain.
- Material for calibration of the lens and camera (see Appendix 9)
- Soft non-fibrous cloth to clean the lens
- Headlamp
- Laptop or notebook to record picture numbers

2.2. Sampling design

The PAI is assessed at the position corresponding to and in between 10 FS trees sampled in the forest patch. For this purpose, two parallel transects could be established, each consisting of 10 photos taken every 15 metres with 30 metres between transects (figure on the left). Depending on the forest stand structure (for example Populus nigra riverine woodlands), the number of transects could be reduced to one while increasing the number of pictures per transect. In another case (such as with a dense understory), photos can be taken to and in between 10 FS trees, at regular intervals (figure on the right). In all cases, it is important to remain representative of stand structure i.e. not avoiding gaps, distance between two photos must never be inferior to 15 m.



2.3. Photo shooting section

Hemispherical photographs must be taken in the absence of direct sunlight, as sunlit foliage may appear as bright pixels and be misclassified as sky. Diffuse light is ideal.

- The best diffuse lighting conditions occur on entirely overcast days (grey skies).
- When overcast days are too scarce, pictures can be taken before sunrise and after sunset, when the sun is hidden behind the horizon and the vegetation is not directly sunlit. Keep in mind that during these times of the day the light environment changes rapidly and camera exposure settings are only valid for a short time.
- Rain must be avoided, because rain droplets on the lens create blurred images.
- Strong wind must be avoided.
- Foggy conditions under the canopy must be avoided.
- Snow on the canopy must be avoided.
- Minimum distance between the lens and the nearest leaf must be 10 × leaf length (e.g., 10-cm leaf - ≥100 cm). Too-close leaves must be removed.
- Minimum distance between the lens and the nearest tree trunk must be > 6 × tree diameter.

2.4. Camera settings

- Set the camera to RAW format and maximum resolution.
- Mode “M” manual.
- Aperture F8.
- Focus set to infinity.
- ISO 400 (up to 800 in light wind or during sunset). Above 800, excessive noise occurs.
- Time delay 2 seconds (or more if needed).
- Shutter speed is determined by field sky conditions, generally between 1/4000 and 1/800.
- Activate the overexposure alert (blinking highlights).
- Save pictures in landscape orientation by default.
- Save images in uncompressed RAW format (e.g., .CR2).
- Set lens to autofocus.

2.5 Shooting steps

- Check weather and light conditions.
- Check battery level and memory card capacity.
- Check date and time (local standard time; disable daylight saving time if possible).
- Install the camera on the tripod.
- Install the camera facing upwards on the tripod; mounting the camera on a gimbal is recommended
- Set lens height to 1.3 m above ground.
- Level the camera perfectly using a 3D bubble level. On sloping ground, the camera must remain strictly vertical (not parallel to the slope). If the mean slope within 7.5 m differs from the local slope, it must be measured.
- Orient the camera so that magnetic north is at the top of the image (flash-shoe direction). Keep the compass away from metallic objects.
- Remove lens cap and protection ring.
- Take the picture and check the preview (see appendix 9): use the histogram and blinking pixels to detect overexposure. Ensure no people, vehicles, or insects are in the frame.
- Record the location (the number of the FS tree or GPS point) and number of the photo. The weather and time.

2.6. Analysis

Output variables (effective PAI, true PAI, effective ALA, true ALA, % of saturated cells, fCover) are derived using the free software CAN-EYE https://can-eye.paca.hub.inrae.fr/ © INRAE. The main variables of interest are:

- True PAI: The True Plant Area Index (m2 of plant/m2 soil); effects of foliage aggregation removed.
- Eff.PAI: Effective Plant Area Index (m2 of plant/m2 soil); accounts for foliage aggregation.
- fCover: Vegetation cover fraction (average fraction of the photos covered with vegetation)

CAN-EYE classifies pixels using the three colour channels. Pixels are classified as sky or vegetation (leaves, branches and trunks). Further methodological details can be found in Weiss et al. (2004) and Demarez et al. (2006)[1]

[1] Weiss, M., Baret, F., Smith, G.J. and Jonckheere, I., 2004. Methods for in situ leaf area index measurement, part II: from gap fraction to leaf area index: retrieval methods and sampling strategies. Agric. For. Meteorol., 121: 17-53. [URL]

Demarez, V., Duthoit, S., Baret, F., Weiss, M. and Dedieu, G., 2008. Estimation of leaf area and clumping indexes of crops with hemispherical photographs. Agricultural and Forest Meteorology, 148(4): 644-655. [URL]

3. Biotic descriptors at tree level

These tree characters are described on the FS trees (10). Only CBH is measured on all GS trees (25).

3.1. Status / Remarks

Specify the status of each tree: dominant - codominant or dominated tree.

Please note in remarks any information that may help in the analysis of the results for physiologic traits. For example, in the case of poplars: proximity to water, collection of branches from vigorous and young branches (shoots) following the breakage of the top …

3.2. Presence of dead tree

Please note the presence or absence of dead trees around each FS tree in a radius of about 10 m.

0 Absence of dead trees

1 Presence of dead trees

3.3. Dieback index

Make a first rough estimation of the level of decline during the selection of forest patches and trees (see I. 3.3.2).

The tree-level assessment consists of a dieback index based on the density of branches and missing growth, and on the degree of defoliation. This defoliation percentage denotes the proportion of needle or leaf loss in comparison to a fully foliated reference tree. In each country, it is important to have a look at this reference tree with the local partner before this operation (See Appendix 7). This assessment is done on the crown out of competition with the crowns of other trees.

Please note the defoliation percentage in the field sheet and note in comment if the tree is damaged due to fungi, pests, bark damage (animal activities, fire, mechanical damage) or affected by parasitic plants (e.g Viscum spp.).

Assess the defoliation of the target species following the scale below:

Class 0 [0-25%] of defoliation

Class 1]25%-50%] of defoliation

Class 2]50%-75%] of defoliation

Class 3]50%-75%] of defoliation

3.4. CBH (cm)

Measure the trunk circumference at 1.30 m, i.e. approximately at an adult's breast height using a circular tape (see Appendix 3: Instructions for CBH measurement).

Specific cases:

- If the tree has more than one trunk all of them must be measured and recorded. Note that the tree is multitrunked in comments (fork, clump, …).

- If the tree is leaning, measure CBH perpendicular to the tree trunk.

- In slope situations, CBH measurement must be realized from the upslope tree side.

- In case of "twin" trees side by side, measure CBH of these trees (insert a line below the FS tree in the spreadsheet in field; see § 5. below).

3.5 .Height

Total height from ground to tallest part of the crown, ideally measured using a clinometer (as the Vertex IV ultrasound instrument) or a telescopic measuring pole (for short trees).

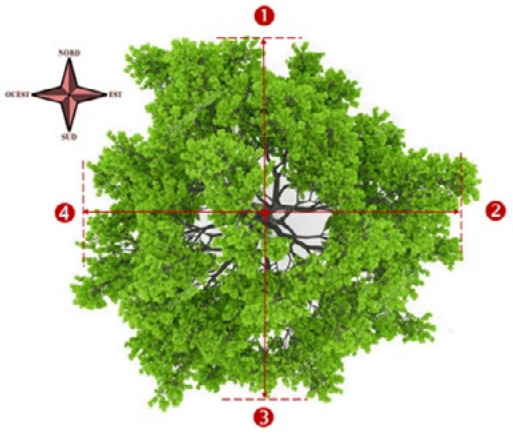
Please note in comments the presence of forked stems and specify approximately the height of this fork. A fork has two leaders of equal importance in thickness and length. Please note the relative position of the fork.

The basal crown height (hbc) is measured using a dendrometer or a telescopic measuring pole. The basal crown is defined as the vertical distance between the ground and the base of the main "functional" green crown envelope.

More details in Appendix 5: Instructions for Vertex Dendrometer and 11.

3.6. Crown size

Crown size is a measure of the footprint or plan area of the crown of a tree expressed as crown width. The simplest estimate is obtained from the average crown width determined by the average of two perpendicular diameters. To measure, the simplest method consists in measuring 4 radii from trunk to the edge of the crown, with the first measurement made along the axis north facing if the land is flat or uphill if the land is sloping. Then, turn clockwise. Use a telemeter to measure the horizontal distance (and not the slope distance). In case of dense understory, use a decimetre if needed. Please note the four radii (in meters to the nearest 10 cm) on the field sheet.



3.7. Materials

- A measuring tape for CBH
- A dendrometer Vertex (to measure height)
- a telemeter (for crown extension)
- A pair of binoculars (for dieback index)

4. Regeneration

Evaluate natural regeneration around each FS tree (on a radius of about 10 m). Assess the presence and abundance of natural regeneration of the target species following the scale in table below:

0 Absence (no recruit visible)

1 Scattered (few/scattered individuals)

2 Groups (presence of scattered groups within the plot)

3 Abundant (regularly spread over the plot, abundant groups, or few groups but with many individuals)

Consider juveniles the young individuals of the target species with a CBH below 23.6 cm and a height greater than 10 cm, as these trees are likely to have survived at least the first year which is normally the first bottleneck.

5. Data management

5.1. Record data in field

In the field, record these biotic descriptors on the specific spreadsheets with Trimble Ranger or digital tablet. See the spreadsheets and use one per GCU:

(i) Template_FS_GS_FIELD_DATA.xlsx which contains:

a. the biotic descriptors at individual tree level including some general descriptors and tree characters,

b. some of the forest stand character biotic descriptors assessed around FS trees like the presence and abundance of natural regeneration, the mortality of the target species and the basal area surrounding FS trees required to estimate the competition level around each FS tree.

(ii) Template_PLOT_INV_FIELD_DATA.xlsx which contains the forest stand characters' biotic descriptors measured at plot level. The data corresponds to the cbh per species measured inside a circular plot via the forestry inventory.

(iii) Template_LAI_FIELD_DATA.xlsx which contains the PAI information: the number of the hemispherical picture taken in the field with the location (GPS point or the FS tree number) and other comments (operators, date, …).

Once the fieldwork is completed, transfer this raw data file to the dedicated folder in the official FORGENIUS repository (/ DATA / Raw_data). Don't forget a backup on your PC.

This file is then formatted (harmonisation of remarks, etc.) and data curated (verification of units, consistency of data, graphic verification, etc.) and saved in the file provide for this purpose: / DATA / Curated_data. Please change the version number accordingly and don't forget a backup on your PC.

5.2. Related document

A report should be provided by the referent of each GCU to ensure traceability of activities within the GCU.

C. Tree ring & wood sampling

I. Tree ring core collection

Check if GCUs are not already described by local partners: soil map, forest stand composition, age …etc.

1. Sampling strategy

On each of the 10 FS trees, two long cores (from the bark to the pith) are collected (1) for micro-density, radial increments and age assessments (2) for δ13C stable isotope analysis. On the same trees, one short core or dust drill is collected for NIRS analysis (chapter C.II.).

Traits assessed	Materials	Methods Identification	Storage
Isotope analysis On the 10 trees	1 long core to the pith	Collection with an increment borer + electric drill. 1 core perpendicular to stem at 1.30 m, aiming the pith on the NORTH face, unless the tree is too damaged or tilted. Note each one with a pencil with GCUCode_TreeCode on the paper containers.	Stored in paper containers individually labelled with GCUCode_TreeCode code 
Micro-density Age Radial increments On the 10 trees	1 long core to the pith	Collection with an increment borer + electric drill. 1 core perpendicular to stem at 1.30 m, aiming the pith on the NORTH face, unless the tree is too damaged or tilted. Note each one with permanent markers with GCUCode_TreeCode code on the panels.	Stored in clear polycarbonate multiwall panels (see NIRS and micro-density protocol) 
near-infrared spectroscopy analysis NIRS On the 10 trees	1 short core 	According to the species, for NIRS analysis, short core (5 cm) for deciduous or dust drill for conifers from a hole drilled at 1.40 m on the north side (12 mm wood drill with 4 points) taking care to remove the bark before drilling.	Collect in paper bags. Aerate in the evening and dry. To be collected at the same week as the Functional Subset branches samples. Note each one with GCUCode_TreeCode on the paper bag.

Ideally, the core should be in a single piece, including bark and pith. If the core breaks into more than two pieces, discard it and sample again at another location. If the bark separates from the core, mark a cross at the bark end to indicate that no rings were lost. If the core is broken in two pieces, please mark the core on either side of the two ends to make it easier to join. Plug the hole with wooden dowel if needed.

2. Material

- Manual Increment borer*
- Electric drill for increment borer and battery **
- PVC cylinder tube and core holders (e.g., paper straws)
- Paper straws and polycarbonate multiwall panels to store
- Waterproof pencil
- Masking tape
- Graduated ruler
- Field-sampling sheets
- Borer cleaning and sharpening set (for borer maintenance)

*Increment borers by Haglöf Sweden ® . https://haglofsweden.com/project/increment-borers/ Borer bit of 5.15 mm inside diameter are preferred. This diameter is recommended to facilitate future work on these cores for microdensity analyses. Length of the borer depends on tree diameter.

** Electric drill/driver is recommended (18 V, with a torque of at least 140 Nm, no impact mode—e.g., Milwaukee M18 FDD3 with 5 Ah or 9 Ah batteries) to reduce physical effort.

3. Method

The wood core samples are collected perpendicular to the stem, at breast-height (1.3 m stem height), aiming the pith using the materials described above. The methods used are described in detail in the appendix 8.

II. Field sampling strategy for NIRS and micro-density

Authors of this section: Delphine Grivet (first version); updated by Nicolas Mariotte and Florence Jean according to Frédéric Lagane, Nassim Belmokhtar and Philippe Rozenberg's advice.

SAMPLING ADULT TREES

1. Sampling and storage for NIRS samples

Ten individuals are sampled in the field for NIRS analyses. Core and drill dust samples are always taken from the south to the north (in flat location) or upstream in slopes. Samples for NIRS analyses and micro-density are taken in the same zone at breast height (<5cm above or below the drill hole). For NIRS calibration it is imperative that the other tree measurements are happening at the same time –field work should be coordinated. In this FORGENIUS project, NIRS was only done on Populus nigra, Pinus sylvestris, Fagus sylvatica and Pinus pinaster but this protocol could be used for other species.

Populus nigra – Pinus sylvestris – Fagus sylvatica

Sample 10 individuals for the short core of the tree using a Pressler drill (see photo below). Only the external 5 cm will be analysed.

The cores are stored immediately in containers (see photo below), closed at one end and individually labelled, following labelling of the Genomic Subset, as soon as they are obtained:

> the 20th tree on site ESP00113_2025_PINUS_SYL is labelled ESP00113_2025_PINUS_SYL_020

To avoid fungus, plastic containers should be avoided, and the samples should be stored in dry conditions. When back to the laboratory, samples are dried in the oven at 30ºC during 48h or more if necessary.

Plastic containers

Paper containers

Pinus pinaster

Sample 10 individuals for drill dust and collect drill samples in a paper bag disposed under the hole to directly collect the sawdust obtained. Use one drill hole of 5 cm depth with a 12 mm drill with a shorter bit pitch to obtain a high amount of drill dust with no need of grinding.

Paper bags containing the samples are individually labelled, following labelling of the Genomic Subset:

> the 20th tree on site ESP00113_2025_PINUS_SYL is labelled ESP00113_2025_PINUS_SYL_020

Dry drill dust samples at 40°C for 48 hours to avoid moisture and send them for processing

2. Sampling and storage of long core for microdensity

Populus nigra - Pinus pinaster - Fagus sylvatica - Pinus sylvestris

Sample 10 individuals in the field for a long core from the bark to the pith of the tree using a Pressler increment borer (see the section C.I). Note the date of collection (months/year) to date the wood cores.

Store the cores immediately in paper containers (see photo above and section C.I), closed and individually labelled, following labelling of the Functional Subset, as already specified. Store the samples in dried conditions.

To correlate core measurements (wood core to the pith) with drill dust NIR spectra (5 cm depth), the same depth for the two samples is compared, i.e. 5 cm length from the outer part of the stem.

D. Functional Subset (FS): Branch sampling protocol

I. Introduction

This section describes criteria for the collection of branch samples to be employed for functional trait assessments in laboratories. This biological material is collected on the 10 FS trees to provide:

(i) a fundamental set of leaf and wood economic spectrum traits that gives information on the performance of trees in the field, i.e., Specific Leaf Area (SLA), Leaf percentage carbon (%C) or nitrogen (%N), carbon or nitrogen stable isotopes (leaf d13C or Leaf d15N) and branch wood density.

(ii) two sets of fundamental water relations and hydraulics traits:

a. Pressure-Volume traits such as osmotic potential at full turgor, turgor loss point, leaf capacitance, fraction of symplasmic volume, bulk leaf elastic modulus, branch capacitance. These traits quantify aspects that relate to water use by plant cells and tissues.

b. Hydraulic traits, such as minimum leaf conductance, xylem vulnerability to embolism, xylem hydraulic conductivity and Huber Value. These traits quantify aspects that relate to plant efficiency in xylem water transport (hydraulic conductivity), xylem resistance to water stress (vulnerability to embolism/cavitation) and leaf water loss under severe stress (minimum leaf conductance).

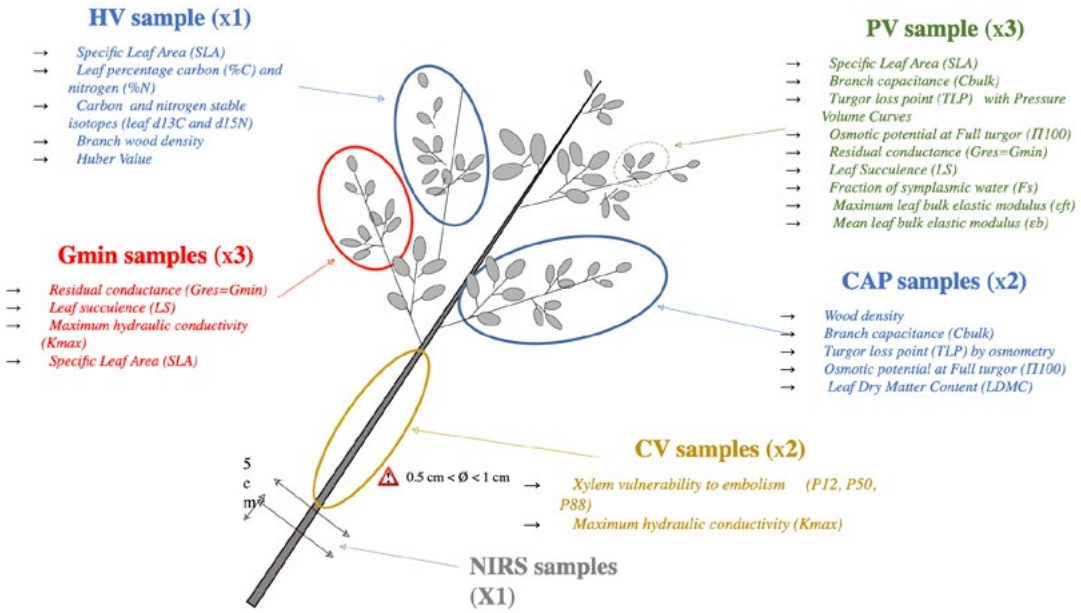
See the scheme below which recaps these traits assessed from the different type of biological material.

2. Branch Sampling strategy

See Figure below illustrating the typical branch that can be used for the collection of samples. See text box on the right for the nomenclature used in this protocol. Six types of samples are collected and described in this section: **CV** (for Vulnerability to Cavitation), **CAP** (for CAPacitance), **HV** (for Huber Value), **Gmin** (Minimum conductance), **PV** (Pressure Volume) and **NIRS** (Near InfraRed Spectroscopy) samples.

Order I = trunck
Order II = Branch
Order III = shoot
Order IV = branchlet

- The branches are collected on the 10 FS individual trees.
- Sun-exposed branches (southern half of the top third of the canopy), out of competition are selected. If trees are isolated, lower canopy branches can be collected, provided they are sun-exposed branches. Whenever possible, avoid branches with damage, or with male/female flowers.



LOCATION OF SUB-SAMPLES ON ONE BRANCH Branch length ≈ 1,50 m

- Sampled branches should be collected preferably early in the morning (if not possible, as late in the afternoon as possible) to limit large water potential gradients.
- For each tree, 2 or 3 large branches should be cut either by climbing or with a long pole pruner. Branches should be about 1 to 2m long, but number and length depend primarily on occurrence of two 40-45-cm long straight segments of <1cm diameter under bark (for CV samples). As far as possible, the samples are collected from the same larger branch or if necessary, from different branches but always from the same tree. Sometimes, CV samples are collected from other branches if the criteria for size and straightness are not met.
- Branches cut down by climbers should immediately be put in water (in a bucket), then prepared in the field as specified in the § below and stored in fresh conditions.
- The samples are identified by the tree number and the sample type.

3. Branch sample characters

Each branch is subsampled to collect stems, shoots or branchlets for the assessment of different traits according to the materials and methods described below. Among all the branches collected on one tree, start first by choosing the CV samples, as it is difficult to comply with the straightness and diameter criteria; follow then with the CAP samples.

Physiological traits assessed	Materials	Methods identification	Storage
Cavitation Vulnerability *CV samples* ***On the 10 trees***	2 segments of branches 40 cm long as straight as possible with a diameter under the bark >0.5 cm & <1 cm	Cut under water, free of needles and branches. Label each one with GCUCode_TreeCode_CV1 for the first segment and $\_CV_2$ for the second one.	Wrapped the 2 samples together with wet paper and cellophane. All CV samples are stored inside one very large black double-bag in fresh conditions until the sample preparation for shipment the day after or 2-3 days after or max 1 week after stored in a fridge at 6°C.
NIRS stem *NIRS samples* ***On the 10 trees***	1 basal segment of branch 10 cm long (1 to 1.5 cm diameter) at the base of one of the CV branch samples.	Label each one with GCUCode_TreeCode_NIRS. Note the tree number on the stem with a felt-tip pen if the tag is lost.	Put all the samples in a paper bag. Store in dry conditions.
Capacitance *CAP samples* ***On the 10 trees***	2 whole shoots of 50/60 cm long, well leafed with 20 or more leaf shoots (~3cm) so they can be put in the pressure chamber. Avoid samples with female or male flowers.	Cut the base of shoots under water. Label each one with GCUCode_TreeCode_CAP_1 and $\_CAP_2$	Put a wet paper towel at the base of the 2 capacitance branches. Put in a black bag (80-100 l) + make a small hole in the bag for ventilation. Then put 4-5 of the bags with branches in a big black plastic bag (150 l) with 2-3 wet paper balls. Keep in fresh conditions (or in the shadow in the field) until the sample preparation for shipment the day after (as far as possible). Be careful with shipments because the samples are easily broken.
Huber-Value *HV samples* ***On the 10 trees***	one long shoot exactly 55 cm.	Remove and DISCARD all the needles and ramifications from the first 5 cm from the base of the branch. Cut a 15 cm segment at the base of the branch (this includes the 5cm that has been peeled in step 2) Label with GCUCode_TreeCode_HV	Take out all remaining leaves or needles and put them in a Ziplock bag, together with the 15cm segment. Store this sample with the EUFGIS code_tree code_HV label, without wet paper inside. Keep in fresh conditions.
Minimum Conductance *Gmin samples* ***On 8 of the 10 trees***	3 shoots (1 + 1 back-up for Gmin + 1 for SLA) with a length of 35-40 cm for conifers and 50-60 cm for angiosperms.	Cut the base of shoots under water. Label each one with GCUCode_TreeCode_$Gmin_1$, $\_Gmin_2$ and $Gmin_3$.	Put a wet paper towel at the base of the 3 Gmin branches. Put it in a 60L garbage bag. Empty the air from the bag and close it tightly. Several Gmin samples are stored inside a large black double-bag on which the GCU code, the sample type (Gmin) and the number of sampled FS trees are written. Store in fresh conditions until the sample preparation for shipment the day after (as far as possible). Avoid storing them for more than 2-3 days.
Pressure Volume Curves *PV samples* ***On 8 of the 10 trees*** ***Same trees as Gmin samples***	3 branchlets 10-15 cm long with 4 cm extra wood at the base for conifers and 10-15cm for broadleaves. 1 for PV + 1 back-up + 1 for SLA	Label each one with GCUCode_TreeCode_PV_1, $\_PV_2$	Put all the branchlets in a Ziplock with 1 ball of wet paper. Store in a cool place.

See appendix 10 for sample illustrations.

Illustration of the preparation of the samples cut under water.

Example of packaging for the shipment of CV samples.

All the labels with the specific codes are prepared in advance to ensure all samples are collected.

4. Material

For branch collection

- Climbing equipment and climb materials
- Pruning shears for collecting samples
- Long pole pruner

For storage

- Bucket
- Water
- shears + saw
- plastic labels
- paper labels
- indelible felt pen
- Ziplock bags
- paper towel
- packaging film
- Paper labels
- Plastic tray

E. Genomic Subset (GS): Branch sampling protocol

Authors of this section: Francesca BAGNOLI, Giovanni Giuseppe VENDRAMIN, Camilla AVANZI and Andrea PIOTTI.

Please see § 1.3 for selection of trees and site codes and tree labelling. In short, at least 25 individual trees are sampled for each GCU. Ideally, trees should be selected along 4-5 parallel transects to ensure a minimum distance of at least 30 meters between trees. If this is not possible, the number of transects should be reduced while increasing the number of trees per transect. Only adult, dominant or codominant trees should be sampled. It is important not to select senescent trees (with evident declining signs) or trees with a defoliation level greater than 50%, unless these characteristics are present in most individuals in the plot. Each selected tree must be tagged and georeferenced individually, and DBH should be measured. Geographical coordinates must be in decimal degrees with 5 decimal units (accuracy 1.1 m), in the WGS 84 reference system format.

1. Sampling and materials

The material used for DNA extraction should be young leaves/needles without evident signs of disease (Fig.1).

Figure 1.

About 5-6 leaves are required for broadleaf tree, 15-20 needles for each conifer with long needles, and 40-50 needles for each conifer with short needles (Fig.2).

Figure 2.

2. Storage of leaves/needles for DNA extraction

Individual samples must be placed into a tea bag (not paper bags or plastic envelopes) (Fig.3): in tea bags the samples can breathe and can dry more quickly without the risk of developing mould.

Figure 3.

Then, individual tea bags with samples must be placed into a plastic bag or another container with enough silica gel (Fig. 4).

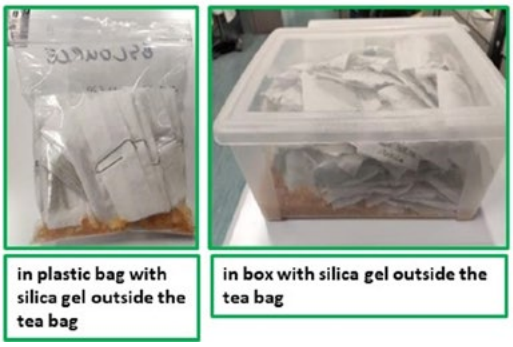


Figure 4.

Silica gel must be changed until the samples are completely dried – in other words – the silica does not change colour anymore! Do not put the plant material directly in contact with silica gel, as the dust can clog up filters during DNA extraction. PLEASE NOTE: we strongly suggest using Silica Gel Rubin which has no toxicity because is cobalt-free, and for which is available both in the bag and in the bulk version (Fig.5).

Figure 5.

Using Silica gel Rubin drying bags can be handy instead of unpackaged silica gel crystals. Change silica gel as many times as necessary (usually once or twice at most) during field activities. Once back in the lab additional oven drying is strongly recommended to ensure the material is completely dry. Oven drying overnight at 50°C is usually sufficient.

Please, make sure that the material is fully desiccated before shipping. Please follow this recommendation rigorously, otherwise the quality of the DNA will not be adequate for obtaining high-quality genomic data. In case of low DNA concentration, if the quality of the material sent is poor, we ask to re-send samples. We recommend that each partner keep a copy of the sampled material in the lab to address any issues that may arise during shipment or DNA extraction.

F. Laboratory Protocols

I. Pressure Volume Curves

1. Introduction

This protocol is dedicated to the sample collection and laboratory measurements of leaf traits related to pressure volume (PV) curves. The targeted measurements allow to derive typical pressure volume traits (Ψ_{tlp}, π_0, F_s, E, C_{bulk}) but also additional related traits (g_{min}, LMA and succulence) on all individual of the site (10 trees/site with 15 sites * 10 species).

2. Sample preparation for lab measurement

a. Measure PV curve traits on the leaf or take shoot samples from one branch per tree.

b. For each branch, use one shoot with two branchlets for LMA and the other one for PV-Curves.

c. Select one of the shoots, put a label (GCUCode_ TreeCode_ PV3) and put it in the cooler (ca. 4ºC).

d. Take the other shoot or an individual leaf (if leaves are big enough) and label it separately (GCUCode_ TreeCode_PV1 and GCUCode_TreeCode_PV2)

e. Recut the basis of the leaf or the shoot under water with a cleaned razor blade and put the cut basis in a tube filled with deionized water. Care should be taken to limit water drops on leaves. Over-rehydration might be an issue and it is hard to control the time needed for each species. Over-hydration is solved by removing the "plateau effect" during the data analysis phase.

f. Put the tubes in a cooler (ca. 4°C) for ca. 12h.

3. PV curves measurements

Build PV curves by measuring the evolution of water potential and weight on leaf or shoot samples (if leaves are too small) dehydrating on a bench. When possible, conduct the different tasks on individual leaves (if leaves are big enough) otherwise (for conifer or small leaves species) conduct the task on small shoots, as much as possible with a similar number of leaves. At each step, record measurement time, air temperature and humidity conditions. Among the two available samples, choose one for the measurement (the other can be used as a replacement in case needed). Record data on a note-sheet and on an Excel spreadsheet.

Start the fan, the temperature and humidity sensor in the area where samples are drying down

Take out one sample from the cooler and note Time, Temperature, Relative Humidity. The base can be re-cut and the bark locally removed at the base to ease plugging the sample in the pressure chamber and ensure a clear reading of water potential.

a. Weight the sample
b. Measure water potential
c. Weight the sample
d. Put the sample (back) in the dry down area. Use a perforated bag if necessary for each sample. We must avoid loss of needles or leaves but let wind from the fan reduce the boundary layer conductance. If needles fall off, set them aside and record the weight (fresh and dry). Note the event.
e. Repeat c) to f) between 8 and 12 times to have water potential steps of ca. 0.3 MPa. Be aware that during the early steps of dehydration water potential decreases quickly.
f. If Gmin is also being measured, continue for as many other steps as necessary until a constant rate of loss of water content per unit of time is obtained. During this phase, measurements of water potentials are not necessary.
g. At the end of the PV curve put the sample in the oven for 48h at 60°C
h. Weight dried samples.
i. Separate leaves and twigs
j. Weight leaves and twigs separately
k. Store the samples in labelled envelopes
l. Fill the dedicated Excel spreadsheet (PressureVolumeCurves_SpreadSheet.xlsx)

4. Measuring Leaf Mass Per Area

a. Take out the LMA sample from the Ziplock bag.
b. Separate the leaves/needles from the twigs.
c. Put the leaves/needles and twigs on the scanner by separating each element (do not overlap leaves) on the scanner.
d. Record images
e. Put the leaves and twigs in two different labelled envelopes.
f. Dry the sample in the oven for 48h at 60°C
g. Fill the dedicated excel spreadsheet (PressureVolumeCurves_SpreadSheet.xlsx)

5. Material

- Bucket
- Water
- shears + saw
- plastic labels
- paper labels
- indelible felt pen
- Ziplock
- paper towel
- Paper labels
- Plastic bag
- Pen
- Note sheet
- Envelopes
- Analytical balance (0.001 g)
- Pressure chamber
- Temperature + Humidity sensor (hobo)
- Fan
- Perforated bags for dehydrating the samples
- Razor blade
- Paper envelope
- Perforated bags
- Pencil
- Hoven
- Desktop scanner for LMA

6. Timing

Time*people required for sample collection and preparation:

1day * 2 people (1/2 day * 2 people without the trips)

Time*people required for lab measurements:

- 1 day * 2 people for PV curve
- 1/2 day * 1 people for leaf area estimation and sample dry weight

Time * people required for data management

- 1 day * 1 people for data management and traits calculation

7. Precautions

Keep the same code for the same individual sample during all the experimentation (field/lab).

Data management: manage and check the validity of data the day after lab measurements as far as possible.

II. G_{min} and desiccation

1. Introduction

This protocol is dedicated to the sample collection and laboratory measurements for residual conductance (G_{min}) and time to full desiccation (T_d) (8 trees/site).

2. Sample collection

See the scheme, section D.2 which illustrates the typical sample called “Gmin samples” that could be used in Functional Subset measurements including sub-sample for G_{min} and T_d.

a. Use individual trees that have been previously identified.

b. For each individual tree, select and sample leafy sun-exposed branches.

c. Samples should be collected at conditions of as low evapotranspiration as possible, (e.g. early in the morning) to limit large water potential gradients.

d. Collect 1 branch for each tree. Use the same branch for another sample.

e. Subsample 3 apical leafy shoots (at least 35-40 cm long for conifers and 50-60cm for angiosperms) from each branch tree for Gmin and Td. From each tree (FS), we need a minimum of 2 shoots per tree (one for measurements and to repeat the measurements at different conditions). The third branch is kept as extra.

f. These branches can be sampled from the same larger branch or from different branches, if necessary, but always from the same tree.

g. Put the subsample in a Ziplock bag with a wet paper towel.

h. Label the Ziplock bag with a unique identifier: GCUCode_TreeCode_Gmin

i. Store the Ziplock bag in a cooler (ca. 4ºC) until the sample preparation at the end of the day.

Resume: A total of 3 leafy shoots per tree, from a minimum of 8 trees per species and site should be collected for G_{min} / T_d.

3. Sample preparation for lab measurement

a. Use two shoots of the three for Gmin / Td measurements and label them as (GCUCode_TreeCode_Gmin1 and Gmin2) and put them back in a Ziplock bag and the bag back into the cooler.

b. Store the third shoot as an extra sample in case needed with any of the other two set of samples. Label the extra sample as (GCUCode_TreeCode_Gmin3) and put it back in a Ziplock bag and the bag back into the cooler.

c. Ship the samples (express shipment) to the lab in charge of the G_{min} / Td measurements

4. G_{min} /T_d measurements

For Gmin / Td measurements, the samples received from each site (8 samples per site from a minimum of 8 different trees per species) should be placed in a DroughtBox and exposed to two different conditions of RH and T while monitoring changes in weight for each branch.

Detailed protocol for Gmin / Td:

Note: We expose the samples to two different conditions of T and RH to determine G_{min} / T_d. Therefore, we need to use two different DroughtBoxes in parallel.

a. Once in the lab, recut the cut end of each shoot and keep it under water overnight while the terminal part of the shoots remains enclosed in plastic bags and stored in dark conditions to allow full rehydration.

b. Once rehydrated and before their installation in the DroughtBoxes, recut each branch ca. 30-40 cm from the tip and measure their psi.

c. Measure the basal diameter and length of the branch (used for estimating stem area or volume etc).

d. Measure the fresh weight of the branch (in grams).

e. Seal the cut ends with paraffin wax (melting point 68ºC, Fluka) and measure its fresh weight immediately.

f. Attach the shoots to each of the eight strain gauge hooks using copper wire. Install a total of 8 shoots in each droughtBox (2 droughtBoxes in total). Close the boxes and allow the branches to dehydrate progressively.

g. Drought-boxes set up for Gmin / Td:

h. Set T and RH at 30ºC and 40% for one DroughtBox and 50ºC and 40% for the other, respectively.

i. Record the weight (g) of each branch at 5 min intervals. The run lasts until registering no major changes in sample weight.

j. Once done with the measurements in the Drought-boxes, take the samples and measure their weight and, if possible, water potential.

k. Separate the leaves and twigs and put them in two different labelled envelopes.

l. Dry the samples in the oven for 48h at 60°C and measure branch Dry Weight (g) and Leaf Dry Weight (g)

m. The two-sided leaf area (LA, m2) is derived from the leaf dry mass of each measured branch and empirical relationships between leaf dry mass and fresh leaf area (i.e., SLA) previously obtained.

n. The surface area of the stem (BA, m2) is determined from measurements of stem length and basal diameter and assuming the shape of a cone. Leaf Area is determined from the leaf dry weight using the SLA values measured by the field team in the field or in a local lab close to the GCU site.

5. Material

- Bucket
- Water
- shears + saw
- plastic labels
- paper labels
- indelible felt pen
- Ziplock bags
- paper towel
- Pen
- Note sheet
- Envelopes
- Analytical balance (0.001 g)
- Pressure chamber
- Drought-Box
- Paraffin wax
- Pencil
- Oven
- Desktop scanner for SLA

6. Timing

Time*people required for sample collection and preparation:

- 1day * 2 people (1/2 day * 2 people without the trips)

Time*people required for lab measurements:

- 3-4 days * 1 person for each run (1 run = 8 samples per DroughtBox, 2 DroughtBoxes).

- 1.5 day * 1 people for leaf area estimation and sample dry weight

Time * people required for data management

- 3 days * 1 people per run for data management and traits calculation

7. Precautions

Keep the same code for the same individual sample during all the experimentation (field/lab).

Data management: managing and checking the validity of data the day after lab measurements as far as possible.

III. Branch capacitance

1. Introduction

Branch capacitance curves are built by measuring the evolution of water potential and weight on branch samples dehydrating on a bench, in controlled conditions of temperature and humidity. This protocol is similar but not identical to the PV protocol described above. At the end of the measurements taken to determine capacitance of the entire branch, the contribution of leaves, xylem and bark to branch dehydration is also measured.

The complete protocol consists of 3 parts and takes a total of 5-6 days to complete (depending on the species), with the second day having most of the workload.

2. Sample preparation in the field

a. From the 2-3 large branches cut from each tree, cut one small branch with at least 20 leaves, or 20-50 needle twigs in case of conifers.

b. Label this branch individually (GCUCode_TreeCode_CAP_1).

c. Put the branches on a black plastic back with a piece of wet paper towel and a small hole to create a saturated atmosphere inside.

d. Keep the bag in cold conditions (~4°C) until sample processing.

3. Parts and timing

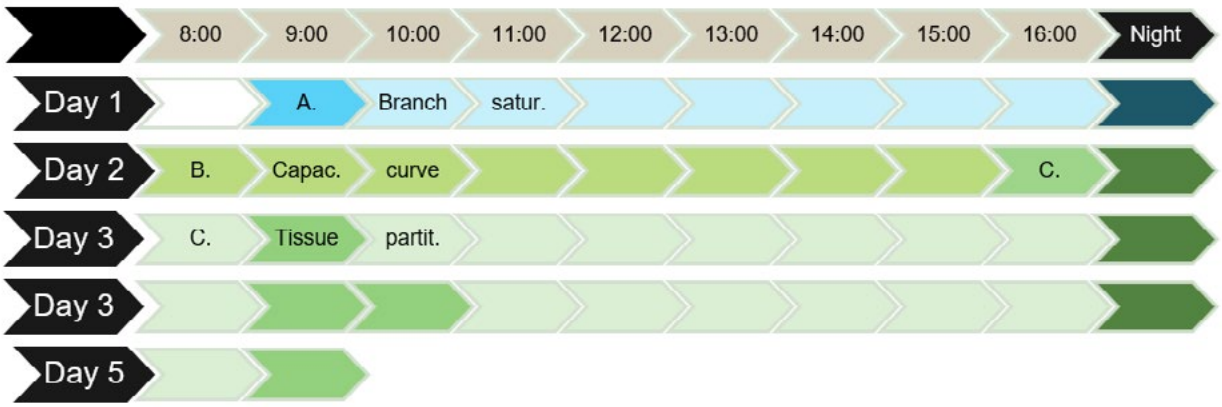
a. Branch saturation: Preparation of the branch sample for steps B+C. This is to be done the day before the Capacitance curve measurements. The objective is to reach water saturation in the branch tissues while keeping it alive.

- ~1h work + 12-24h waiting time. 12 hours is preferred to limit the over-saturation of the branch.

b. Capacitance curve: Capacitance curve measurements (water potential + branch mass), taken periodically during a controlled process of drying.

- 8h-16h work (depending on species, see specifications).

c. Tissue partitioning: Measurements related to water and mass contribution by the different plant tissues. Consist of separating the different tissues (leaves, xylem, bark) and measuring water-saturated and dry weights of the parts.



- ~3-4h work + ~72h waiting time.

4. Material

A. Branch Saturation

- Bucket (1 or 2 depending on size, at least 30cm deep)
- Shears
- Big black plastic bag
- Distilled water
- Cold chamber (4ºC)

B. Capacitance curve

- Pressure chamber [Model: Scholander pressure chamber (PMS-1505D)].
- Gas valve [Reg Presión Adaptador HPR800-BF-V1/4 –DIN6-200/200 (5100411)].
- Compressed Nitrogen gas.
- Temperature/Humidity sensor [HOBO MX Temperature and Relative Humidity Data Logger for Mobile Devices].
- Precision balance [SARTORIUS Series SECURA 5102-1S (5000g/0.01g)].
- Magnifying glass.
- Lamp.
- Shears.
- Razors.
- Big black plastic bags (1 per sample, renewable).
- Zip plastic bags (11 per sample, renewable).
- Acclimatized room + fan + dryer oven (depending on species, see specifications).
- Hanger.

C. Tissue partitioning

- Precision balance [SARTORIUS Series SECURA 5102-1S (5000g/0.01g)].
- Vacuum pump [LINEALAB Bomba de membrana ME 4R NT].
- Rubber/silicone tube (connector for vacuum tube).
- Vacuum bottle or flask.
- Shears.
- Razors.
- Plastic tubes (FALCON Tubes 50ml; 1 per sample, renewable).
- Paper bags (20x30cm; 5-6 per sample, non-renewable).
- Dryer oven.

5. Protocol

Branch Saturation

a. Select 1 to 5 branches to be processed simultaneously. Check that they are correctly labelled and have the correct size and characteristics (see species specifications).

b. Fill 1 or 2 buckets (depending on number and size of branches to work on) with distilled water, up to ~20cm.

c. Put the branches in the water so that their base is submerged. Re-cut the bottom 5cm of the branch under the water.

d. Cover the bucket with the branches with a big black plastic bag and leave them in a cold chamber (4-10ºC) for at least 12h (and not more than 48h). 12 hours are preferred to limit over-saturation.

Capacitance Curve

e. Set the AC fan and or oven at the corresponding temperature (depending on species, see specifications) and put the T/H sensor in it, switched on.

f. Take the branches prepared in section <A> out of the bucket and put each one in a black plastic bag with a small ball of wet paper towel.

g. Leave each branch equilibrating 10-15 min inside the black plastic bag.

h. Take a leaf or a small twig (see species specifications) with the shears from each branch and measure its water potential in the pressure chamber. Recut base with sharp blade. The water potential of the branch will be equal to the pressure reached in the chamber when water starts leaking out of the base of the leaf/twig.

- For a proper Water Potential (WP) measurement, cut a thin slice at the base of the leaf/twig to have a clean cut to observe in the magnifying glass.
- Note that some species (especially conifers) have resiniferous vases that can drip resin before Water Potential has been reached. For these, have a small piece of paper towel ready to periodically clean the base of the leaf/twig.

Example of the moment WP equilibrates to the pressure in the Pressure Chamber and water leaks from the xylem (on *Quercus robur*).

Resiniferous conducts (present in all conifers) which can leak resin at any pressure (example on *Pinus halepensis)*.

i. Weigh the fresh mass of the branch prior to and following the water potential measurement and keep each leaf/twig used for the Water Potential measurement in a zip bag.

j. Hang the branches in the drying chamber or in the oven for the corresponding drying time (see species specifications). After the corresponding time has passed, note the temperature and humidity at the chamber/oven and put the back to the black plastic bags for equilibration.

k. Repeat steps <g> to <j> until you get the desired number of measurements per branch (usually 11-12 measurements).

l. If branch G_{min} is also being measured, continue as long as necessary until a constant rate of water loss per unit of time is obtained. No measurements of water loss are required during this phase.

Tissue partitioning

m. After the final measurement point for each branch, take one extra leaf/twig from each branch, weigh it, and put it in an open tube with the base submerged in water. After that, bring them in a cold chamber in the dark and leave them to saturate 16-24h.

n. From each branch measured, cut a small piece of stem (3-5cm), without ramifications or scars, from the base of the branch and another from at least 20cm from the base. Note which segment comes from the base (LOW) and which one from the upper part (UP).

o. Separate the bark from the xylem of the segments with a razor and weight each component separately.

p. Put the bark and xylem parts in a vacuum bottle filled with distilled water and leave them overnight saturating in the vacuum using the vacuum pump. Make sure each segment is properly separated and labelled to avoid later confusions.

q. Cut the rest of the branch in smaller parts and put them in labelled paper bags (usually 2-3 per branch). Collect also all the leaves/twigs used for Water Potential measurements from their zip bags and put them together in a separate paper bag. After that put all these paper

bags in the drying oven at 70ºC for 40-48h.

r. The next day, measure the water potential and weight of the same leaf/twig from step <m>.

s. Take the woody components off the vacuum bottle (step <o> & <p>), remove the excess water with a towel and measure the mass of each component separately.

t. Put the components from steps <r> & <s> in labelled paper bags and put them in the drying oven at 70ºC for 40-48h.

u. After drying, take the branches from step <q>, separate the leaves from the stems, and weigh each part separately. Weight also the bag with dried leaves/twigs used for Water Potential.

v. After drying, take the components from step <t> and weigh them separately.

6. Species specifications

Branch sample size per species

For a proper measurement of capacitance, the branches used should be healthy (avoiding decaying or very defoliated branches) and have a certain mass and number of leaves/twigs that allows for the removal of one of them each measurement without it causing a significant loss of mass or leaves.

As a rule, a good branch should be at least 40cm and have at least 20 measurable leaves/twigs. Here are more specific sizes observed for various species:

- ***Fagus sylvatica:*** 70-80cm branch with >20 measurable twigs.
- ***Malus sylvestris:*** 40-50cm branch with >20 measurable brachyblasts.
- ***Quercus robur:*** 60-80cm branch with >20 measurable twigs.
- ***Populus nigra:*** 40-50cm branch with >20 measurable leaves.
- ***Abies alba:*** 40-60cm branch with >20 measurable twigs.
- ***Picea abies:*** 40-60cm branch with >20 measurable twigs.
- ***Pinus pinaster:*** 40-50cm branch with >20 measurable brachyblasts.
- ***Pinus halepensis:*** 40-60cm branch with >20 measurable brachyblasts.
- ***Pinus pinea:*** 40-60cm branch with >20 measurable brachyblasts.
- ***Pinus sylvestris:*** 40-60cm branch with >20 measurable twigs.

Unit of Water Potential measurement per species

				
Fagus sylvatica	***Malus sylvestris***	***Pinus halepensis***	***Quercus robur***	***Populus nigra***
				
Abies alba	***Picea abies***	***Pinus pinaster***	***Pinus pinea***	***Pinus sylvestris***

Source: EUFORGEN / *Quercus rubur* © Hugh Asher, Silvotherapy

Drying timings and specifications per species

Tables indicating the drying times (min-max), as well as the temperature and RH values recorded for each measurement and each species in the FORGENIUS project. The time ranges are due to intraspecific variation in the drought resistance of the samples from different populations. The timings must be adjusted per species and population to obtain enough significant points in the capacitance curve.

Broad-leaf species + *Abies alba*

	Fagus sylvatica		*Malus sylvestris*		*Quercus ilex*		*Quercus robur*		*Abies alba*	
Measurement	Time (min)	Temp (°C)	Time (min)	Temp (°C)	Time (min)	Temp (°C)	Time (min)	Temp (°C)	Time (min)	Temp (°C)
	Space	Humidity %	Space	Humidity %	Space	Humidity %	Space	Humidity %	Space	Humidity %
1	0	-	0	-	0	-	0	-	0	-
	-	-	-	-	-	-	-	-	-	-
2	2	24-30	2	24-30	1-8	23-33	1-8	23-33	5	25-30
	AC	35-70	AC	35-70	AC	40-75	AC	40-75	AC(+F)	30-55
3	2-4	24-30	2-4	24-30	1-10	23-33	1-10	23-33	5-10	27-33
	AC	35-70	AC	35-70	AC	40-75	AC	40-75	AC(+F)	30-50
4	2-5	24-30	2-5	24-30	1-10	23-33	1-10	23-33	5-15	29-33
	AC	35-70	AC	35-70	AC	40-75	AC	40-75	AC(+F)	25-55
5	2-10	24-30	2-10	24-30	1-10	23-33	1-10	23-33	5-20	29-33
	AC	35-70	AC	35-70	AC	40-75	AC	40-75	AC(+F)	25-55
6	2-10	24-30	2-10	24-30	2-12	23-33	2-12	23-33	5-30	28-35
	AC(+F)	35-70	AC(+F)	35-70	AC	40-75	AC	40-75	AC+F(O)	25-55
7	2-10	24-30	2-10	24-30	4-17	23-33	4-17	23-33	10-60	30-40
	AC(+F)	35-70	AC(+F)	35-70	AC	40-75	AC	40-75	AC+F(O)	30-60
8	10-45	28-30	10-45	28-30	5-45	23-33	5-45	23-33	15-60	30-41
	AC(+F)	35-60	AC+F	35-60	AC	40-75	AC	40-75	O	35-60
9	15-60	28-30	15-60	28-30	10-60	23-33	10-60	23-33	20-60	30-50
	AC(+F)	35-60	AC+F	35-60	AC	40-75	AC	40-75	O	25-50
10	20-60	28-30	20-60	28-30	10-80	23-33	10-80	23-33	30-60	35-50
	AC(+F)	35-60	AC+F	35-60	AC	40-75	AC	40-75	O	25-50
11	20-90	28-30	20-90	28-30	15-90	23-33	15-90	23-33	30-90	30-50
	AC(+F)	35-60	AC+F	35-60	AC	40-75	AC	40-75	O	20-50

* AC = AC room; F = Fan turned on; O = Oven

Conifer species

Measurement	*Picea abies*		*Pinus halepensis*		*Pinus pinaster*		*Pinus pinea*		*Pinus sylvestris*	
	Time (min)	Temp (ºC)	Time (min)	Temp (ºC)	Time (min)	Temp (ºC)	Time (min)	Temp (ºC)	Time (min)	Temp (ºC)
	Space	Humidity %	Space	Humidity %	Space	Humidity %	Space	Humidity %	Space	Humidity %
1	0 -	- -	0 -	- -	0 -	- -	0 -	- -	0 -	- -
2	5-10 AC+F	25-30 40-60	15 AC+F	30-40 30-70	15 AC+F	30-32 20-50	10 AC+F	30-32 60-80	5-30 AC	25-31 35-60
3	5-10 AC+F	25-30 40-60	20-30 O	30-35 35-60	15-60 AC+F	30-32 20-50	15 O	30-35 60-75	5-30 AC(+F)	27-31 35-60
4	5-15 AC+F	25-30 40-60	30 O	30-35 35-60	15-60 AC+F	30-32 20-50	20-30 O	30-35 60-70	5-45 AC+F(O)	27-35 35-60
5	5-20 AC+F(O)	25-30 40-60	30 O	30-40 35-50	30-120 AC+F	30-32 20-50	30 O	30 50-60	2-10 O	28-38 20-55
6	5-20 AC+F(O)	25-30 40-60	30 O	35-40 30-50	60-120 AC+F	30-32 20-50	30 O	35 50-60	4-15 O	28-40 20-55
7	5-30 O	30-35 40-60	30 O	40-45 30-50	120-120 AC+F	30-32 20-50	30 O	35-37 40-50	5-20 O	28-40 20-55
8	10-45 O	30-35 40-60	<night> AC	Room T Room H	<night> AC	Room T Room H	30 AC	40 30-50	5-40 O	28-40 20-55
9	15-80 O	30-35 40-60	40-60 O	40-45 30-45	120-120 O	38-41 20-30	45 O	40 30-50	5-40 O	28-40 20-55
10	20-80 O	30-35 40-60	60-70 O	40-45 30-45	120-120 O	38-41 20-30	45 O	45 30-50	5-45 O	28-40 20-55
11	20-90 O	30-35 40-60	60-90 O	40-45 30-45	120-120 O	38-41 20-30	60-90 O	45 30-50	10-60 O	28-40 20-55

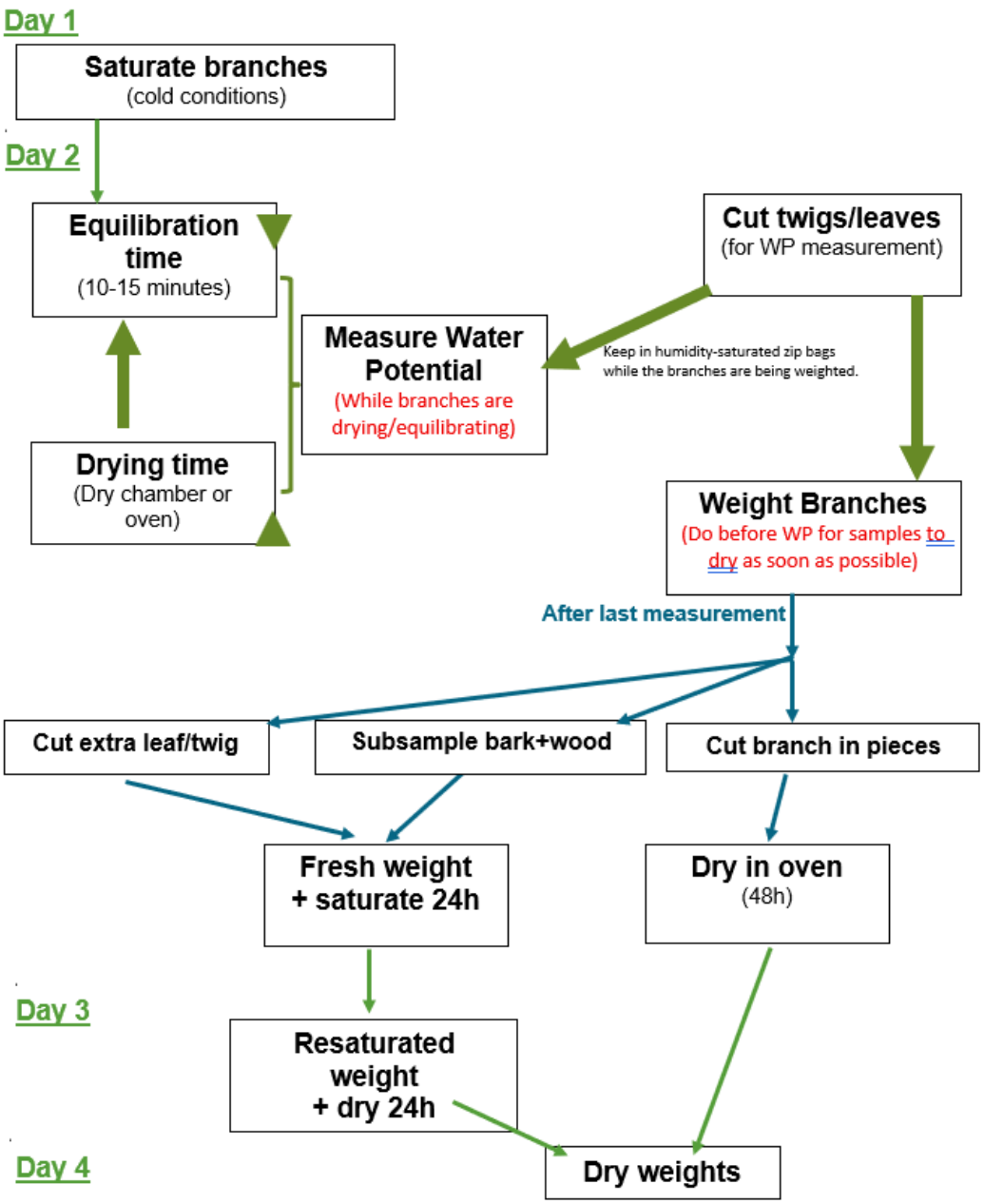
* AC = AC room; F = Fan turned on; O = Oven

7. Related Documents

The following capacitance record sheets (excel sheets) are available for leaves and twigs at portal.eufgis.org/Guides/protocols:

* Capacitance_record_sheet_leaves.xlsx
* Capacitance_record_sheet_twigs.xlsx

8. Overview of a workday



IV. Volumetric Wood Density

1. Introduction

Wood density was measured both on a 10cm wood segment at the end of a 50cm terminal branch, and on one core, from under the bark to the pith.

The general protocol for WD measurements follows the GenTree protocol and, if required, the protocols given in the PrometheusWiki (http://prometheuswiki.org/tiki-custom_home.php).

2. Sample preparation in the field

The branch segment to be used can be obtained from the base of one of the capacitance samples, or by taking an extra 10cm segment with no ramifications (or 15cm with some ramifications) from the base of an extra 50cm branch in the field. In that case store these samples in a zip plastic bag with a wet piece of paper towel.

For the core wood density, use the one collected for isotope analysis (Section C.I.), before proceeding with that protocol.

3. Branch Wood Density

a. Cut a branchless 10 cm segment from the bottom section of the branch stem. If only a segment shorter than 10cm length is available, cut various segments of shorter lengths while avoiding the ramifications. Annotate the actual lengths with a micrometre.

b. Measure the over-bark diameter of the segment in the centre of the segment.

c. Separate bark from xylem, taking precautions to prevent to break the bark into many small pieces. Measure the under-bark diameter of the segment in the centre of the segment.

d. Measure the fresh volume of both bark and wood using the immersion technique and Archimede's principle in a 2-decimal or 3-decimal balance: Submerge the wood and bark segments (separately) in a container filled with water resting on the scale. Make sure the segment is fully immersed and does not touch the walls or the bottom of the container. Record the weight in g from the balance. Since water density is 1 g/cm3, the figures of the weight in g equate the volume of the object inside the water in cm3.

e. Dry both bark and wood at 70ºC for 48 hours and measure the dry mass of both in g.

f. Calculate wood density as the ratio of dry mass to fresh volume (g/cm3) for both bark and wood. If the segments had been broken down into two smaller pieces, sum the respective volumes and dry masses before calculating the final density ratio.

4. Core Wood Density

a. Use the dry core sampled for isotope analysis. Cut the core precisely under the bark and at the pith.

b. Obtain the dry weight of the core on a precision scale (0.0001g) and note it.

c. To calculate the fresh volume of the core, us the total length recorded in the field (from under the bark to the pith). The diameter can be obtained from the borer used to obtain it. The final formula for the density will be calculated as:

Core Wood Density (g/cm3) = Dry Weight x Core Length x 0.25π x (Core Diameter)2

5. Materials

- Precision scales (0.01g and 0.0001g).
- Razor blades.
- Pruning shears.
- Small water container.
- Support to submerge the sample into the water.
- Paper bags.
- Oven.

V. Turgor loss point by osmometry

1. Introduction

Branch turgor point is determined by extracting the sap from leaves following membrane rupture and measuring the resulting osmotic concentration of the liquid solution in an osmometre. We also test whether equivalent estimates can be obtained by directly employing leaf punches (which bypass the needs for sap extraction). The following protocol follows the first route.

2. Sample preparation in the field

a. From the 2-3 large branches cut from each tree, cut a small branch with at least 10 leaves, or 20-50 needles in case of conifers.

b. Label this branch individually (GCUCode_TreeCode_TLP_1).

c. Put the branches on a black plastic back with a piece of wet paper towel and a small hole to create a saturated atmosphere inside.

d. Keep the bag in cold conditions (~4ºC) until sample processing.

3. Branch TLP measurements

a. Rehydration of the leaves: Once in the lab, take at least 10 leaves from the branch (at least 20-50 for conifers), fill Falcon tubes with distilled water, and set the leaves so that their petiole is submerged in the water (avoid submerging the whole leaf if possible).

b. Put the leaves to rehydrate for 12-24h, at 4ºC in darkness.

Populus nigra leaves, ready for rehydration.

c. The next morning, take the leaves out of the vial, and quickly and carefully blot dry with a paper towel.

d. Transfer leaves to paper bags previously labelled and store the samples at - 80ºC. Leave them in storage whenever ready for analysis.

If space is only available in -20ºC, an extra freezing step is required to ensure breakage of the cell membranes. Freeze the samples by submerging them inside liquid N for 60s, or alternatively by placing them in the -80ºC deep freezer for several days.

Measuring TLP with a freeze-point Osmometre

e. Leave the leaves to defrost inside a black plastic envelope to avoid dehydration. This should require in the order of 10 min (carry out preliminary tests if beforehand to check if this is appropriate).

f. Turn on the osmometre and begin the self-calibrating routine, do a manual calibration if necessary (follow any cleaning-calibration specifications from the osmometre's manual).

g. Cut out and discard the main and secondary nerves of the leaves. Put the remaining material inside a syringe.

h. Extract the sap of the syringe into an eppendorf.

i. Empty the remaining contents of the syringe into a paper bag and put into the oven to dry for 48h at 70ºC.

j. Weigh the dried leaf material and note it.

The following steps assume the use of the model - ADVANCED INSTRUMENTS 3320 MICRO-OSMOMETRE ANALYZER.

k. Extract 20 µl of sap using the osmometre's pipette, clean any excess from the tip (check image) and load it carefully into the reading chamber (don't let the spring from the loader set the pipette on its own as it is a bit rough and increases the risk of leaks, leading to error readings and contamination of the chamber.

l. Wait for the Osmometre to give a reading (~2min). Note it, extract the pipette, discard the pipette tip and clean both pipette and reading chamber with the provided cotton swabs (use a bit of alcohol for the cleaning from time to time).

m. If the Osmometre gives a reading outside of its resolution range, prepare a diluted sample of the sap and read again. Correct later for the dilution ratio used.

n. Apply formula to transform reading into TLP values. First convert from mosm/kg to MPa multiplying by -0.002469 to obtain osmotic potential at full turgor in MPa units. Then convert osmotic potential at full turgor to turgor loss point using the formula: TLP=0.832 * ▯▯0 -0.631, where ▯0 is osmotic potential at full turgor and TLP is turgor loss point.

Measuring TLP with a Vapour Pressure Osmometre

Measurements with the osmometre (following Barlett et al 2012, paper in Methods in Ecology and Evolution, for the use of equations to estimate TLP from the osmotic potential at saturation).

a. Turn on the osmometre and begin the self-calibrating routine. Wait for temperature stabilization.

b. If cell sap is employed for the measurements, put the leaves in a syringe and push the plunger until you extract about 10 µl of sap (following steps g-j on the freeze-point osmometre method). Choose one filter paper disk and place the required amount of sap on the surface of the disk. Follow the instrument instructions regarding quantities and getting rid of excess moisture.

c. Alternatively, if leaf disks are employed, with the leaf still frozen, use a sharp leaf puncher to cut the exact size of the leaf to fit the loading cell of the osmometre. Use a sharp needle to make about 15 holes on the upper surface of the punch to speed up vapour equilibration. Leaves are left to defreeze inside the osmometre chamber and measurements are repeated until equilibrium is reached.

d. Load in the osmometre cell, wait for equilibration and record measurements of the osmometre. Repeat the reading a second time without opening the cell to ensure stability.

e. Apply the same formula as above, i.e., first convert to MPa using the conversion factor, then calculate TLP from osmotic potential at full turgor as: TLP=0.832 * ▯▯0 -0.631 to transform the reading into TLP values.

f. At the end of the experiment, put the sample leaves in the oven for 48H00 at 70°C

g. Weigh the dry masses of the samples from both TLP_1 and TLP_2.

h. Store the samples in labelled envelopes for subsequent LES traits (%N, d13C, SLA)

i. Fill the dedicated Excel spreadsheet (TugorLossPoint_SpreadSheet.xlsx)

j. You can compute parameters based on the R script available at https://github.com/mmencuccini/FORGENIUS-routines/Capacitance_calculation_partitionin_MM.R

4. Material

- Falcon tubes
- 10 ml syringes
- Plastic labels
- Indelible felt pen
- Ziplock
- 10 µl pipette
- Pipette tips
- Pipette tip holder
- Paper labels
- Paper bags and envelopes
- Pens and pencils
- Note sheet
- Vapour pressure osmometre/ Freezing-point Osmometre (+ accessories)
- Razor blade
- Large black plastic bags
- Oven

5. Timing

Time*persons required for sample collection and preparation:

- 1day * 2 persons (1/2 day * 2 persons without the trips)

Time*people required for lab measurements:

- 1 person - 1 days for 10 branches/GCU, i.e., 20 branches overall.

- 0.5 day * 1 people for leaf sample dry weights

Time * people required for data management

- 1 day * 1 people for data management and traits calculation

6. Precautions

- Since the samples are stored in deep-freeze freezers, these measurements can be done during periods of no fieldwork

- Make sure the osmometre calibration is always carried out reliably.

- Keep the same code for the same individual sample during all the experimentation (field/lab).

- Data management: manage and check the validity of data the day after lab measurements as far as possible.

7. Related documents

- TugorLossPoint_SpreadSheet.xlsx

- References: Bartlett et al., 2012. Rapid determination of comparative drought tolerance traits: Using an osmometre to predict turgor loss point. Methods in Ecology and Evolution, 3: 880-888.

VI. SLA, %N, d13C and Huber value

1. Introduction

Huber Value (HV) is a plant functional trait measured as the ratio between the cross-section area containing the active vases of a branch and the total leaf area that those vases irrigate. Specific Leaf Area (SLA) is the ratio between the leaf area and the total dry weight of those leaves. It is obtained as part of the HV protocol.

The protocol consists of two parts: the measurement of leaf area (which is done differently for broad-leaved species and linear-leaved species), and the measurement of the cross-section active for water transport.

2. Sample preparation in the field

a. From the 3-4 large branches cut from each tree, choose one that is NOT employed for Capacitance and turgor loss point.

b. Use a tape measure to determine the distance of 50cm from the tip of the branch that is most distal from the branch base.

c. Recut the stem at 50cm distance from the apex and throw away the rest. Make sure there's no ramifications on the basal 5cm of the branch (and preferably 10cm). If there are, look for another branch.

d. Detach all the leaves remaining on the 50-cm-long branch and put them in a zip bag (plastic bag if leaves are wet).

e. Label the zip bag with the following code: GCUCode_TreeCode_HV.

f. Cut a 15cm long segment at the base of the 50-cm-long branch and insert it in the same bag.

g. Discard the rest of the branch.

h. Keep the bagged samples in cold (~4°C) for preservation until laboratory work.

i. Ship to the laboratory with the rest of the sample material.

3. Parts and timing

a. Leaf Area and SLA: Obtained by directly measuring the area of a subsample of fresh leaves (by scanning in broad-leaved species, or by longitude/thickness/diameter measurement in linear-leaved species) and later

extrapolating it to the whole sample using SLA measured on the subsample.

- 1-2h work (broad-lv.) or ~8h work (linear-lv.), + 48h waiting time.

b. Active Cross-Sectional Area: Measurement of the branch wood section (15cm), that is stained due to the presence of active vases and measurement of the active area.

- ~8h work.

4. Laboratory branch HV and SLA measurements

Leaf Area and SLA

a. Take all fresh leaves from the sample and separate a random subsample of them.

Subsamples: 10 leaves for broad-leaved species and 30 leaves for linear-leaved species (60 for Abies alba).

b. Put all leaves not used for the subsample in a paper bag.

Broad-leaved species

c. Take the 10 leaves of the subsample and scan them in a digital scanner. Make sure to have a good contrasting background (either white or black, depending on the species).

- Recommended to set the leaves between two transparent plastic sheets to minimize the scanner getting dirty.

- Have a known size reference through the scanning (know the scanner area dimensions or scan a ruler with the leaves).

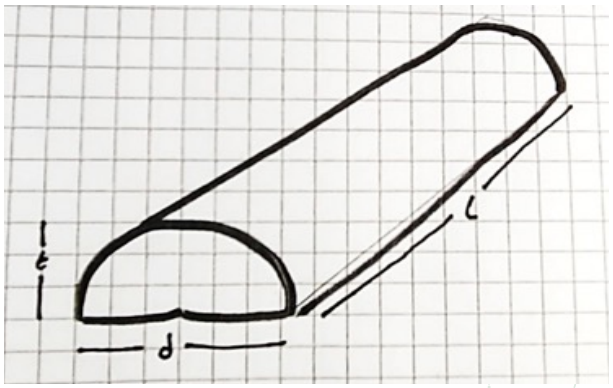
Scanned *Quercus robur* leaves

Linear-leaved species (*Pinus* and *Picea*)

c. Take 30 leaves and measure, using a ruler and a precision calliper, their individual lengths (l), thickness (t) and width (d). These will be used to calculate each individual leaf area, assuming a semicircle-prism shape, using the formula:

$$Leaf\ area = [((2t + d)/4 \times \pi + d) \times l]$$

- For some species other assumed shapes will work better (like square or triangular prisms), and a different formula



must be used in consequence.

- For modelling we will assume projected leaf area is equal to half total leaf area as in Modis (https://modis-land.gsfc.nasa.gov/lai.html)

- NB2: In case thickness is not recorded we will apply the following formula where everything is expressed as a function of diameter (d):

Leaf Area =1 + π/2 l × d

d. After measuring, put the subsample leaves in a paper bag (separate from the rest of the sample).

e. Calculate the total leaf area of the subsample by adding together each individual area.

Abies alba

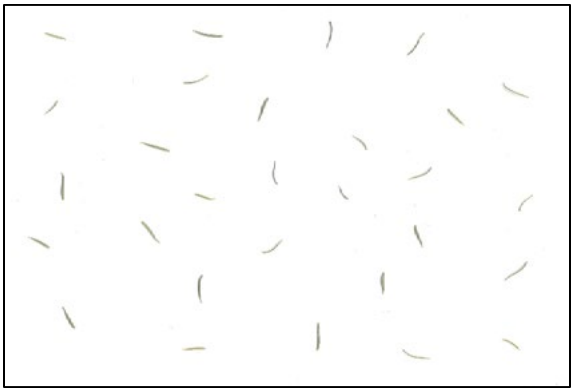
a. Take two separate random sets of 30 leaves and measure, using a ruler and a precision calliper, their individual thickness (t) and length (l).

b. Take the 10 leaves of the subsample and scan them in a digital scanner. Make sure to have a good contrasting background (either white or black, depending on the species).

- Recommended to set the leaves between two transparent plastic sheets to minimize the scanner getting dirty, and set the upper side to be scanned (the lower side has two white strips that could make calculating the area difficult).

- Have a known size reference through the scanning (know the scanner area dimensions or scan a ruler with the leaves).

c. Take the leaves out of the scanner and put them in a paper bag (separate from the rest of the sample).



d. Calculate the projected leaf area of the subsample with an image analysis program (like ImageJ).

e. Assuming the needles to have a coarsely triangular cross-section (based on various anatomical cross-sections examined), the formula for the calculation of the total leaf area is:

Leaf Area = √([Projected Leaf Area]^2 + 4 x l^2 x b^2) + [Projected Leaf Area]

f. Dry all leaves in an oven at 70ºC for 48h.

g. Weight the dried leaves.

h. Calculate the total leaf area of the branch by comparing the total weight of the leaves with the subsample, and assuming a linear relation between dry weight and leaf area:

Leaf area (Total) = Leaf area (Subs.) × (Dry weight (Total))/(Dry weight (Subs.))

i. SLA can also be calculated from this data, with the formula:

SLA = Leaf area (Subs.) × (Dry weight (Total))/(Dry weight (Subs.))

Vase section area

a. Use a digital precision calliper to determine the diameter of the base of the wood segment. Measure the total diameter (over the bark), the hardwood diameter (under the bark), and the pith's diameter if easily visible.

b. Prepare (filter and dilute appropriately) a 1 litre of 0.5% toluidine blue solution in a flask or IV bag.

c. Cut under water 5cm on each end of the woody segment (sample). From now on all steps involving this sample must be done under water.

d. Attach the IV bag to polypropylene tubing and fit it to the base of the segment under gravity pressure.

e. For resinous species, wait for 5 minutes after cutting the sample under water, and cut a thin slice (~1mm) on each end before attaching the tubing. This is to remove the resin that might have been secreted by the sample in the initial cut, which could be blocking some of the vases.

f. Leave the stained water to pass through the stem for several minutes-hours (depending on the species) in a drain.

g. After the sample has been stained, remove it from the tubing and put it in the oven to dry for 48h at 70ºC.

h. Recut the base of the stem using sharp razor blades and determine the proportion of the xylem area that is conductive from the total xylem area (visually or using an image analysis program).

5. %N and d13C measurements

At the end of the HV and SLA measurements, take a random sample from the dried branch leaves and grind them to a powder using the grinding machine. Follow the protocols from the selected analytical lab to determine the quantities to be sent for external analysis.

6. Material

- Leaf Area / SLA
- Ruler.
- Paper Bags (2 per sample).

- Digital calliper (0.01mm precision).
- Digital scanner.
- Transparent plastic sheets (x2).
- Oven

Vase section Area

- IV infusion bags.
- Silicone tubes (compatible with the IV tubes).
- Plastic tray (5-10cm deep).
- Shears.
- Razors.
- 1L glass flask.
- Small spatula
- Filtering system (filter paper + funnel or other systems).
- Toluidine blue.
- Distilled water.

7. Timing

Time*persons required for sample collection and preparation:

*- 1day * 2 persons (1/2 day * 2 persons without the trips)*

Time*people required for lab measurements:

- Leaf areas:1 person - 1 days for 10 branches/GCU, i.e., 20 branches overall.

- Dye experiment:1 person - 1 days for 5 wood segments /GCU.

*- 0.5 day * 1 people for leaf sample dry weights*

Time * people required for data management

*- 1 day * 1 people for data management and traits calculation*

8. Precautions

Keep the same code for the same individual sample during all the experimentation (field/lab).

Data management: manage and check the validity of data the day after lab measurements as far as possible.

VII. Vulnerability curves measurements with a cavitron

1. Cavitron Preparation

1. Turn on the computer
2. Turn on the speed control box and camera
3. Turn on the centrifuge
4. Turn on the light source
5. Make sure there is enough measurement solution in the water tank; make more if needed

2. Sample Preparation in the lab

***It is possible to store samples in the fridge for 10-20+ days before taking measurements (depending on species) ***

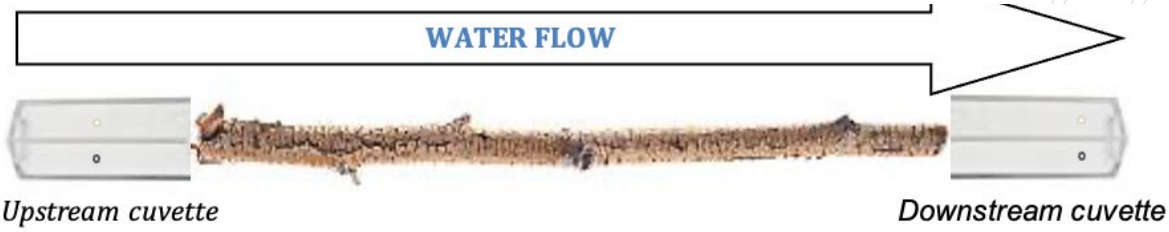
1. Just before taking measurements, cut your sample underwater a little above the needed length (16.5, 27.5 (standard rotor), 42.5 or 100cm depending on the rotor used)
2. To avoid crushing vessels, use a sharp razor blade to shave off both extremities to the exact needed length
3. Gently remove a few centimetres of bark at both ends for angiosperms and debark the whole branche for conifers

** If your samples are curved, you might also consider cutting them a little shorter, as they tend to straighten as you spin faster, and can break the reservoirs.*

*** For resin- (all conifer species) or latex-producing species, it is recommended to remove the entire bark to avoid resin/ latex accumulation in the reservoirs (from centrifugation).*

4. Measure the average diameter of the sample, below bark, at each extremity. Enter both values in the software (PARAMETERS tab > Input stem diameter)

5. Install the sample in the central part of the rotor with both extremities enclosed in the reservoir (proximal part of the sample in the upstream reservoir / distal part of the sample in the downstream reservoir). Make sure that the holes of the reservoirs are placed sideways (or downwards for the Cavi1000)



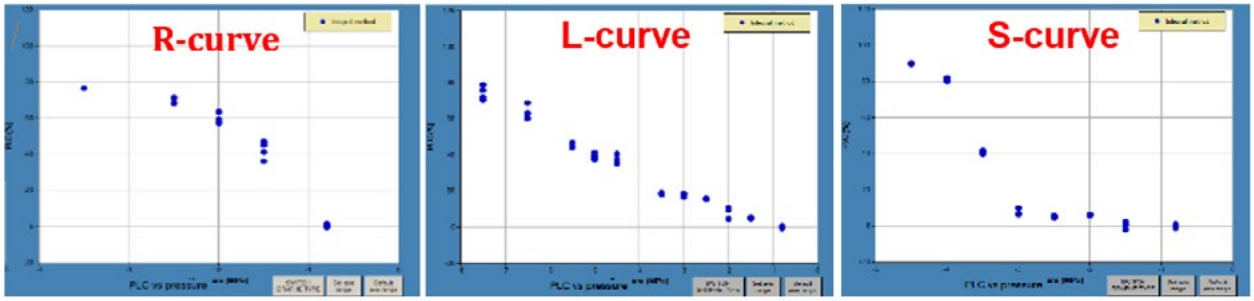
6. Lock the lid of the rotor with all screws making sure that the lid is flush with the rotor but without over-tightening the screws

7- Close the centrifuge lid. You are now ready to spin!

Figure 1 The CaviSoft window with the location of each step of the Measurement protocol

Figure 2 Keyboard keys needed for measurements

Figure 3 Possible curve shapes obtained. An R-curve indicates that vessels are too long for proper measurement with this rotor-size. An S-curve is what we aim for.



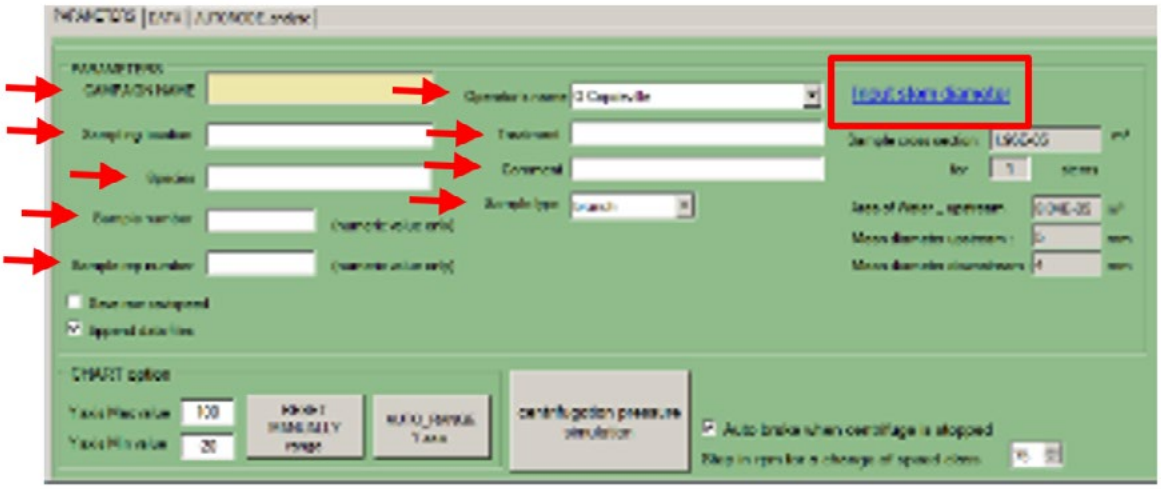
3. Measurement

Double-click the CaviSoft v5.2 icon to launch the software

All operations are now performed safely and remotely using the software

1. Connect the camera by clicking the “camera connected” checkbox

2. Enter all your sample information in the PARAMETERS tab



*** Do not forget to enter the sample diameters!**

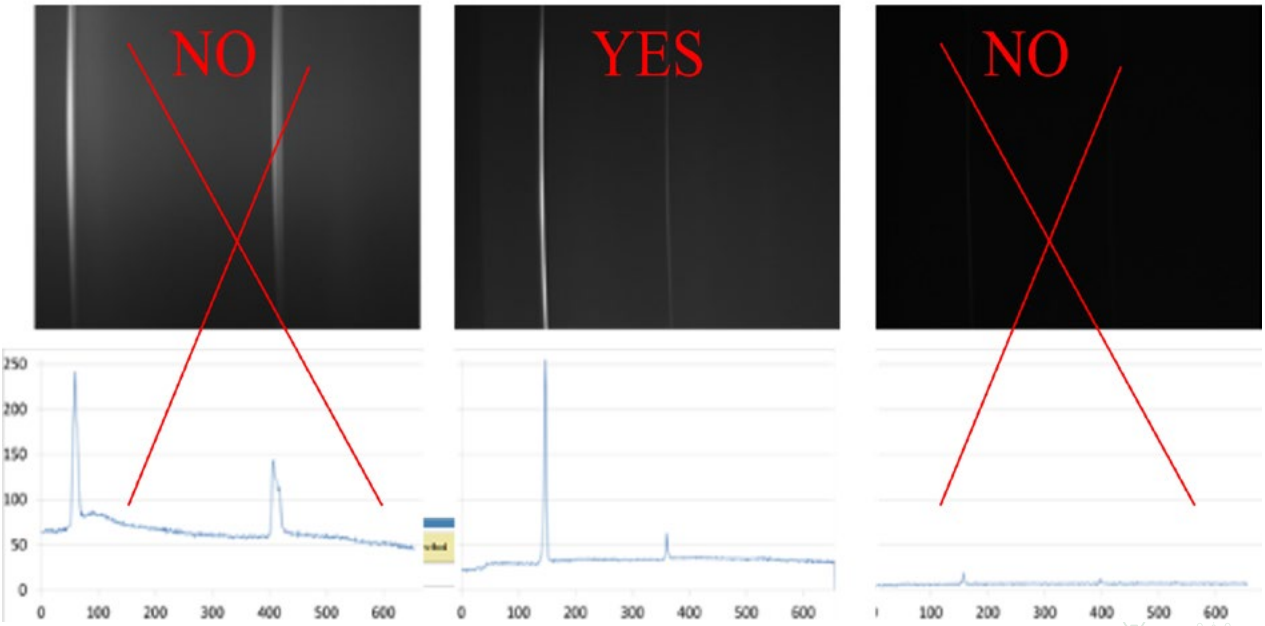
3. Set the desired pressure by clicking on the “Set cavitron pressure MPa” button, and press the “START” button to start the centrifuge

*** For the Cavi1000, press the “START” button before setting the pressure**

4. a. Activate measurement mode by pressing the icon 

   b. Click in the “MEASURE MODE ACTIVATED” textbox to add water in the reservoirs by pressing the downwards arrow on the keyboard until both menisci appear

5. As best as you can, adjust the menisci by playing with the light source and camera exposure time to obtain 2 single, sharp and bright menisci



6. Click on the "Automatic ROI" button to automatically detect the menisci in the Region of Interest (ROI) windows

**If the menisci aren't ideal, click on the "manually define downstream ROI" buttons and then double click on the downstream and upstream menisci. The width of the ROI windows can also be increased (if menisci move quickly) or decreased (if downstream meniscus doubles or if menisci are not very bright).*

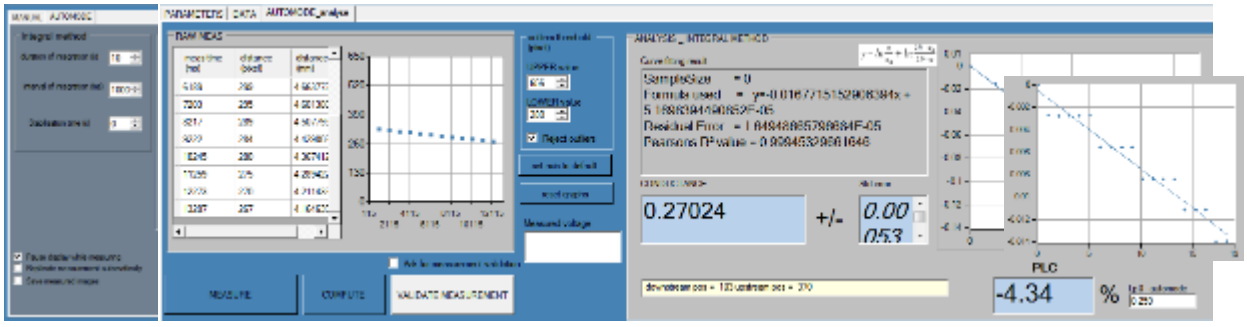
***The measured line can be adjusted by increasing or decreasing its value to move it to the brightest/clearest part of the menisci*

7. a. Once the upstream meniscus is in the upstream ROI, press on the spacebar to start automatic measurement

b. At the end of the first measurement, a window appears to name and save the data as a .csv file in the desired campaign folder

**From then on, the software automatically monitors the distance between both menisci, compute the conductivity, and save all parameters, raw data and variables of interest in the current file.*

8. Check that the measure is OK in the "AUTOMODE_analysis" tab; the bottom right graph should show a perfect negative linear regression, with minimum scatter. If not, adjust the Integral method options (duration of integration and interval of integration seen in figure below left) until such a trend is achieved



Ex. For a branch with good conductivity, the upstream meniscus usually moves well, therefore a 10s duration of integration and 500-1000ms interval of integration setting is appropriate.

However, when a branch has low conductivity, the upstream meniscus moves much more slowly. Therefore, the duration and interval of integration should be increased until it forms decreasing plateaus in a descending diagonal line (ex. 50s duration and 1000-2000ms interval of integration settings).

9. Add water to the reservoirs before taking a new measurement so that the data points are taken at the same place

10. Once measurements are stable and there are at least 3 points with similar conductivity and PLC values, press Ctrl+W compute finish to create the reference of your PLC

11. Let the upstream meniscus reach the downstream meniscus before setting a new Pressure level and wait a full 2 minutes before adding water to start your new measurements (until the Countdown Timer box isn't red anymore)

** If the upstream meniscus doesn't reach the downstream meniscus, measure the difference in X pixels between both menisci by placing the cursor above them. Enter that difference by clicking on the "Minimum meniscus position (grad)" link. This is especially important at very negative pressures as that difference can change the pressure used in the equations!*

12. Repeat steps 9 and 11 until measurements at a certain pressure level indicate a PLC of at least 80%

13. Press "STOP" button

14. Press File Append_data, and double click on the appended data file in the current campaign folder

15. Remove the sample from the rotor and dry the centrifuge reservoir if needed.

VIII. Soil Texture Analysis

1. Introduction

The separation of the soil into fractions by size is done in the laboratory on the samples collected in the field (See Section B.I). After the protocol the following fractions will be obtained as percentages in dry mass:

- Coarse elements: >2mm.
- Coarse sands: 0.2mm - 2mm.
- Fine sands: 0.05mm - 0.2mm.
- Silts: 0.002mm - 0.05mm.
- Clays: <0.002mm.

2. Soil Texture Analysis

a. Take at least 500g of the air-dried soil sample and put it in a paper bag. Then put it into the oven to dry for 48h at 70ºC. Obtain the dry weight of the sample using a precision scale (0.01g minimum).

b. Separate the coarse elements: The sample is placed on a rubber mat and a wooden roller is passed over it to break any aggregates that may be present. The sample is then placed on a 2 mm diameter steel sieve to separate the two fractions. Check if any agregates remain on the coarse element fraction and repeat this step if so.

c. Weigh each fraction to determine the percentage of coarse elements and store them separately in zip plastic bags.

Determination of the residual moisture of the samples

d. Weigh about 20g of fine fraction from step c (note the exact weight at 0.0001g resolution) and place it in an aluminium container of known weight.

e. Dry the sample at 105ºC for 24 hours and obtain the dry weight after that. The ratio between the weights before and after the drying will be applied to correct for residual moisture in the subsample obtained in step f.

Destruction of organic aggregates

f. Weigh 20g of fine fraction from step c (note the exact weight at 0.0001g resolution and correct it for residual moisture from the ratio obtained in step e). Add it into a stainless-steel jar, making sure to clean all residue from the container used for weighing into the jar using distilled water.

g. Add ~30mL of distilled water and 5-10mL of 30% hydrogen peroxide. An oxidizing reaction will start.

h. Keep adding 5mL of hydrogen peroxide every time the reaction stops, until there's no more noticeable reaction or 30mL of total hydrogen peroxide have been added. Leave the jars for 6h (or overnight) at room temperature.

Separation of coarse and fine sands

i. Weigh the 0.2mm and 0.05mm sieves.

j. Put the 0.2mm sieve on top of the 0.05mm sieve and filter the solution of soil through them. Use a funnel to collect the filtered sollution into a 1L glass bottle.

- Wash the soil retained in the filters constantly with distilled water while removing them softly with a stir bar to make sure all the silt and clay get through the sieves. Stop once the water passing through the filters is clear.

k. Put the sieves with the substrate on an aluminium container and into the oven to dry at 105ºC for 24h.

l. Weigh the dried containers with the retained sands. Substract the empty container's weight (step i) to obtain the sand's dry weight.

Separation of silt and clay

m. Add 15 mL of sodium hexametaphosphate (check Section F.VIII.3 for the preparation of this reactive) into the the 1L glass bottle containing the filtered soil suspension. Place the bottle on an automatic shaker for at least 6h. This will dissolve any remaining aggregates.

n. Empty the bottle into a 1000 mL test tube and fill it to exactly 1L with distilled water.

o. Stir the suspension thoroughly for a few seconds and keep the test tube completely still from now on.

p. Let the solution settle for exactly 46 seconds (assuming 20ºC room temperature, or 41 seconds at 25ºC) from the moment the stirrer is removed. During that time, slowly insert the Robinson's 10cm into the solution (recommended marking the 10cm point on the pipette with indelible marker).

q. Once the time is up, extract 20mL of the suspension and empty them into a previously weighted (to 0.0001g precision) aluminium container.

r. Clean the pippete with distilled water and put the aluminum container into the oven at 105ºC for 24h.

s. Weigh the dry container that will now contain the fraction of silt and clay. Subtract the weight of the empty container and multiply by 50 to know the whole fraction of silt and clay in the 1000mL test tube.

t. After 6h, a new extraction is carried at a depth of 7.5cm (assuming 20ºC room temperature, or 8.5cm at 25ºC) with the Robinson's pipette to obtain the clay fraction in 20 mL of suspension, to be put into another aluminium container, also previously weighted.

u. Follow steps r and s for this new sample, which will give the clay fraction in the 1000mL test tube.

v. Calculate the percentage of each fraction keeping in mind that the sand (coarse and fine), silt and clay fractions have been calculated on a subsample of ~20g (step f), while the coarse elements were calculated form the total weight obtained in step a.

3. Preparation of Sodium hexametaphosphate

a. Mix 35.74g of (NaPO3)6 (R10204640 Fisher Chemical - Code: S/4160/53) with 7.94g/L of Na2CO3 (Ref 10264545 Fisher Chemical - Code: S/2920/53) into a beaker with distilled water.

b. Stir until dissolved, heat the solution if necessary to facilitate the mixture.

c. Add the sollution into a 1L volumetric flask and fill up to exactly 1L.

4. Materials

- Paper bags.
- Zip plastic bags.
- Oven.
- Rubber mat.
- Wooden roller.
- 2mm, 0.2mm and 0.05mm steel sieves.
- Stir bars.
- 10cm funnels.
- 1L test tubes.
- 1L glass bottles.
- 10mL pippette.
- 20mL Robinson's pipette.
- 1L volumetric flask.
- Glass beaker.
- Stainless steel jar (0.5L - 1L).
- Precision scale (0.0001g).
- Aluminium containers.
- Sodium hexametaphosphate.
- Hydrogen peroxide 30%.
- Distilled water.

G. Appendices

I. Appendix 1. Printable materials list for FORGENIUS GCU sampling protocol

Materials List (*not allowed in case of traveling by plane)	**Check-list**
Site, Plot and Tree Selection	
• Handheld GPS or DGPS (Differential Global Positioning System)	
• Android tablet	
• Laser distance measuring system (e.g., telemeter or Vertex), better and easier than decametre	
• Compass for direction	
• Durable metal or plastic tree labels to identify and mark the tree + Dymo	
• Staples and stapler	
• Spray of recyclable forest paint or works ribbon*	
• Construction tape or milestones for delimiting virtual plot*	
• Construction tape for selecting tree*	
• Camera	
• Serp / hand saw	
Abiotic Environmental Descriptors	
• Clinometer	
• Compass	
• Soil auger*	
• Crow bar*	
• Pickaxe*	
• Folding shovel*	
• Rigid tape measure*	
• Knife	
• Screwdriver	
• Pruning shears (for cutting roots)	
• Basin (to collect soil)	
• Photo camera	
• Plastic bags (to carry soil sample)	
• Record sheet + pencil	
• Munsell soil colour chart	
• Hydrochloric acid	

Materials List (*not allowed in case of traveling by plane)	**Check-list**
Site, Plot and Tree Selection	
• Handheld GPS or DGPS (Differential Global Positioning System)	
• Android tablet	
• Laser distance measuring system (e.g., telemeter or Vertex), better and easier than decametre	
• Compass for direction	
• Durable metal or plastic tree labels to identify and mark the tree + Dymo	
• Staples and stapler	
• Spray of recyclable forest paint or works ribbon*	
• Construction tape or milestones for delimiting virtual plot*	
• Construction tape for selecting tree*	
• Camera	
• Serp / hand saw	
Abiotic Environmental Descriptors	
• Clinometer	
• Compass	
• Soil auger*	
• Crow bar*	
• Pickaxe*	
• Folding shovel*	
• Rigid tape measure*	
• Knife	
• Screwdriver	
• Pruning shears (for cutting roots)	
• Basin (to collect soil)	
• Photo camera	
• Plastic bags (to carry soil sample)	
• Record sheet + pencil	
• Munsell soil colour chart	
• Hydrochloric acid	
Biotic Environmental Descriptors	
• Measuring tape for DBH	
• Dendrometer Vertex or decametre for checking radius	

• Bitterlich relascope	
• Field sheet and pencil	
• Chalks (to mark inventoried trees)	
LAI Equipment	
• Digital camera	
• Digital single-lens reflex camera (≥12.3 MP, no compact camera)	
• Fish-eye lens compatible with camera	
• Field of view: 180°	
• 4.5mm lens for APS-C cameras, 8mm for full-frame cameras	
• Tripod or telescopic monopod with levelling head	
• Measuring tape or stick (1.3m height)	
• 3D bubble level or self-levelling mount	
• Compass (for camera alignment to true North)	
• Inclinometer (for slope measurement)	
• Material for calibration of lens and camera	
Sample Collection – Tree Cores	
Manual Increment borer	
Electric drill for increment borer *	
Battery for electric drill for increment borer*	
PVC cylinder tube and core holders (e.g., paper straws)	
Waterproof pencil	
Masking tape	
Graduated ruler	
Field-sampling sheets	
Borer cleaning and sharpening set	
Sample Collection – Branches	
Climbing equipment and materials	
Pruning shears (2 or 3) for collecting samples	
Long pole pruner (~18m)	
Shears and saw	
Sample Storage	
Bucket*	
Plastic box (60cm × 40cm × 20cm)*	
Water	

Manual shears*	
Plastic labels	
Paper labels	
Indelible felt or pencil	
Zip lock bags	
Cotton balls	
Cellophane	
Plastic tubes	
Plastic tray	
Pen	
Note sheet (for data management)	
Laboratory Protocols	
Small tube (for rehydrating samples for 12 hours)	
Sopalin (absorbent paper)	
Shears	
Zip lock bags	
Alcohol	
Analytical balance (0.001g)	
Pressure chamber	
Gas cylinder	
Temperature and humidity sensor (e.g., Hobo ProV2)	
Fan	
Perforated bags (for dehydrating samples)	
Oven	
Razor blade	
Paper envelopes	
Desktop scanner (for LMA)	
Pencil	
Notebook (for data entry)	
Safety Equipment	
Toolbox	
First aid kit	

II. Appendix 2: Check-list for the organisation of the campaign with local partners

	GENERAL POINTS TO CONSIDER BEFORE SAMPLING				
	Country/ GCUs visited:	**To-do list before field campaign (column D and E)**			
		not concerned = "X"	yes	no	
			in progress		
			To be modified by:		
	TO CHECK	**MILESTONES**	**FIELD**	**PARTNER**	**COMMENTS**
1-	**Choice of GCU(s)**				
	Provide all GCUs shapefiles	*≈ Months-8*	X		Make sure that you have all the GCU shapefiles at hand. For the remote sensing and Functional Subset (FS) analyses, review the size, accessibility, and fragmentation of the GCUs.
	Confirm the pre-selected GCU with the local partner.	*≈ Months-6*			Contact the EUFGIS National Focal Points to confirm whether the selected GCU(s) are appropriate, based on their
2-	**Preliminary visit of GCU / information of forest stand**				**Check whether the selected GCUs are suitable.**
	Preliminary visit	*before field campaign*	X		Ensure the feasibility* of the GCU through a preliminary field visit and/or by contacting local forest managers. *Feasibility includes the location and boundaries of the GCU,
	Information on the forest stand:	*≈ Months-2*			Information to optimise the organisation of the fieldwork (people, equipment, etc.):
	Tree height		X		Will tree climbing be necessary to collect sun-exposed
	Understorey density		X		
	Site accessibility		X		**Important: The site must be accessible by car, as approximately 30 litres of water will need to be transported to the GCU for sample collection and preparation, unless water is available nearby (e.g. lake,**
	Date of last thinning		X		For the objectives of the Functional Subset, avoid sites that have undergone thinning in the past five years.
3-	**Planning and Organization**				**Information to optimise the organisation of the field**
	Propose an initial pre-schedule 3-4 months in advance	*≈ Months-4*		X	Prepare a provisional schedule for each National Focal Point to plan the week of measurements and sample collection with the local partner. Reserve 2 or more days for GCU visits, tree selection, and assistance with NIRS, microdensity and
	Confirm the schedule with all involved parties	*≈ Months-1*			Confirm the week of measurements and collection with the local partner, depending on phenology or specific constraints (e.g. COVID, forest manager availability).
5-	*Specify the meeting point or accommodation for the first day*	*≈ Week-2*			Provide a shapefile, waypoint via Google Maps, or address (either at the GCU or at the accommodation, e.g. hotel/guest
4-	**Permissions to enter the site (national rules,**				
	Invitation/letter from the EUFGIS National Focal Point	*≈ Months-1*	X		**Prepare a letter or document in the local language explaining that the mobile team is conducting a research project, including contact details (to show to authorities at**
	Permission to access to the GCU by car		X		if necessary
	Forest managers/ owners permissions		X		
	to collect samples				Make local arrangements to obtain sample collection permits, if necessary.
	to ship samples				Check the Nagoya protocol, particularly for non-EU countries.
5-	**Specific logistics issues**				
	Means of transport	*≈ Months-1*			Specify the mode of transport: by car (specify plate number), by train, by plane
	Materials	*≈ Months-1*			Indicate whether the materials will be transported by the mobile team or need to be provided by local partners due to
	big shoot				
	pole pruner				
	Sample shipment	*≈ Months-1*			Samples should be prepared in the field and stored in black bags on the same day of collection.
6-	**Phone contact list**				Include the phone contact details of each person involved in
	to be completed				
	...				
7-	**Other points**				
	Mosquito presence ?				
	...				

III. Appendix 3: Instructions for CBH measurement

Here are the step-by-step guidelines to assess the « CBH or DBH» (Circumference or Diameter at Breast Height) of trees using a girthing tape. Indeed, use of a girthing tape is preferred over the caliper for measurements on tree stems. A girthing tape measures circumference, integrating stem diameter in all radial directions and eliminating diameter variability caused by direction when the stem shape deviates from circularity.

> Determine the breast height as that at 1.30 m

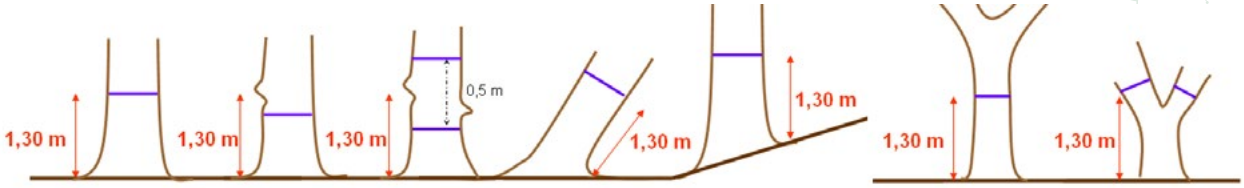
> Determine the ground level. This is the level where the stem touches the soil. The soil includes the upper layer but not

the woody debris that might accumulate above it over time. On sloping terrain, breast height is measured starting from the up-slope of the stem, i.e. the highest point at which the stem meets the ground.

> Determine the breast height as that at 1.30 m above the ground level, measured along the longitudinal axis of the stem.

> If breast height falls on a branch, a start of whorls or an uncharacteristic bulge close to 1.30 m, it's better to shift this level. Beyond 20 cm of offset, DBH should be measured above and below this point at equal distances from breast height and the average of the two measurements should be the one noted.

> If a tree forks below 1.30 m, it is considered as 2 (or more) separate trees. Each branch gets its own tree ID, but to indicate that they share the same root system, you provide them with the same stump ID. Measure DBH of each branch and note as separate trees. If a tree forks at or above 1.30 m, consider it as a single tree. If the crotch of the fork falls at 1.3 m, measure DBH just below the fork.



Niveau de mesure de la circonférence à 1,30 m - (1) un arbre vertical (2) un arbre avec un défaut à 1,30 m (3) un arbre avec défaut sur 50 cm autour de 1,30 m : 2 niveaux de mesure avec moyenne des 2 valeurs (4) un arbre penché (5) un arbre sur terrain pentu (6) arbre fourchu : fourche à plus de 1,30 m de haut (7) arbre fourchu : fourche à moins de 1,30 m de haut : 2 brins à mesurer

> If a tree has multiple suckers (e.g. in coppice) measure all of them at 1.30 meter and record that they are coming from the same stump and have the same root system.

Measurements

Material

> Girthing tape . The tape has one side normally graduated in circumference and the other one graduated for direct reading of the diameter (photo n°2). Usually, the thinnest graduation is in mm. An extra length at the end of the tape (beyond 0) makes it easier to handle and read (see photo n°2).

> 90° alcohol (or turpentine) for cleaning tape (from resin in particular).

Method

> Before measurement, remove ivy, moss, twigs or anything else that may skew the measurement.

> Place the girthing tape or measurement tape perpendicular to the central axis of the stem (photo n1, 3, 4) at the breast height level (see above).

> Make sure the tape is not kinked so as not to bias the measurement. For this, hold the case of the girthing tape with your left hand and wrap the trunck with your left arm, then wrap the trunk on the other side with your right arm and retrieve the end of the tape with your right hand. Then position your thumbs between the tape and the trunk to detect any irregularity on the trunk when unwinding the tape. Then, pull on the tape by bringing both hands towards you, without ever releasing the tape tension, to wrap the tree. The box and the end of the ribbon are then pulled back towards you. If necessary, another person could check the tape placement.

https://www.zimmersa.com/ruban-de-cubage/200-chevillere-de-cubage-3m-a-rappel-automatique.html

> Read circumference to the nearest mm directly from the girthing tape with a 1 mm accuracy. To do so, we have to superimposes the two ends of the tape to read the measurement (photo n°1) at the level of the zero line (photo n°2).

Photo n°1: positioning of fingers to tension the tape

Photo n°2: reading of the measurement (top photo: circumference side; bottom photo: diameter side)

Photo n°3: example of tape positioning error

Photo 4: Offset of the theoretical measurement level at 1.30 m due to branch insertion at 1.30 m (maximum offset +/- 20 cm around 1,30m.

(Possible) marking of the measuring level

If the tree is to be measured successively (every year for example), the chosen measurement level must be materialised (immediately after the measurement) by a line of paint all around the tree or only on part of it. During an inventory on a plot, it can be interesting to orient the marking to facilitate the verification of the trees measured by the field operators.

IV. Appendix 4: Bitterlich relascope instructions

In English: https://web5.silvanus.at/media/26/bb/c8/1695700772/Spiegel-Relaskop_1.%20Teil%20Englisch%20MS,CP.pdf

In French: Microsoft Word - DENDRO10-2013.doc (jymassenet-foret.fr)

https://www.onf.fr/outils/ressources/281fe1ac-ef4d-4a4f-adfd-16dd4af1cf33/++versions++/4/++paras++/2/++ass++/1/++i18n++data:fr?_=1544440664.955191&download=

V. Appendix 5: Instructions for Vertex Dendrometer and height measurements

The operation consists of measuring the total height of a tree with a “Vertex” dendrometer. This operating procedure is largely based on the forest technical note of Gembloux (D. Pawels, Faculté universitaire des sciences agronomiques, gembloux, Belgium).

Instructions Haglöf for Vertex Dendrometer

For more details, see the instructions below:

Vertex III et IV: https://share.google/o3Z33qvTxqJ4MFRMg

Vertex Laser Geo: https://www.forestry-suppliers.com/Documents/4504_msds.pdf

Positioning of the transponder on the tree and the operator

The height measured at the vertex corresponds to the distance separating the ground from the intersection between the operator’s line of sight and the vertical passing through the transponder. This remark helps to avoid errors when measuring broad-crown deciduous or leaning trees.

General precautions for transponder positioning

The transponder must be oriented towards the measurer and slightly inclined in steep terrain. As a rule, it must be positioned at a height of 1.30 m from ground level. This height is predefined: TRP Height. If for visibility problems, one wishes to fix the transponder at another level, it is necessary to change the preset height (TRP).

General precautions for positioning the measurer

To correctly determine the total height of a tree, the operator must locate himself:

- in a direction allowing good visibility of the top of the tree,
- at a distance slightly greater than the presumed height of the tree,
- in the case of a tilted tree, in a direction perpendicular to the plane of inclination of the tree,
- and in the case of steep terrain, preferably place yourself upstream from the tree or between the upstream and the contour line but in no case downstream from the tree (diagram 1).

Beware of lateral branches that hinder the aim of the tree crown; to avoid overestimating the measurement, it is necessary either to back up (flat ground) or to climb a little further upstream (steep ground) to better “overhang” the tree (see diagram 2).

Different cases encountered

Flat land, straight tree:

The transponder is fixed against the tree to be measured at a height of 1.3 m above ground level.

The measurer is positioned in a direction allowing the best visibility of the top of the tree and at a distance slightly greater than the presumed height of the tree.

Flat land, leaning tree:

The transponder is positioned at a height of 1.30 m above ground level. It must be above the tree crown and in the plane of greatest inclination of the tree.

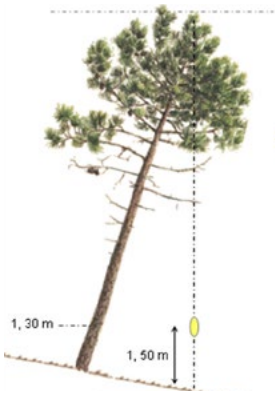
The measurer favours one of two directions perpendicular to the tree’s inclination plane allowing a good visibility of the top of the tree. It must be at a distance slightly greater than the presumed height of the tree.

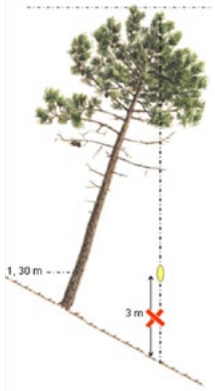
Sloping land, straight tree:

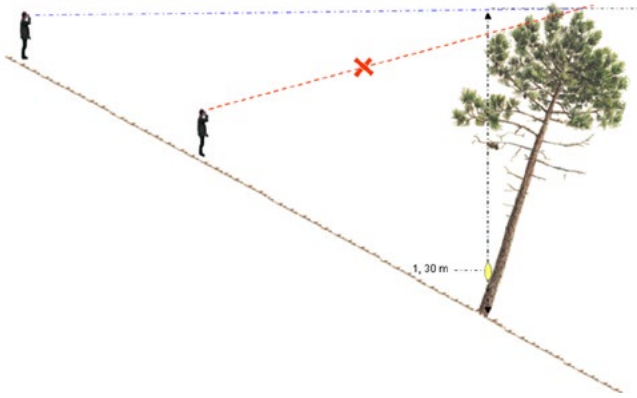
The transponder is fixed against the tree to be measured at a height of 1.3 m from ground level, upstream side.

The meter is preferably positioned upstream of the tree or between the upstream and the contour line but in no case downstream of the tree. It must be at a distance slightly greater than the presumed height of the tree.

Steep terrain, tilted tree:







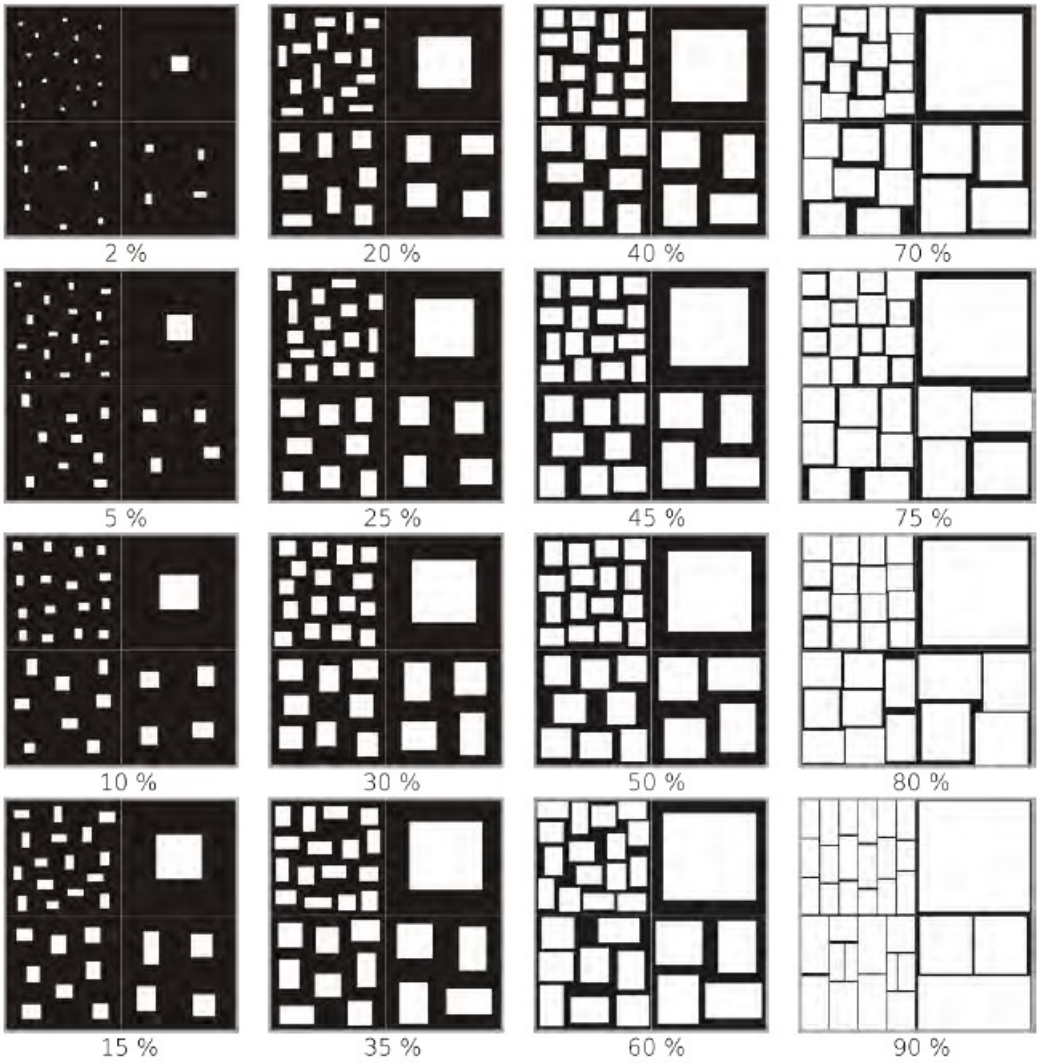
As far as possible:

The transponder is positioned above the tree crown, in the plane of the steepest inclination of the tree. It must be positioned at a level equivalent to that of the 1.30 m materialized on the trunk (Fig. on the left). In this case, the measurer must be pwositioned in a direction perpendicular to the inclination plane and it must be at a distance slightly greater than the presumed height of the tree.

Otherwise:

If the transponder cannot be positioned above the treetop due to too much elevation (fig. in the middle), the transponder must be positioned on the trunk at the 1.30 m level but in this case, the measurer has to position himself well upstream from the tree to ‘overhang’ the summit. It certainly is at a greater distance than the presumed tree height (fig. on the right; The measurer (red line) underestimates the tree’s height).

VI.Appendix 6: Soil descriptors: estimation of percentage of coarse elements, rock outcrop or coarse elements on surface



Charts for estimating percentages of coarse fragments, FAO (2006), Figure 5, modifiée par B. Repe. (Sources: World Reference Base for Soil Resources – International soil classification system for naming soils and creating legends for soil maps, the edition, 2022).

VII. Appendix 7: Dieback index

Thanks to Bernard Boutte (from DSF, Département de la Santé des Forêts, France) for his support.

General considerations

The dieback index is based on the density of branches and missing growth, and on the degree of defoliation. This defoliation percentage denotes the proportion of needle or leaf loss in comparison to a fully foliated reference tree. This assessment is done on the crown out of competition with the crowns of other trees.

We assess this defoliation of the target species following the scale below:

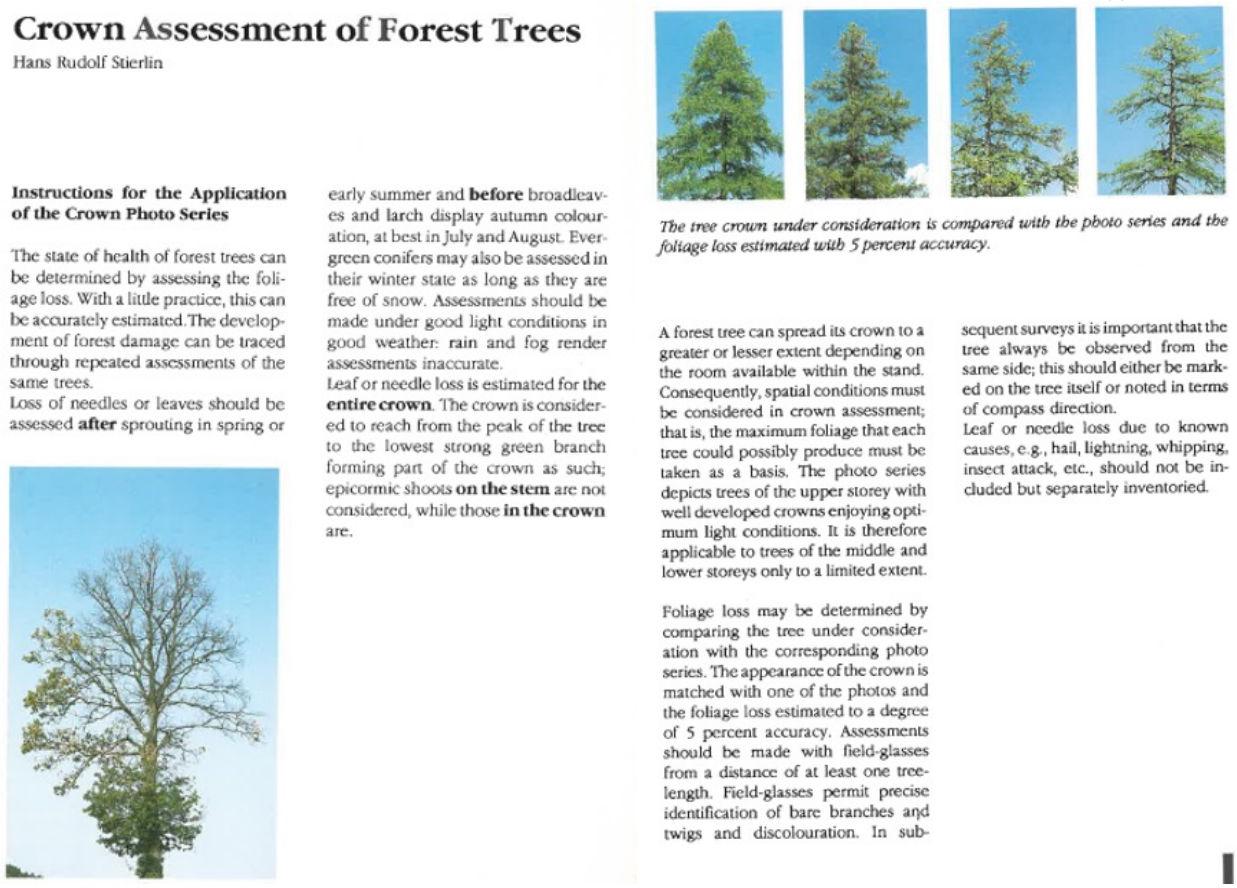
Class 0 [0-25%] of defoliation

Class 1]25%-50%] of defoliation

Class 2]50%-75%] of defoliation

Class 3]50%-75%] of defoliation

Take the opportunity of climbing the tree to take a look at the crown to confirm (or not) the dieback class assessed from the ground.



Crown Assessment of Forest Trees

Hans Rudolf Stierlin

Instructions for the Application of the Crown Photo Series

The state of health of forest trees can be determined by assessing the foliage loss. With a little practice, this can be accurately estimated. The development of forest damage can be traced through repeated assessments of the same trees.
Loss of needles or leaves should be assessed **after** sprouting in spring or early summer and **before** broadleaves and larch display autumn colouration, at best in July and August. Evergreen conifers may also be assessed in their winter state as long as they are free of snow. Assessments should be made under good light conditions in good weather: rain and fog render assessments inaccurate.
Leaf or needle loss is estimated for the **entire crown**. The crown is considered to reach from the peak of the tree to the lowest strong green branch forming part of the crown as such; epicormic shoots **on the stem** are not considered, while those **in the crown** are.

The tree crown under consideration is compared with the photo series and the foliage loss estimated with 5 percent accuracy.

A forest tree can spread its crown to a greater or lesser extent depending on the room available within the stand. Consequently, spatial conditions must be considered in crown assessment; that is, the maximum foliage that each tree could possibly produce must be taken as a basis. The photo series depicts trees of the upper storey with well developed crowns enjoying optimum light conditions. It is therefore applicable to trees of the middle and lower storeys only to a limited extent.

Foliage loss may be determined by comparing the tree under consideration with the corresponding photo series. The appearance of the crown is matched with one of the photos and the foliage loss estimated to a degree of 5 percent accuracy. Assessments should be made with field-glasses from a distance of at least one tree-length. Field-glasses permit precise identification of bare branches and twigs and discolouration. In subsequent surveys it is important that the tree always be observed from the same side; this should either be marked on the tree itself or noted in terms of compass direction.
Leaf or needle loss due to known causes, e.g., hail, lightning, whipping, insect attack, etc., should not be included but separately inventoried.

17

(Sources: WSL-FNP- Sanasilva - Tree crown photos – Peter Brassel)

Example: *Pinus pinaster*

Pinus pinaster Aiton 2.10

Photographs and Description:
Italy *(LINNÆA ambiente)*

Shape of the crown
Pyramidal, especially in young trees, or irregular (Fig. 1).

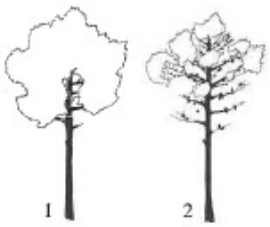
Type of transparency
Mainly starting from the bottom and moving upwards, or from the central section moving outwards (Fig. 2). In many cases only the upper section of the crown continues to be in good condition.

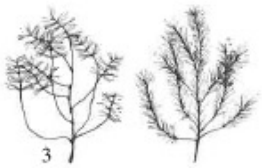
Elements which influence the transparency
Ramification alterations
These consist primarily in the destruction of branches and branchlets (Fig. 2); reduction (see *Pinus pinea*) is rare. No regeneration had been observed.

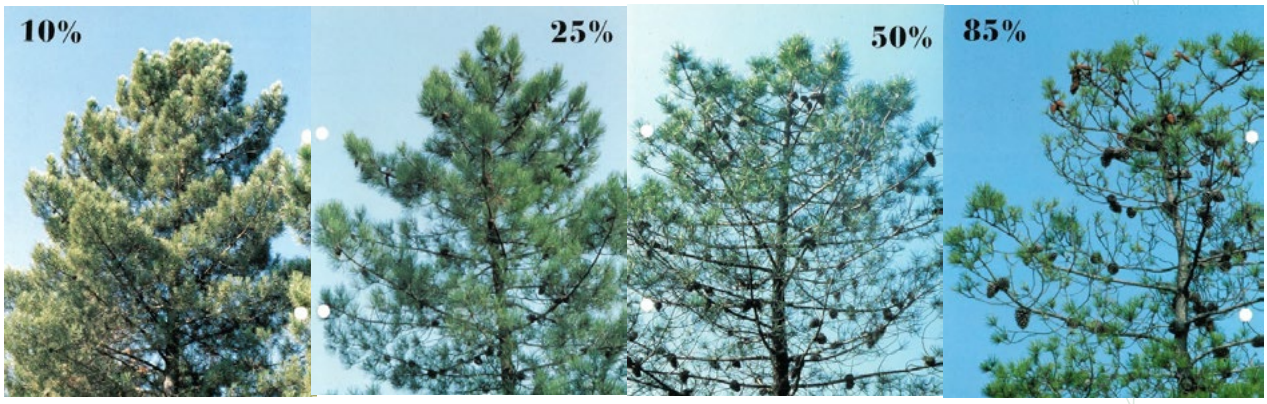
Needle retention
In healthy trees there should be three (four) needle years present. The number of needle years can be greater when there is a flowering effect. In cases of transparency starting from the central section of the crown the number of needle years present will be lower throughout the crown; in cases of transparency starting from the bottom, the upper section may still have a normal number of needle years.

Flowering effect
Frequent; may affect the entire crown in certain trees (Fig. 3, comparison between branches with and without flowering effect).

Microphyllia
Frequent, although less so than in *Pinus pinea* (Fig. 4, comparison between branches with and without microphyllia).

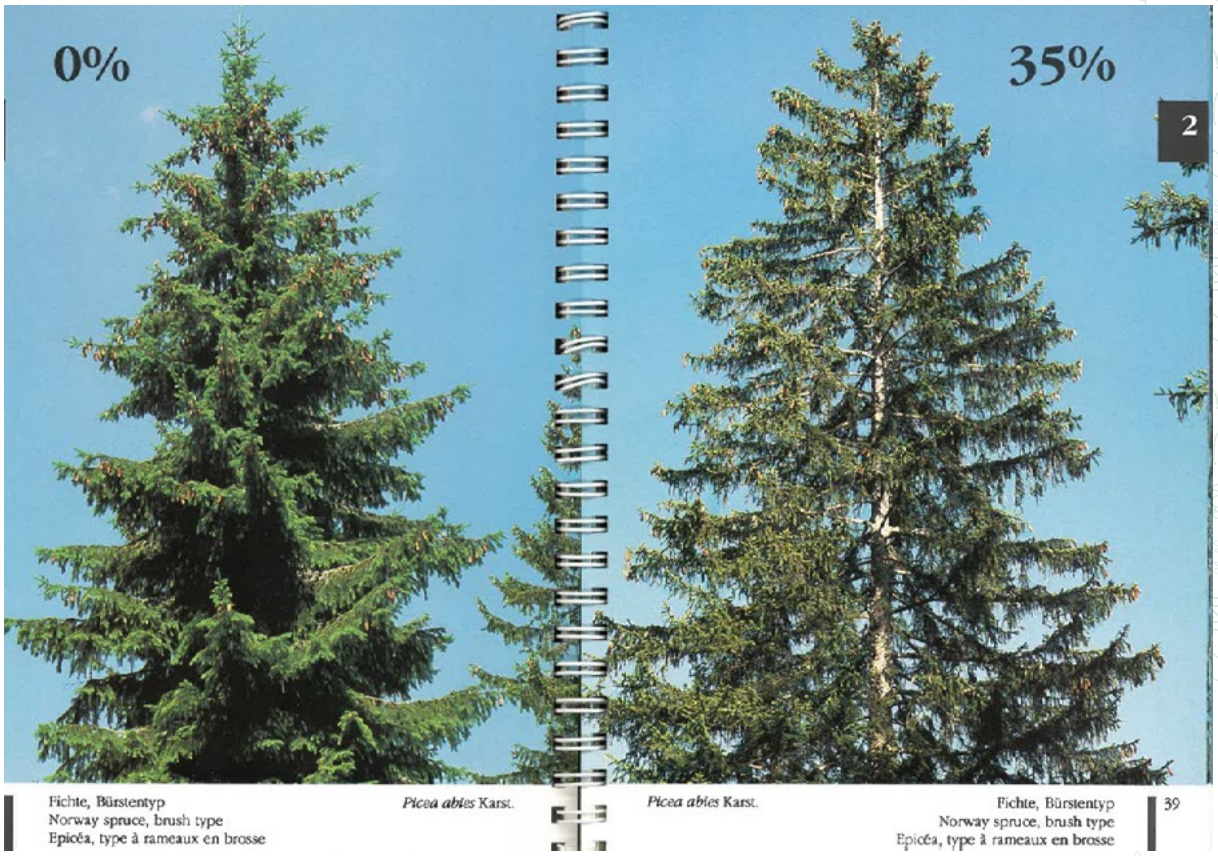






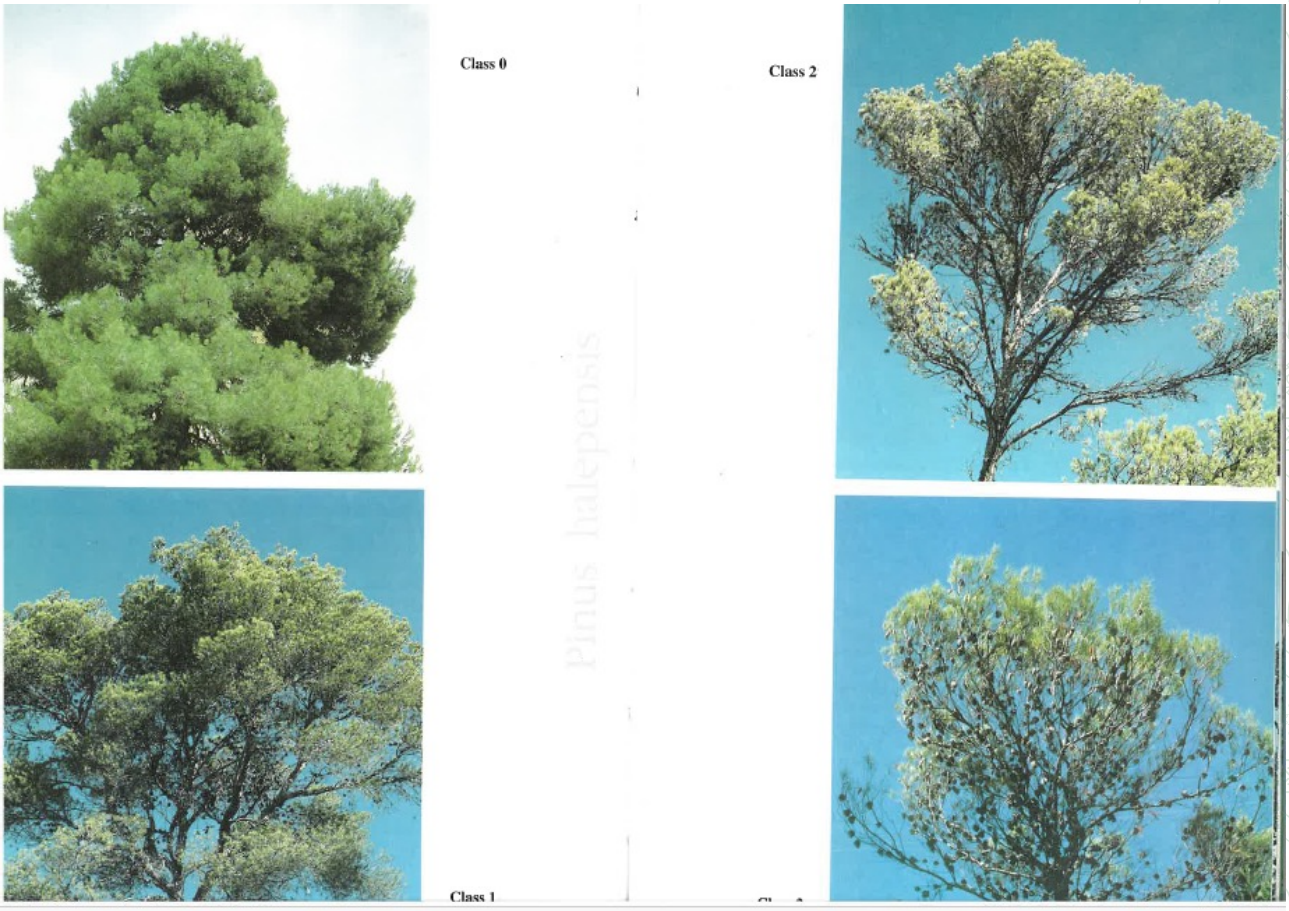
Example: *Fagus sylvatica*

Example: *Picea abies*



Example: *Quercus robur*

Example: *Pinus halepensis*



Class 0 [0-25%] of defoliation

Class 1]25%-50%] of defoliation

Class 2]50%-75%] of defoliation

Class 3]50%-75%] of defoliation

Example: *Pinus pinea*

Class 0 [0-25%] of defoliation

Class 1]25%-50%] of defoliation

Class 2]50%-75%] of defoliation

Class 3]50%-75%] of defoliation

Example: *Pinus sylvestris*

VIII. Appendix 8: Materials and methods for tree ring core collection

The operation consists of collecting wood samples (called a 'wood core') using a Pressler increment borer (manual or motorised). Core samples are generally taken at breast height (1.30 m) for radial growth analysis, as this is a reference height in forestry and the effect of the root system is reduced. This technique has the advantage of quickly acquiring a large amount of growth data without being destructive.

Preliminary precautions

Remember to check beforehand that the manager has given their approval for this wood core collection.

Materials

- Manual increment borer
- Electric drill adapted for increment borers*
- Battery for electric drill for increment borer**
- PVC cylinder tube and core holders (e.g., paper straws)
- Paper bags and polycarbonate multiwall panels for storage
- Waterproof pencil
- Masking tape
- Graduated ruler
- Field-sampling sheets
- Borer cleaning and sharpening kit

*Increment borers by Haglöf Sweden ® . https://haglofsweden.com/project/increment-borers/ Borer bit of 5.15 mm inside diameter are preferred. This core diameter is recommended to facilitate future work on these cores for microdensity analyses. Length of the borer depends on trunk diameter.

** Electric drill/driver is recommended (18 V, with a torque of at least 140 Nm, no impact mode—e.g., Milwaukee M18 FDD3 with 5 Ah or 9 Ah batteries) to reduce physical effort.

Haglöf increment borers are 2- or 3 threaded.

- 2-threaded borers are recommended when drilling in hardwood, with a slower penetration in the wood and less friction and stress onto the material.
- 3-threaded borers will penetrate the wood faster and is suitable for most types of wood.

Sources: https://haglofsweden.com/project/increment-borers/

Methods

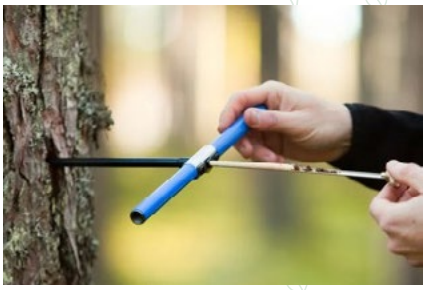
Step 1: Assembling the equipment

- The length of the borer depends on the diameter of the trunk.
- Unscrew the extractor.
- Insert the square end of the bit into the centre of the handle (ensure correct orientation).
- Lock the bit using the small pin on the handle.
- The increment borer is now ready for use.

Step 2: Locating the sampling point

Identify the exact sampling location. Avoid visible or likely defects (branches, frost cracks, wound of the crust, etc.) and reaction wood (compression or tension). On steep slopes, the core must be taken parallel to the contour lines to avoid reaction wood.

Step 3: Core extraction



Sources: https://haglofsweden.com/project/increment-borers/

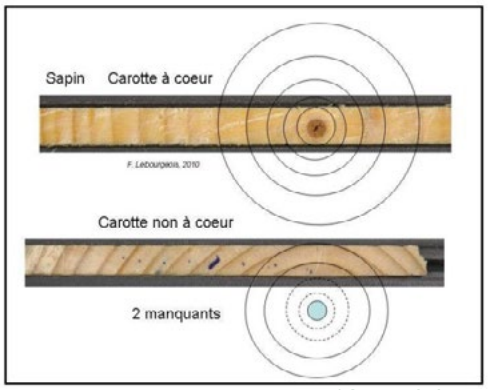
- Position the barrel shaped tip of the borer at the chosen location.
- The borer bit must be perpendicular to the stem axis.
- In this horizontal plane, choose the correct direction to aim to the pith (which may be off-centre). Branch insertion patterns may provide clues.
- Apply strong forward pressure to the stem in a straight 90° angle while simultaneously screwing the bit until the threaded borer engage through the bark and anchor firmly in the wood.
- It is difficult but essential to maintain the correct axis without tilting up or down to reduce breakage on the wood core.
- When the borer has penetrated the tree stem, you can stop putting pressure. Simply turn the handle in full circles until the two-thirds of the trunk diameter is reached. Then, rotate the handle to a horizontal position.
- Insert the extractor into the borer bit, sliding it under the wood core while keeping it strictly horizontal.
- Press the extractor inside the borer core tube applying appropriate force depending on wood hardness. Be careful to support the outer end of the extractor to avoid bending it (common among beginners).
- Then, turn the borer handle with the extractor one turn counterclockwise to detach the wood core. A full turn preserves the original orientation of the core, which may be useful if the pith has not been reached.
- Carefully pull the extractor out, with the core securely kept in place by the toothed end of the extractor. Support the core by placing a hand beneath the extractor.

- Ideally, the core should be in a single piece, including bark and pith. If the core breaks into more than two pieces, discard it and sample again at another location.
- If the bark separates from the core, mark a cross at the bark end to indicate that no rings were lost.
- If the core is broken in two pieces, please mark the core on either side of the two ends to make it easier to join.

The end of the borehole may be plugged with a wooden dowel (beech dowel) regarding the forest managers'requests.

Step 3 bis: If the pith is not reached

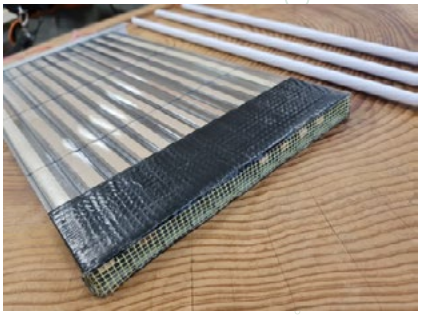
It is common not to reach the pith, usually because of its eccentricity.



© F. Lebourgeois - INRAE

- Observe the core, holding it in the same orientation as it stood in the tree to determine on which side the pith lies.
- The curvature of the rings indicates the direction and distance to the pith. Visualize a full circle based on the innermost visible rings.
- Then, insert the extractor or a straight tool (e.g., a pencil) into the first hole to identify its axis.
- Take a new wood core parallel to the first one, offset from the vertical axis by the previously determined distance according to the curvature of the rings. If this distance is less than 2–3 cm, shift at least 2–3 cm above or below the first attempt to avoid drilling into the same hole.
- A maximum of three core collection may be made to avoid excessive damage to the tree.

Step 4: Core storage and identification



- Carefully detach the core from the toothed end of the extractor with a slight rotation.
- Store the cores for micro-density, radial increments and age assessments in a clear polycarbonate multiwall panels holding up to 20 cores (several air inlets are made to ventilate and prevent fungal growth) and store the core for isotope analysis in paper containers individually.
- Label each core on its storage support (paper bags or polycarbonate multiwall panels) using water-proof pencil or permanent marker.
- Mark each with EUFGIS code_tree code.
- Make an inventory to ensure that all required cores have been sampled.
- In the field, close the end of the polycarbonate multiwall panels immediately after storing all the samples.
- Handle and transport the polycarbonate panels or the paper containers in safety place during the transport
- Store these samples in dried conditions.

Regularly check core storage conditions. Mold must be avoided. If needed, the polycarbonate panels may be opened carefully to prevent condensation and promote drying.

Step 5: Measurement

Measure bark, sapwood and total lenght of each core. The transition between sapwood and hardwood is recognizable by colour difference or by a difference in core wetness or by a difference in light transmittance. In cases where this is impossible, note NA in the datafile.

Step 7: Borer maintenance

- Materials: Borer cleaning kit including a cleaning rod designed for .22 calibre rifles
- Clean the equipment (the borer bit and extractor) with 90° alcohol to remove resin in particular,
- And clean the borer bit with the cleaning rod that holds a small cotton pad soaked in WD-40 or equivalent spray lubricant and rust preventative) and lightly lubricate the outside of the entire bit and extractor.
- Store in a dry place.

For more details on borers, their handling and maintenance, read the attached Grissino-Mayer (2003) and/or product sheet from the Haglöf Increment Borers (Haglöf Increment Borers Product sheet ENU.pdf).

References: Grissino-Meyer, H. 2003. A manual and tutorial for the proper use of an increment borer. Tree-ring research: 59(2), 63-79.

IX. Appendix 9: PAI section: what is a good picture?

Diffuse light is ideal to get a good hemispherical picture. The best diffuse lighting conditions occur on entirely overcast days (grey skies). When overcast days are not available, we have to take pictures on sunrise or sunset days.

During these times, conditions change rapidly and sensitivity and exposure may have to be changed by the operator.

Rain, strong wind, snow and foggy conditions within the canopy must be avoided.

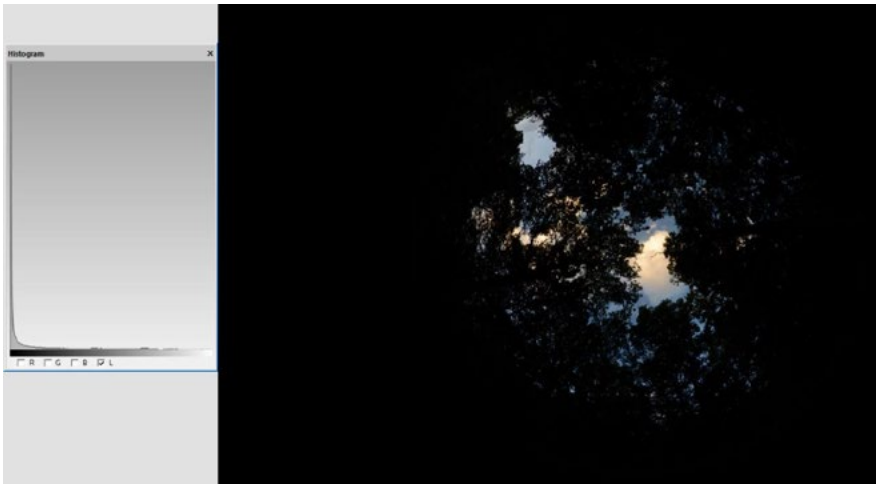


Fig1: DHP examples of different lighting conditions. Unsuitable sky condition: There is no overexposure, but the light is not diffuse and the sky is not completely overcast. The contrast between the trees and the blue sky will be different compared to the white cloud. In addition, some parts of the clouds reflect the sunlight creating an orange glow (from ICOS, Ancillary Data Forest,

Version 20200330). Fig2: DHP examples of different lighting conditions. Unsuitable sky condition: There is no overexposure and the light is fairly diffuse (notice the even distribution in the light histogram), yet the contrast between the trees and the blue sky will still be different compared to the white cloud (from ICOS, Ancillary Data Forest, Version 20200330).

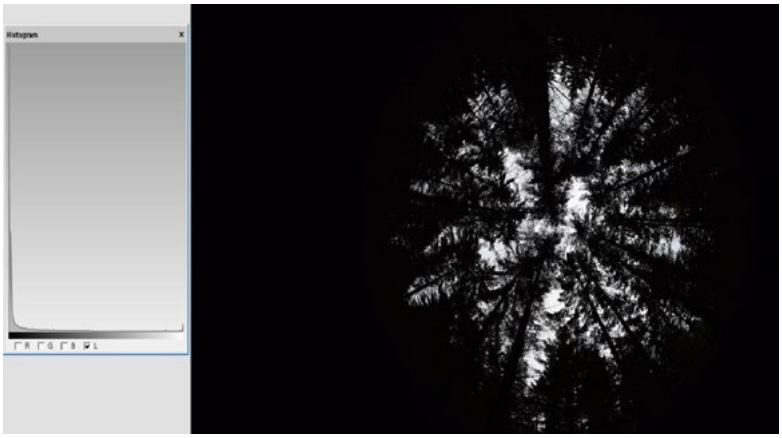


Fig3: DHP examples of different lighting conditions. Suitable sky condition with incorrect camera setting: There is an entirely grey/white, yet probably very thin cloud cover. With insufficient shutter speed, this creates overexposure (notice the small peak in the histogram on the right side), which will discard the picture for analysis (from ICOS, Ancillary Data Forest, Version 20200330).

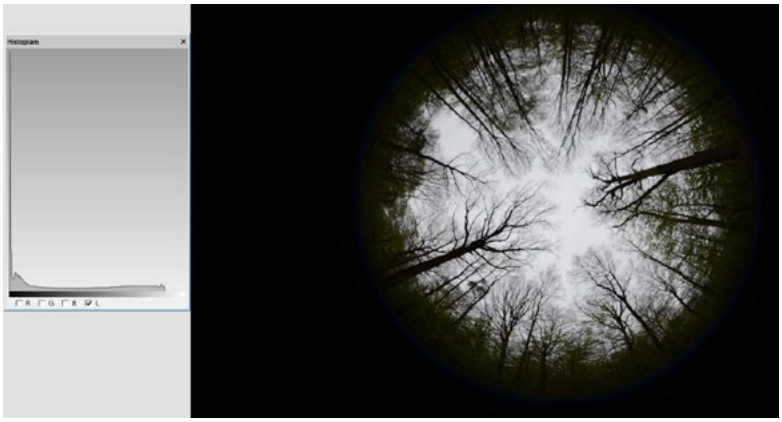


Fig 4: DHP examples of different lighting conditions. Suitable sky condition with correct camera settings: This is a perfect example (from ICOS, Ancillary Data Forest, Version 20200330).

Fig 5: Here is a photography (bad) of the screen of a Canon reflex. Showed black zones were in fact twinkling pixels indicating beams of light. Note the right part of the histogram: too much bright pixels indicating overexposure. Shutter speed is too low.

A picture can be overexposed in one part and underexposed in another; however the absence of overexposure must be favoured.

RAW THERAPEE is a free program that can help in analysing Digital Hemispherical Pictures.

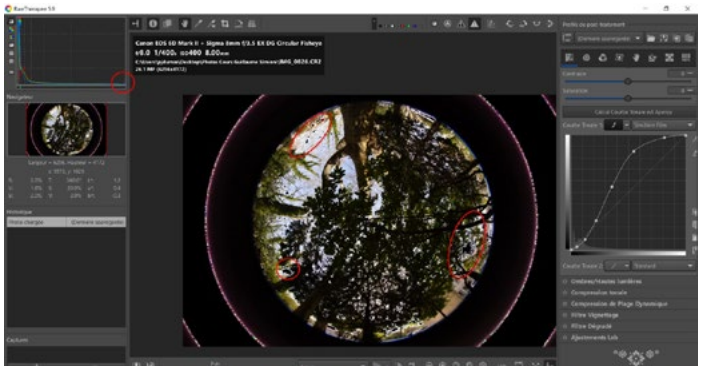


Fig 6: Overexposed picture. Next trial with higher shutter speed.

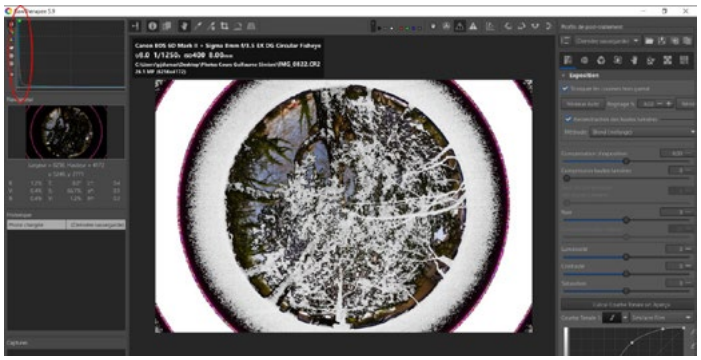


Fig 7: Underexposed picture. See the histogram completely left skewed and the diagnostic tool in the application. No good compromise was found in these conditions, neither (1/800; overexposed and underexposed, nor 1/1000; underexposed). The sun is high in the sky behind the branches. In such case absence of overexposure must be favoured (1/1000 or 1/1250).

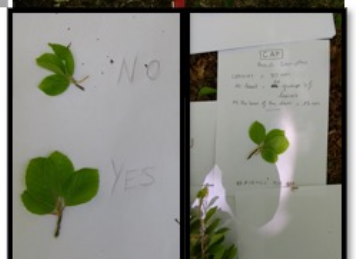
X. Appendix 10: Biological material samples

An illustration of the biological sample collected for each target species

Fig 7: Underexposed picture. See the histogram completely left skewed and the diagnostic tool in the application. No good compromise was found in these conditions, neither (1/800; overexposed and underexposed, nor 1/1000; underexposed). The sun is high in the sky behind the branches. In such case absence of overexposure must be favoured (1/1000 or 1/1250).

EXAMPLE FOR *FAGUS SYLVATICA*

HV	CAPacitance	Gmin *(Drought box)*	CV / NIRS (segment)



EXAMPLE FOR *PINUS SYLVESTRIS*

CAPacitance	Gmin *(Drought box)*	CV (+ NIRS at the base)	HV

EXAMPLE FOR *QUERCUS ROBUR*

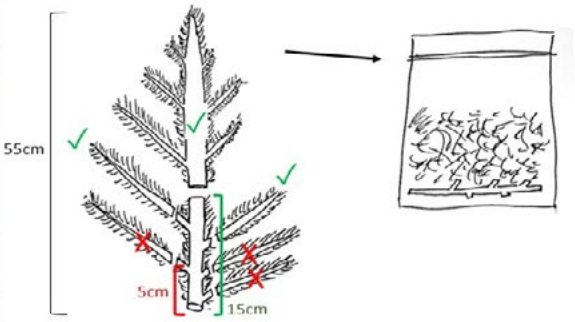
CAP	HV	Gmin
		

PV →

CV	To avoid: big ramification
	

EXAMPLE FOR *PICEA ABIES*

CAP	HV

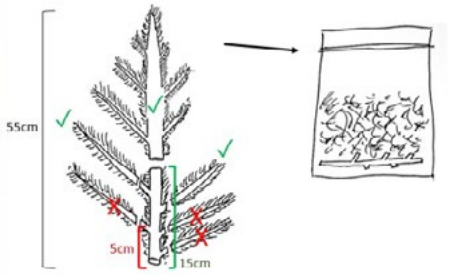


PV →

Gmin	CV

EXAMPLE FOR *ABIES ALBA*

CAP	HV
	



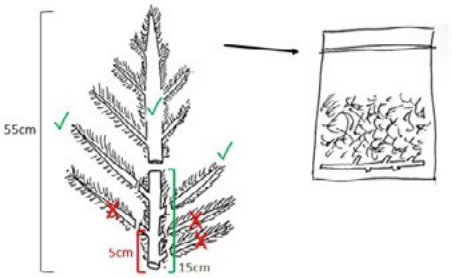
Gmin	CV	PV
		

EXAMPLE FOR *PINUS HALEPENSIS*

CAP	HV

20 twigs per branches

HV Sample similar as CAP sample



Male or female cones:
to avoid on all samples

Gmin	CV

XI. Appendix 11: Methods for the measurement of the crown basal height

The crown basal height (CBH) is defined as the vertical distance between the ground and the base of the live crown i.e the main “functional” green crown envelope and not the insertion base of the lowest green branches. This phenotypic trait is essential to assess the photosynthetic capacity of the tree. It is measured using a dendrometer as Vertex according to the materials and methods described in the appendix 5.

In practice and to ensure the homogeneity of the measurements, we have decided to objectify our measurement criteria. This protocol is based on an initial document from P. Dreyfus, INRAE, URFM, Avignon.

6. Methods for conifers

3 criteria are used and must be combined: (1) sanitary one (2) continuity criteria (3) dissymetry. Indeed, the assessement of the basal crown must consider the healthy status of the tree, the continuity of the branches along the trunk and the dissymmetry of the basal crown envelope.

1) Sanitary: Regarding this first criteriion, the basal crown height corresponds to the height of the base of the representative branches of the green and healthy crown envelope. If the tree is declining (dieback noted 2), the level of the branches retained may not be completely healthy.

2) Contiguous: we have to consider the discontinuity (or not) between the lower branches (potentially candidate for the base of the crown) and the upper ones.

- In the case of young forest stands, the definition we usually use for the base of the crown is as follows: height of the completely green whorl (without taking into account any “small” dry branches) located lowest in the tree but contiguous with the rest of the crown, i.e. not separated by a completely dead whorl.

- In taller, older forest stands, it is not easy to distinguish the whorls, in height and at a great distance: the previous definition is then adapted by retaining the height at which the crown is green on all sides and excluding a level of branches that would be “clearly” separated from the rest of the crown (the distance separating this branch from those above must not exceed half the length of the longest branch). If not, we give up the candidate branch and continue climbing, this time considering the branch above the discontinuity (see photo opposite of Picea abies).

3) Dissymmetry: In the case of a marked dissymmetry of the crown between upstream and downstream of a tree (particularly on sloping land, in windy conditions, or because of the tree competition between trees), the level of measurement retained is an average of these respective dissymmetric heights.

7. Methods for broadleaves

For the basal crown assessment on broadleaves, the same criteria as above are retained but should be completed by a new one taking into account the inclination of the potentially candidate branch.

1) Sanitary criteria: Measure the height of the base of the representative branches of the crown in general green crown envelope. If the tree is declining (dieback noted 2), the level of the branches retained may not be completely healthy.

2) Non-discontinuity criteria: 2 branches or series of branches are continuous if the distance separating them does not exceed half the length of the shortest of the two branches in the area. This limit makes it possible to exclude the

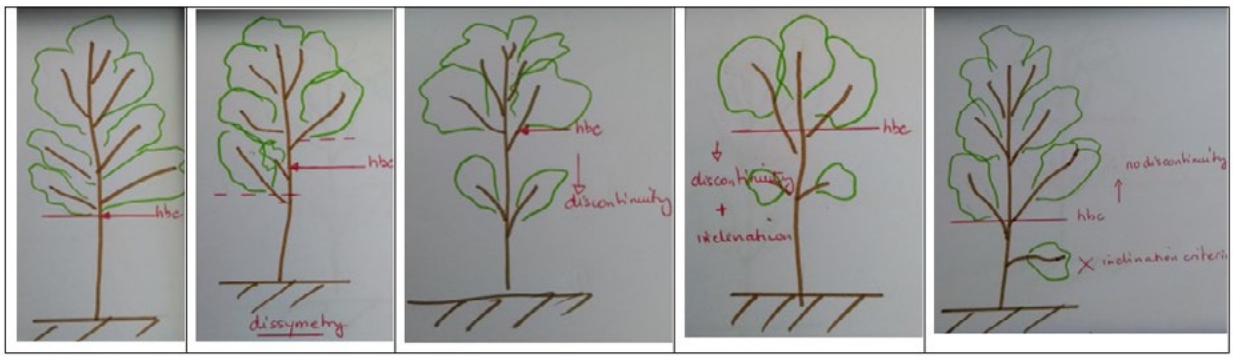
series of small branches inserted at the bottom of the trunk and which do not correspond to the base of the crown (whether from a “forestry” point of view or by considering the part of the foliage which seems a priori likely to contribute significantly to the growth of the tree, and although this is quite subjective).

3) Inclination criteria: a branch that is almost horizontal (or even slightly descending) is not taken into account as defining the base of the crown if all the branches above it shows a clear tendency to grow upwards (indicating that they belong “actively” to the crown). However, in certain conditions or for certain individuals, the tree may have a crown where all the branches are almost horizontal; in such cases, this 3rd criteria is not considered.

4) In the case of a marked dissymmetry of the crown (between upstream and downstream of a tree, or because of the competition of a tree), the level of measurement retained is an average of these respective dissymmetric heights.

In the case of very long branches with green leaves only at the end of the branch, the level of measurement retained is not the level of insertion of the branch but the level of the location of the needles/green leaves located on these same branches, thus defining the lower envelope of the “functional” green crown.

Example for *Quercus robur*



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