Samuel Bouchut
PhD student
under the direction of Fabio Gennaretti

Dendroecology course

Quantitative Wood Anatomy

UQAT

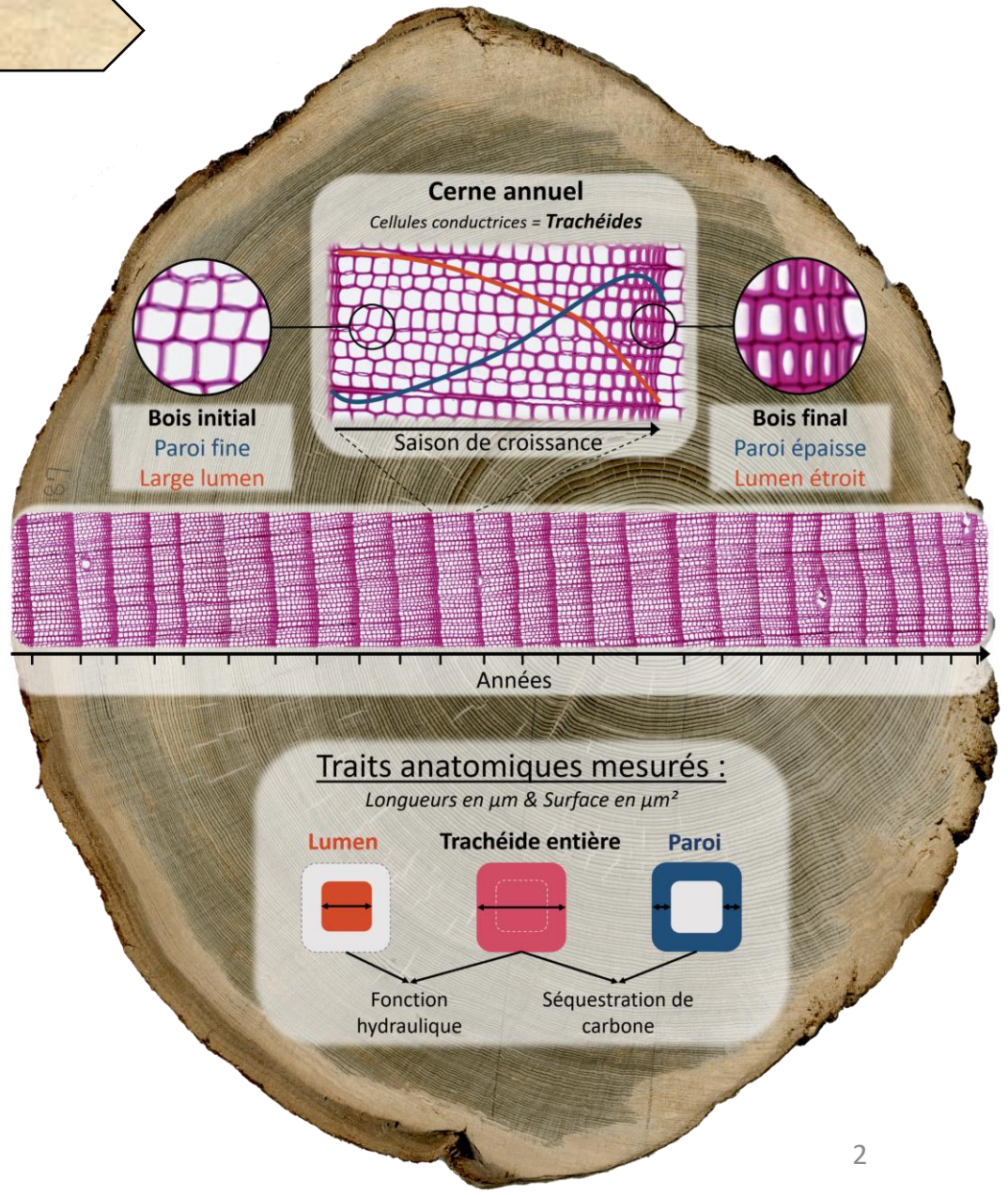
GREMA

Chaire Dendro-eco

Quantitative Wood Anatomy

Anatomical traits

- QWA = “numeric analysis of xylem anatomical traits (WAT) of plants and their relationship to plant functioning, growth, environment, wood quality and species identification” (Von Arx, 2021)
- **Fine temporal resolution :** Inter-annual to subseasonal
- Large number of measurable traits
- Each trait is specifically influenced by the environment
- Direct **Structure-Function** relationships

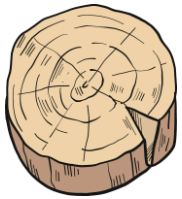


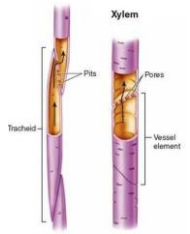
Quantitative Wood Anatomy

Anatomical traits

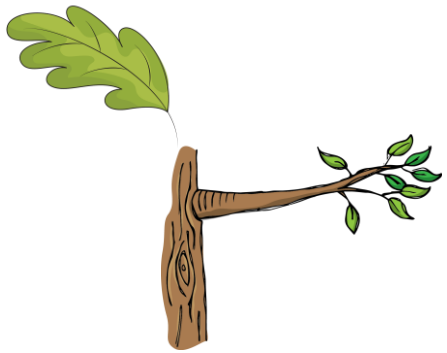
- All **measurements** of :
  - dimensions
  - quantities
  - proportions
  - absence/presence of a structure

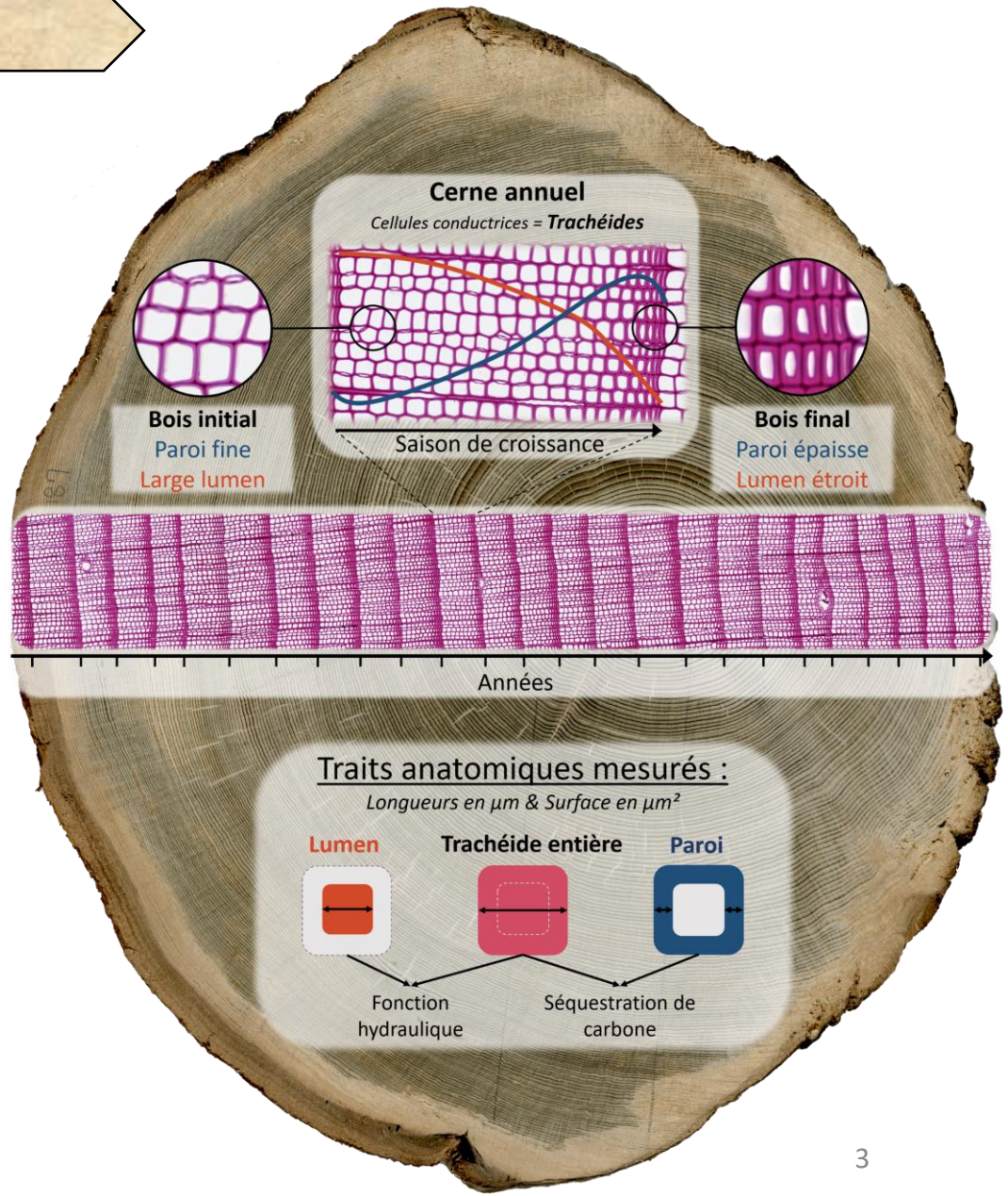
- At the **scale** of :
  - Organs
  - Growth rings
  - Constitutive cells





- Xylem is present in :
  - Roots
  - Stems
  - Branches
  - Leaf veins (primary xylem)



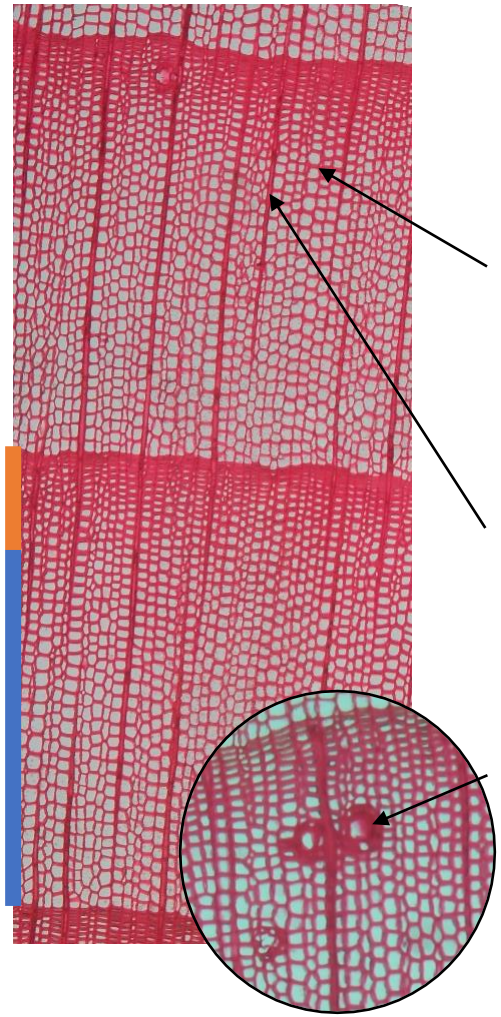


Quantitative Wood Anatomy

Structure/Function relationships

Wood anatomical traits as indicators of functional properties

Picea mariana



Conifers

Tracheids
Water transport
Mechanical support
Carbon sequestration

Rays
Storage
Radial transport

Resin ducts
Defense

Latewood
Earlywood

Quercus acutissima

Angiosperms

Vessels
Water transport

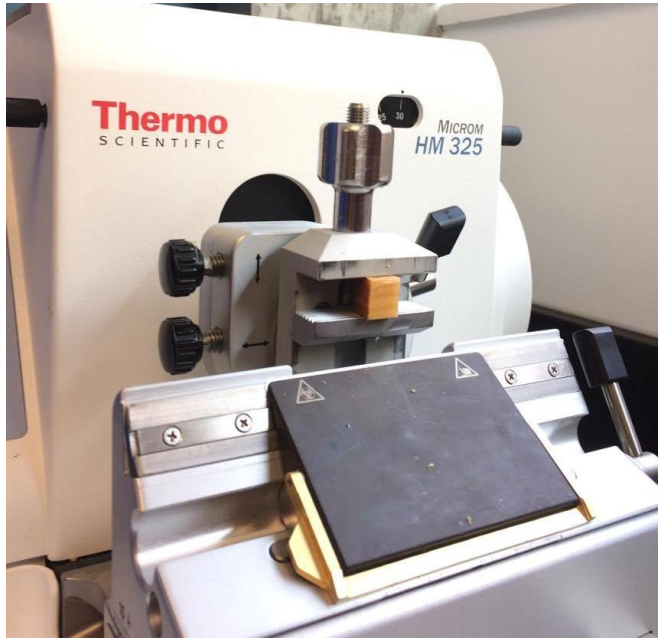
Fibers
Mechanical support
Carbon sequestration

Axial parenchyma
Storage

Rays
Storage
Radial transport

Quantitative Wood Anatomy

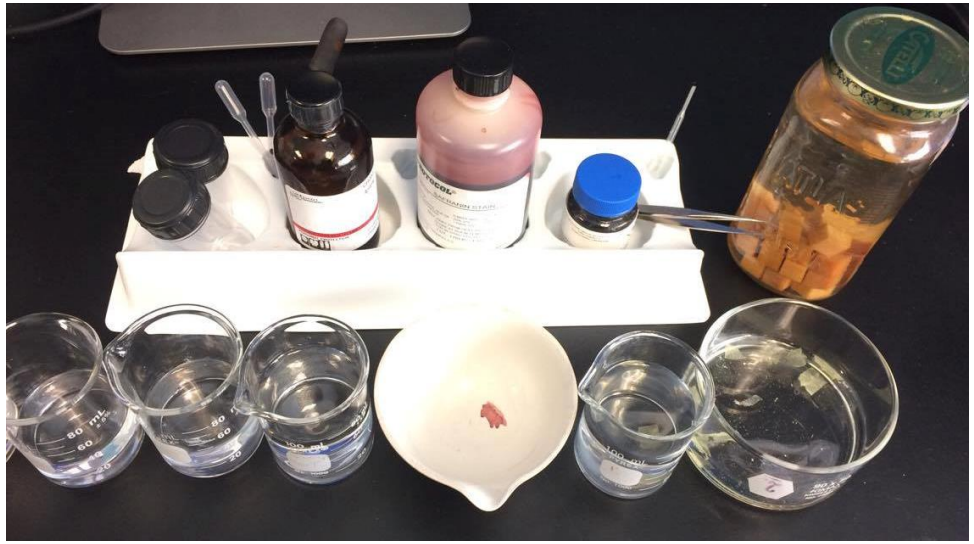
Lab methods



Step 1 : Cutting with a Microtome → thin sections of 6 to 10 µm

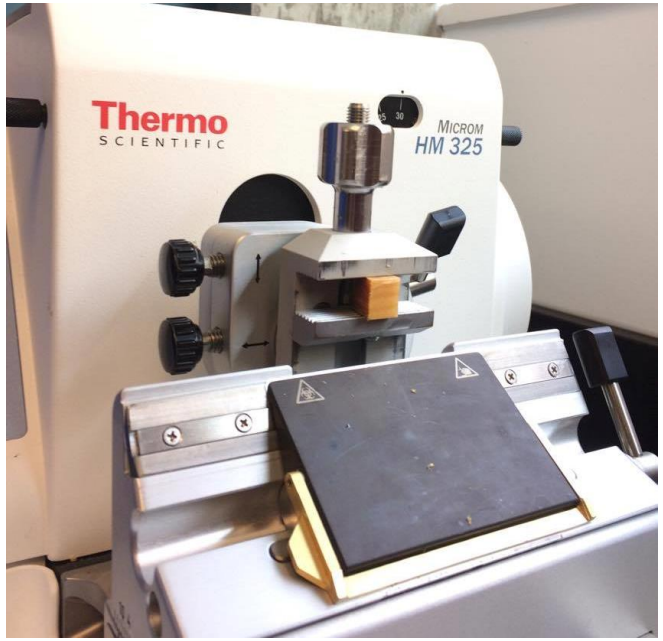
Step 2 : → Dehydration with increasing concentrations of alcohol,
→ staining to increase contrast,
→ mounting of permanent slides

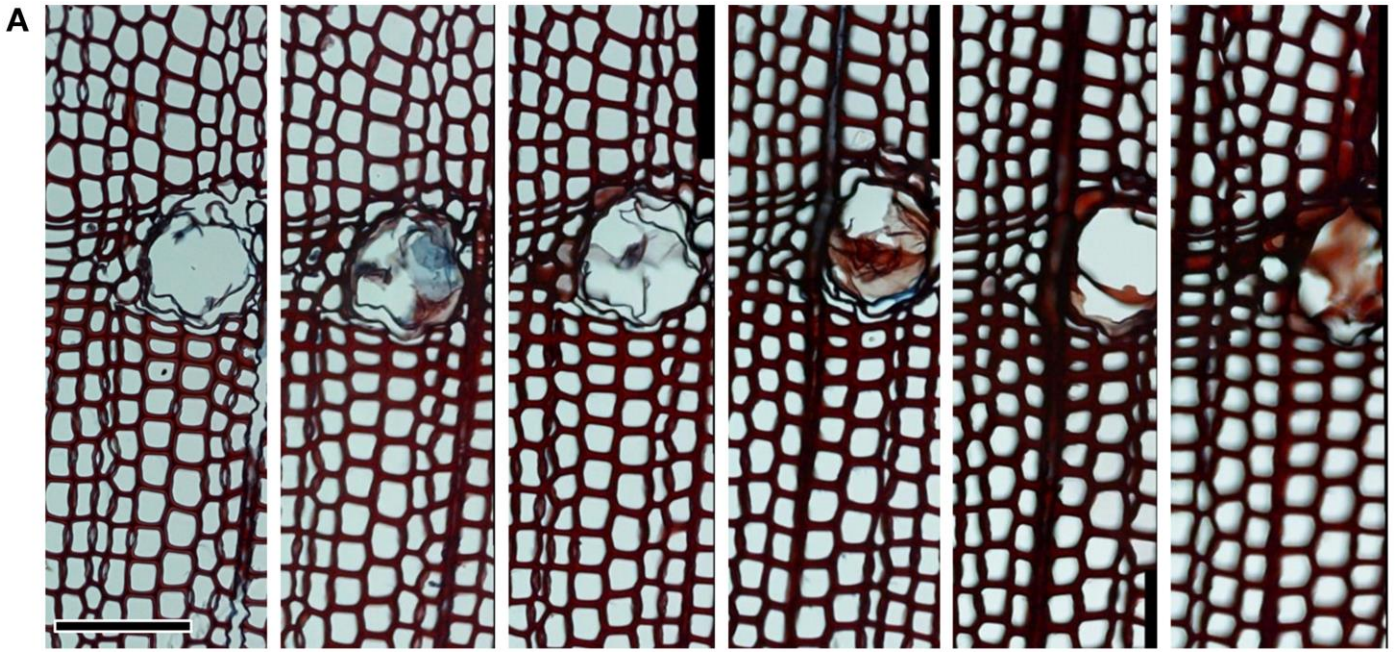
Step 3 : Image Numerization
→ Slide scanner
→ Camera with microscope +image stitching

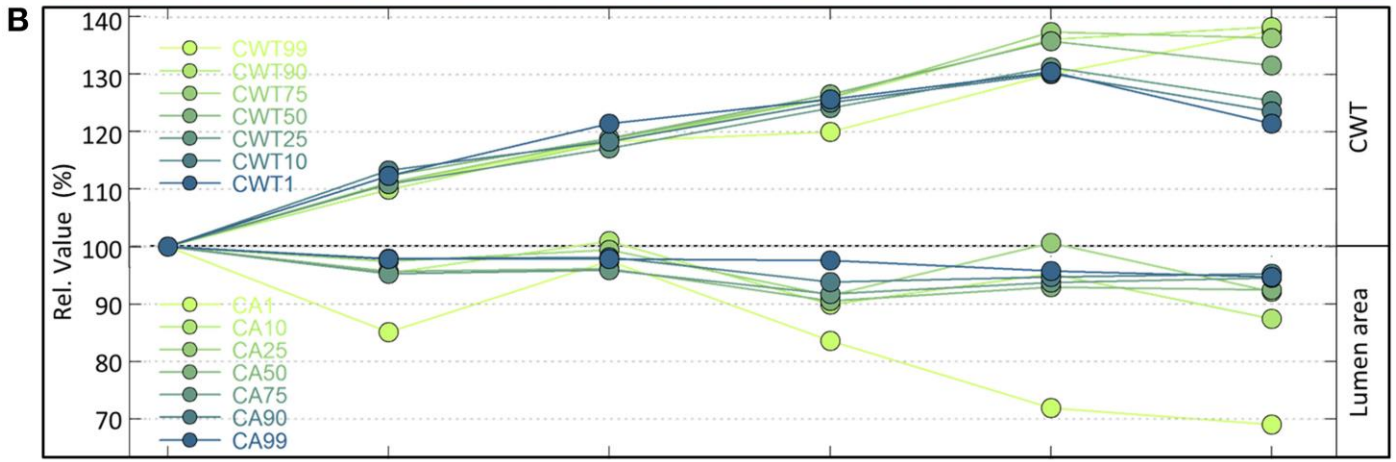


Quantitative Wood Anatomy

Lab methods







Step 1 : Cutting with a Microtome

Sources of bias :

→ Section thickness

→ Sample orientation

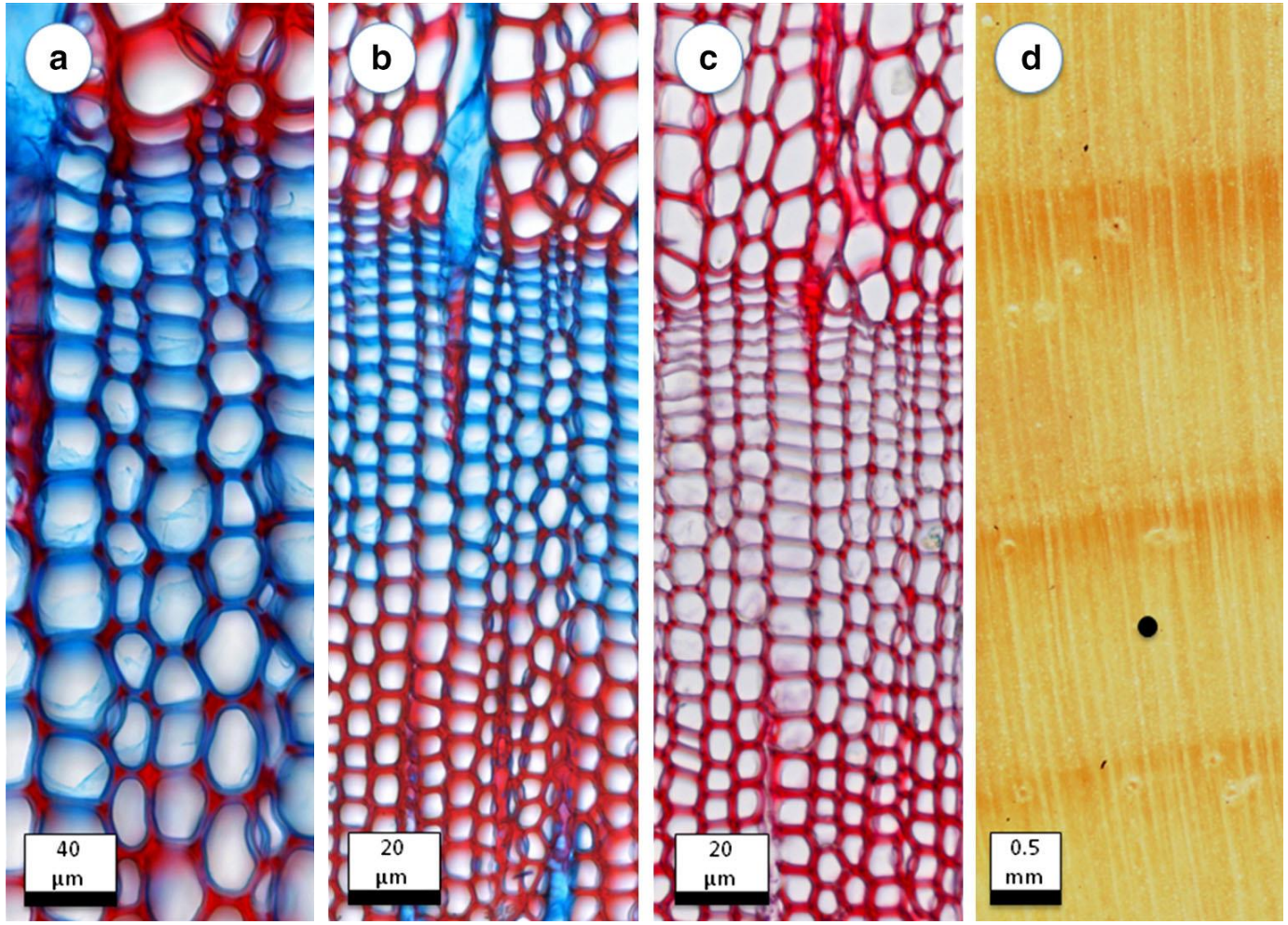
→ Blade quality

Von Arx, 2016

Quantitative Wood Anatomy

Lab methods

Staining



Piermattei, 2015

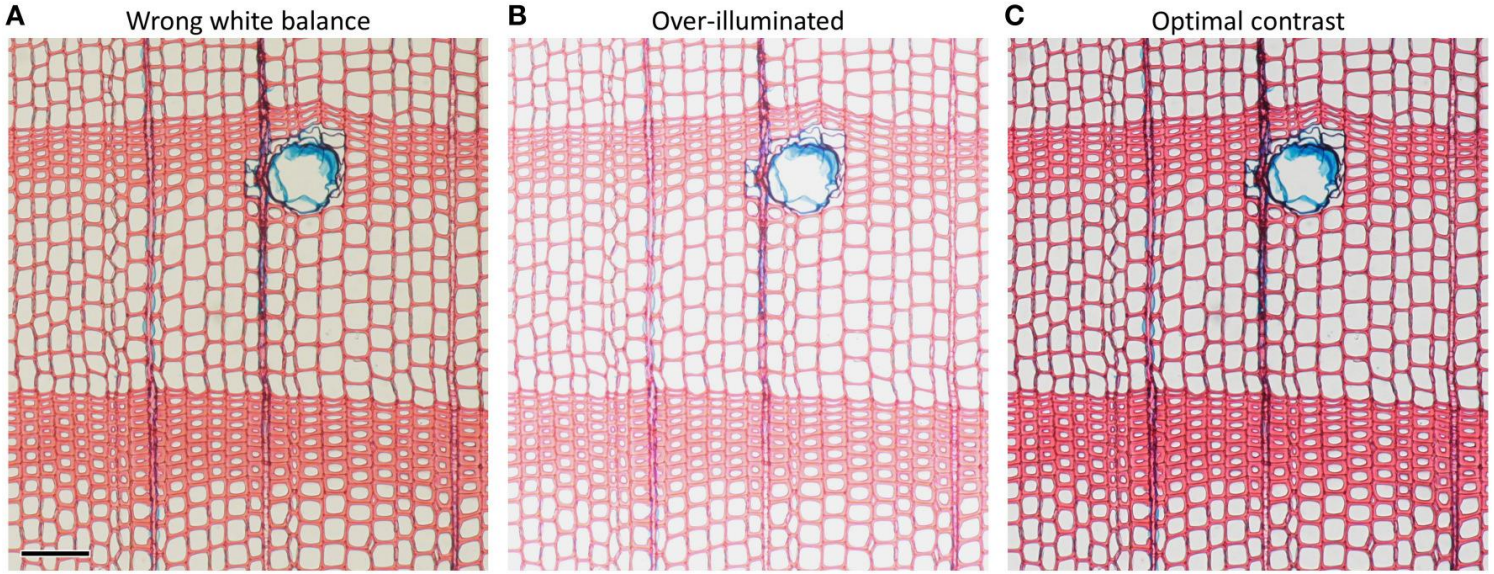
a – b) Double staining with **Safranin + Astrablue**
→ non-lignified cells are stained in blue

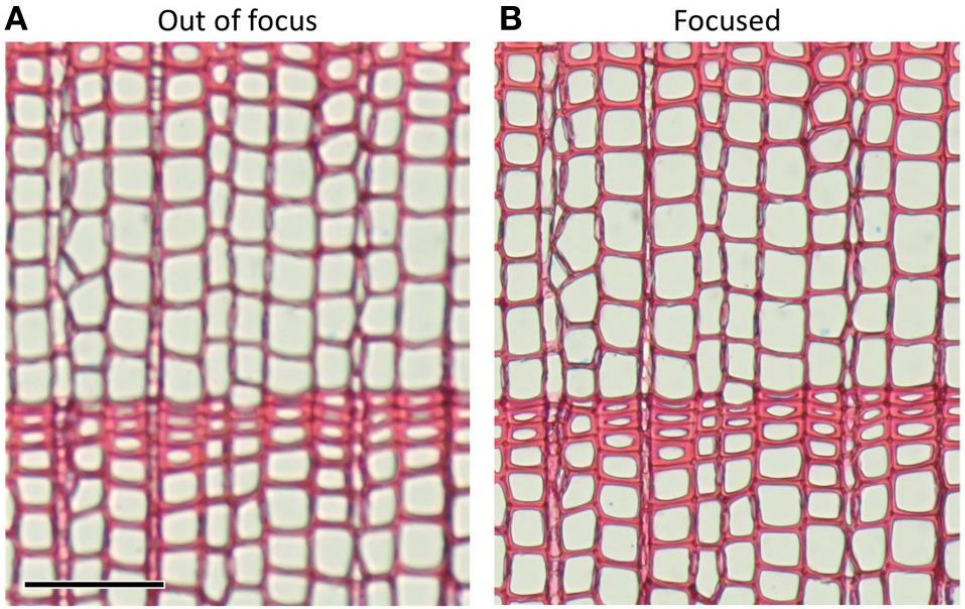
c) Simple Safranin staining

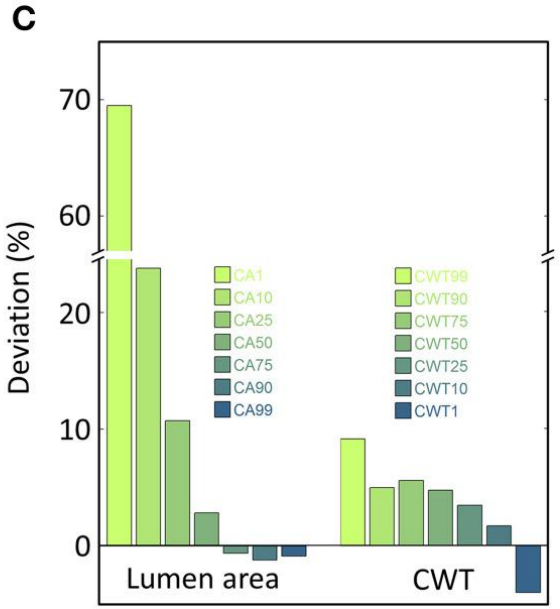
Quantitative Wood Anatomy

Lab methods

Step 3 : Image Numerization
→ 10x objective are usually sufficient
Giving a resolution of 1.7–2.5 pixels/µm
→ 4x objective can be used for angiosperm vessels





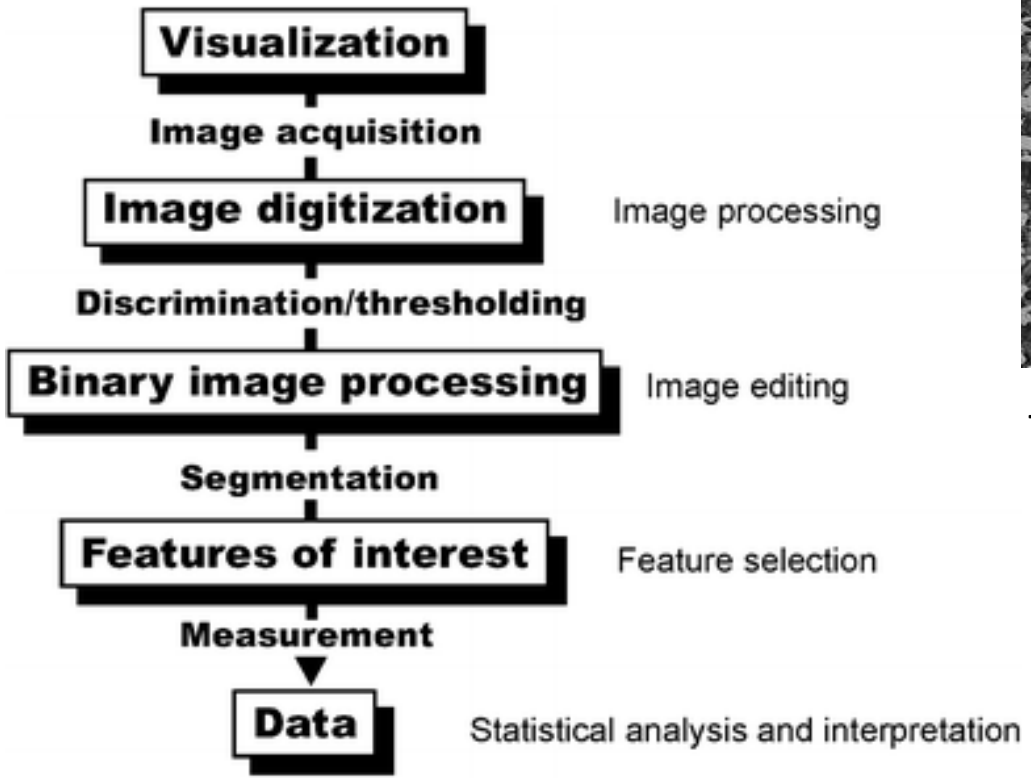


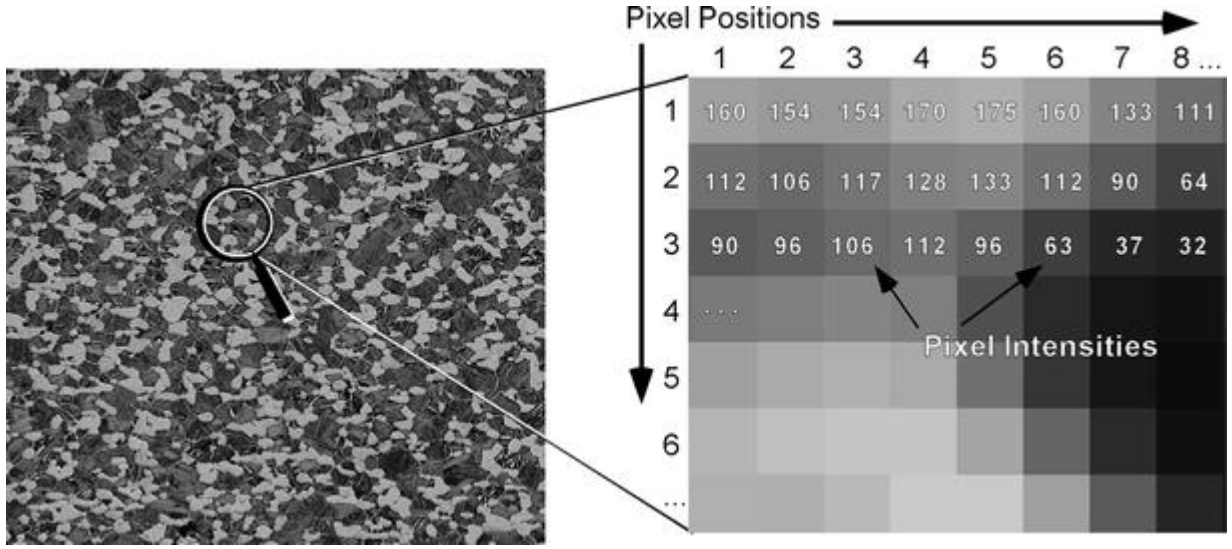
Von Arx, 2016

Quantitative Wood Anatomy

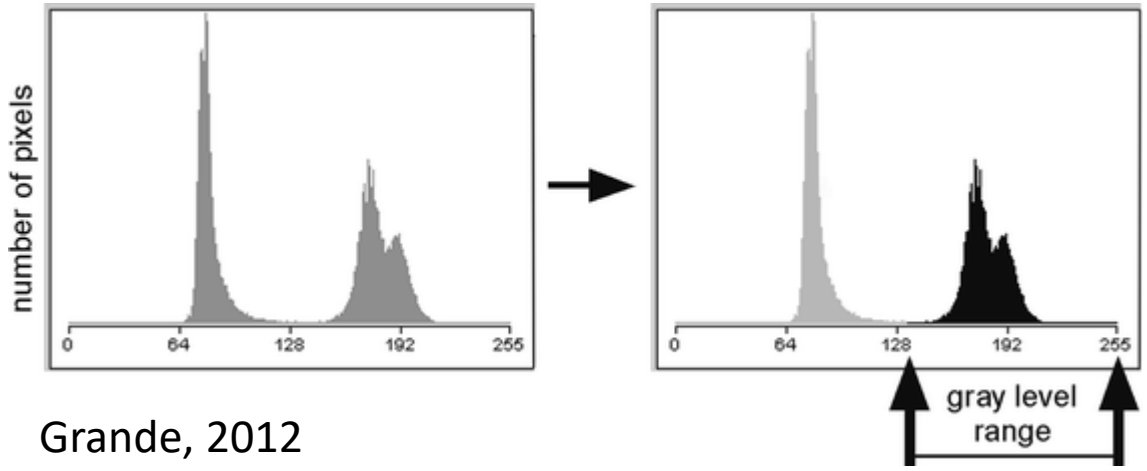
Image Analysis Basics

Step 4 : Image Analysis





Thresholding gray levels in an image by selecting the gray-level peaks that are characteristic of the features of interest



Grande, 2012

Quantitative Wood Anatomy

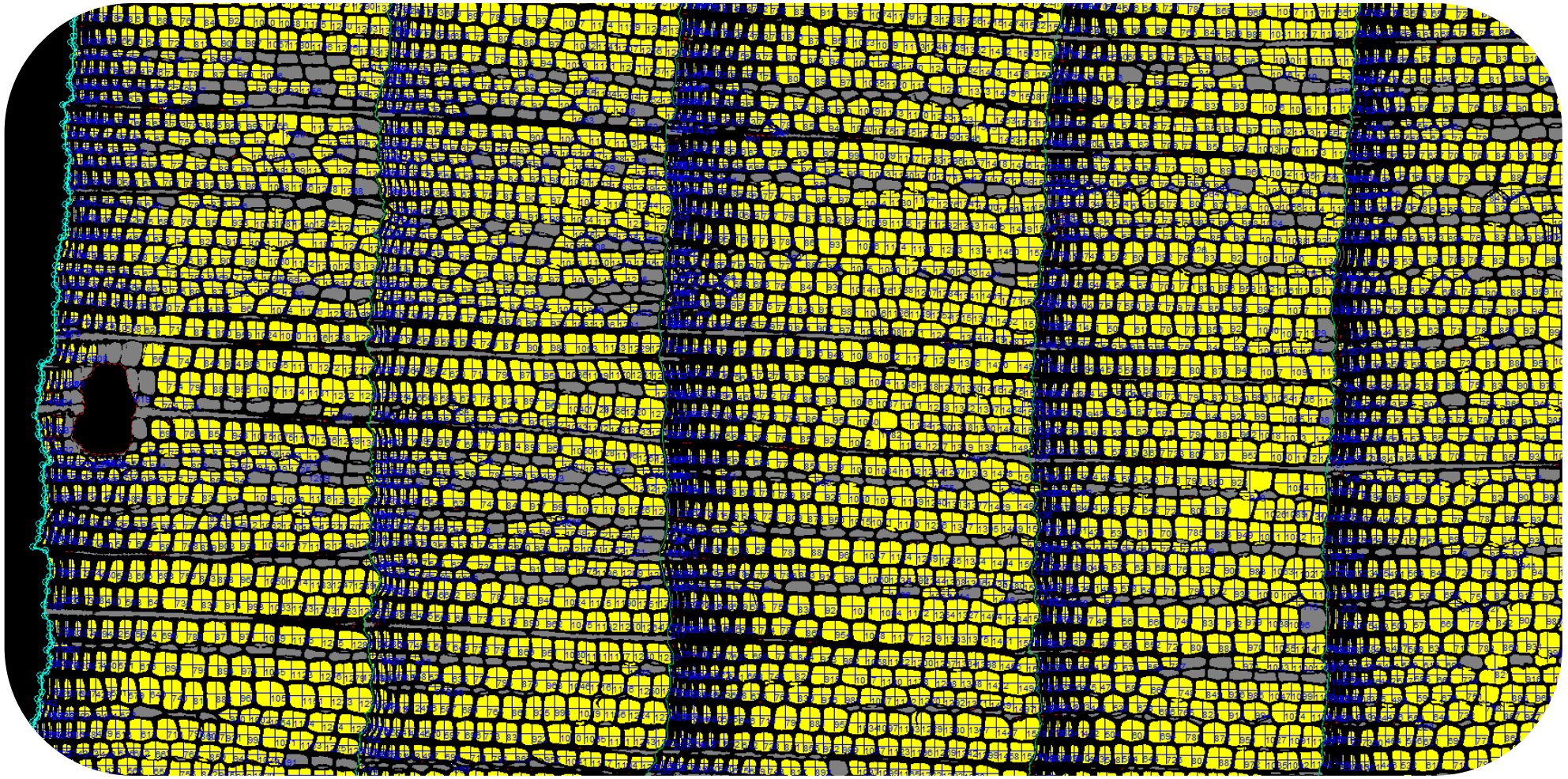
Image Analysis Basics

Step 4 : Image Analysis

→ Semi-automated cell recognition & measurements

→ Filters, color or B&W thresholds, form coefficients

WinCELL™
Wood Cell Anatomy



Quantitative Wood Anatomy

Image Analysis Basics

Guidelines for Best Practices in Image Processing

→ When it's possible : **avoid image edition**

Treating Images as Data: Digital scientific images should be treated as data

Filters Degrade Data: Use of software filters to improve image quality is usually not recommended for biological images.

Comparing Images: Digital images that will be compared to one another should be acquired under identical conditions.

Saving the Original: Manipulations of digital images should always be done on a copy of the raw image data. The original must be retained.

Cloning Degrades Data: Cloning objects into an image or from other parts of the image is very questionable.

Manipulating the Entire Image: Manipulations that are specific to one area of an image and are not performed on other areas are questionable.

Making Simple Adjustments: Simple adjustments to the entire image are usually acceptable.

Making Intensity Measurements: Intensity measurements of digital images should be performed on raw data and the data should be calibrated to a known standard.

Issues With Magnification: Magnification and resolution issues are important.

Cropping is usually OK: Cropping an image is usually acceptable.

Lossy Compression Degrades Data: Avoid the use of lossy compression.

Issues With Pixels: Be careful when changing the size (in pixels) of a digital image.

https://ori.hhs.gov/education/products/RIandImages/default.html

Quantitative Wood Anatomy

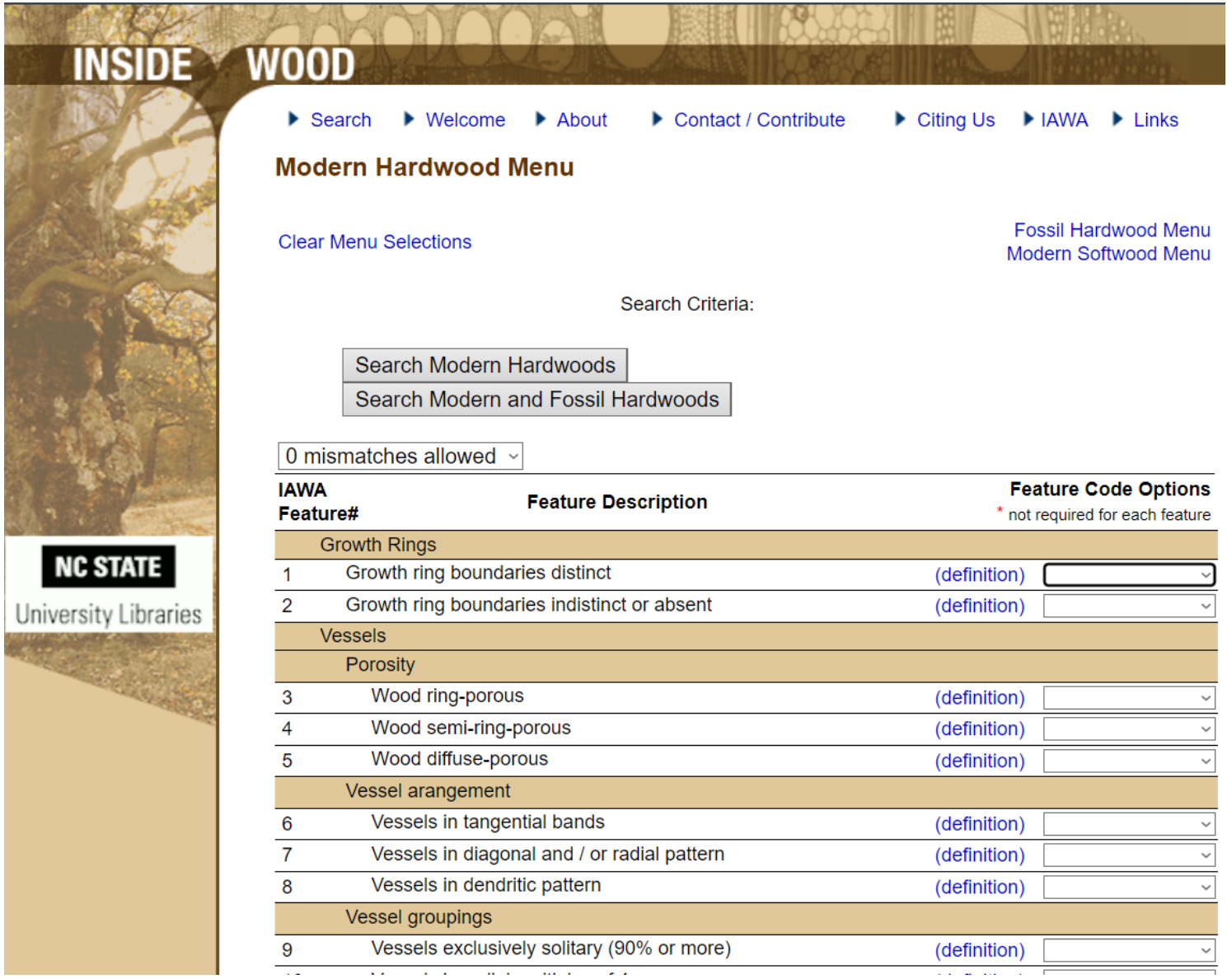
Game of the Day

Identify the 5 hidden species

Give the species where you can find the following features :

- Axial parenchyma in vertical files
- Wood ring-porous
- Wood diffuse-porous
- Helical thickenings
- Frost ring
- Tyloses
- Prismatic crystals
- Scalariform perforation plates
- Gums and other deposits in heartwood vessels

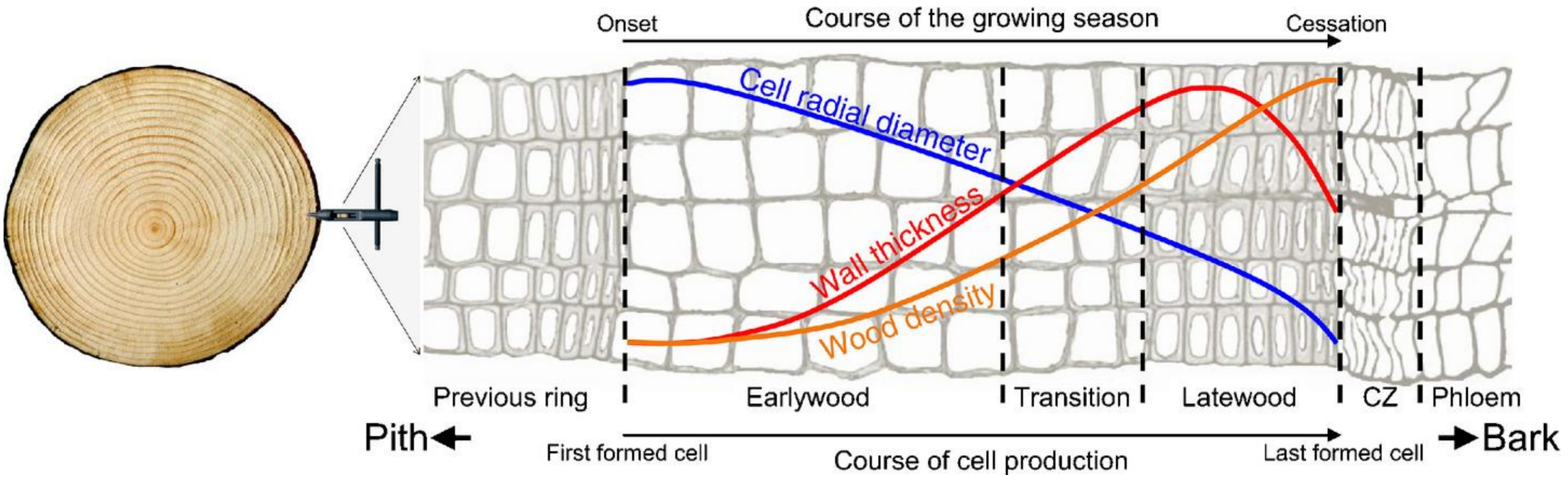
→ Get help with the Inside Wood Web Database & the IAWA lists of microscopic features



Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms



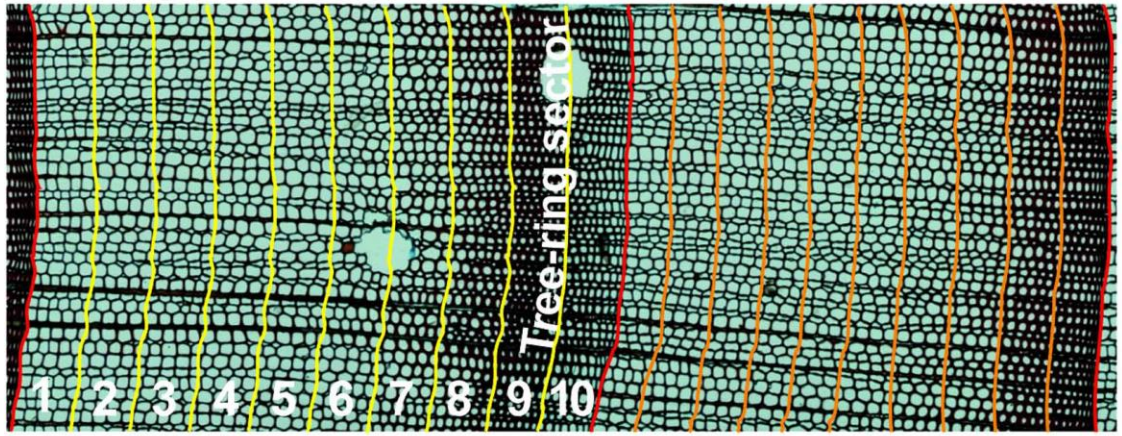
Cuny, 2014

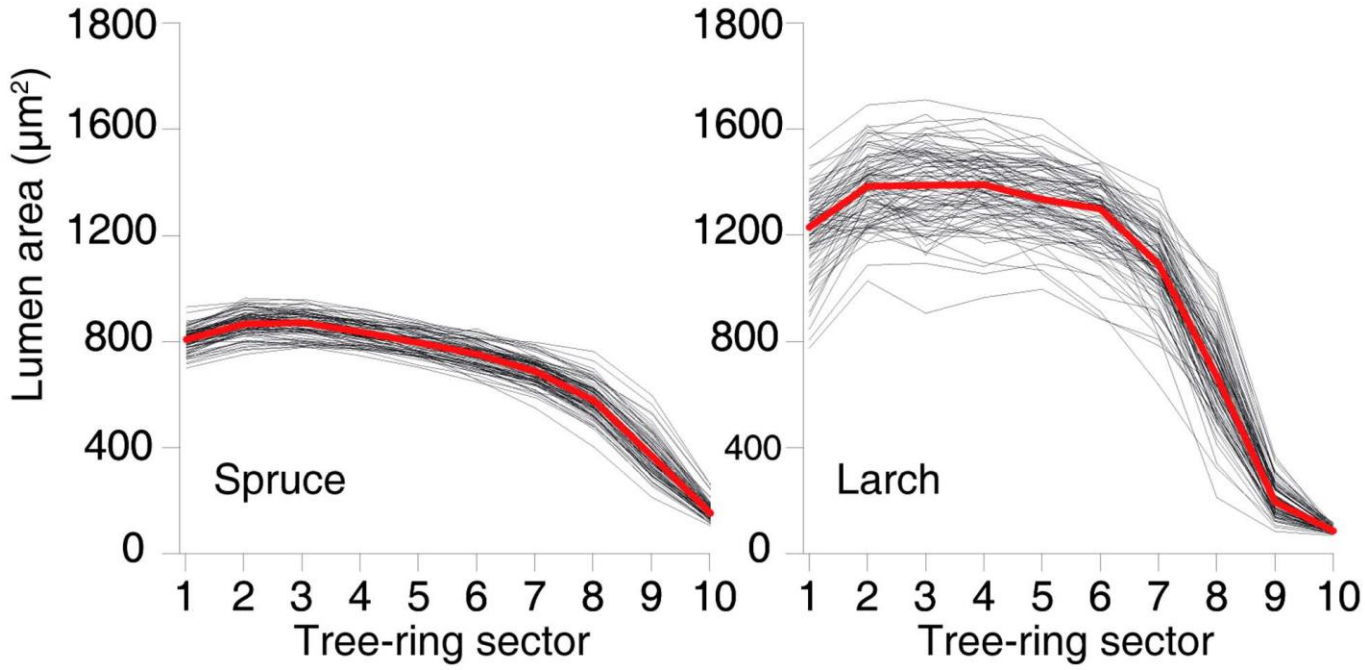
Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms

Ring divided in a fixed number of sectors of equal width
→ Sectors of different rings have different widths





Carrer, 2017

Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms

Rings are divided in sectors of equal width

→ Differents rings can have a different number of sectors

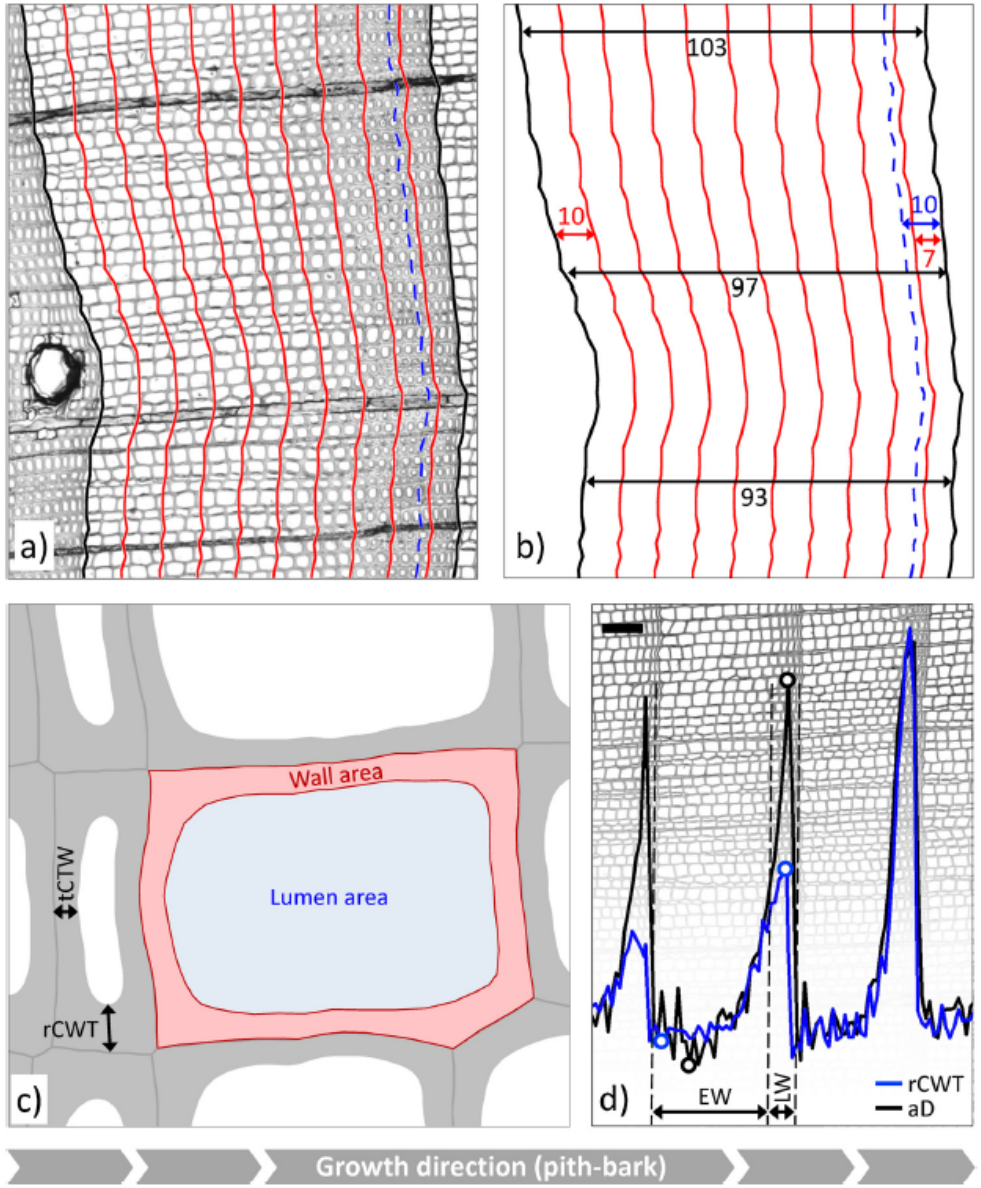


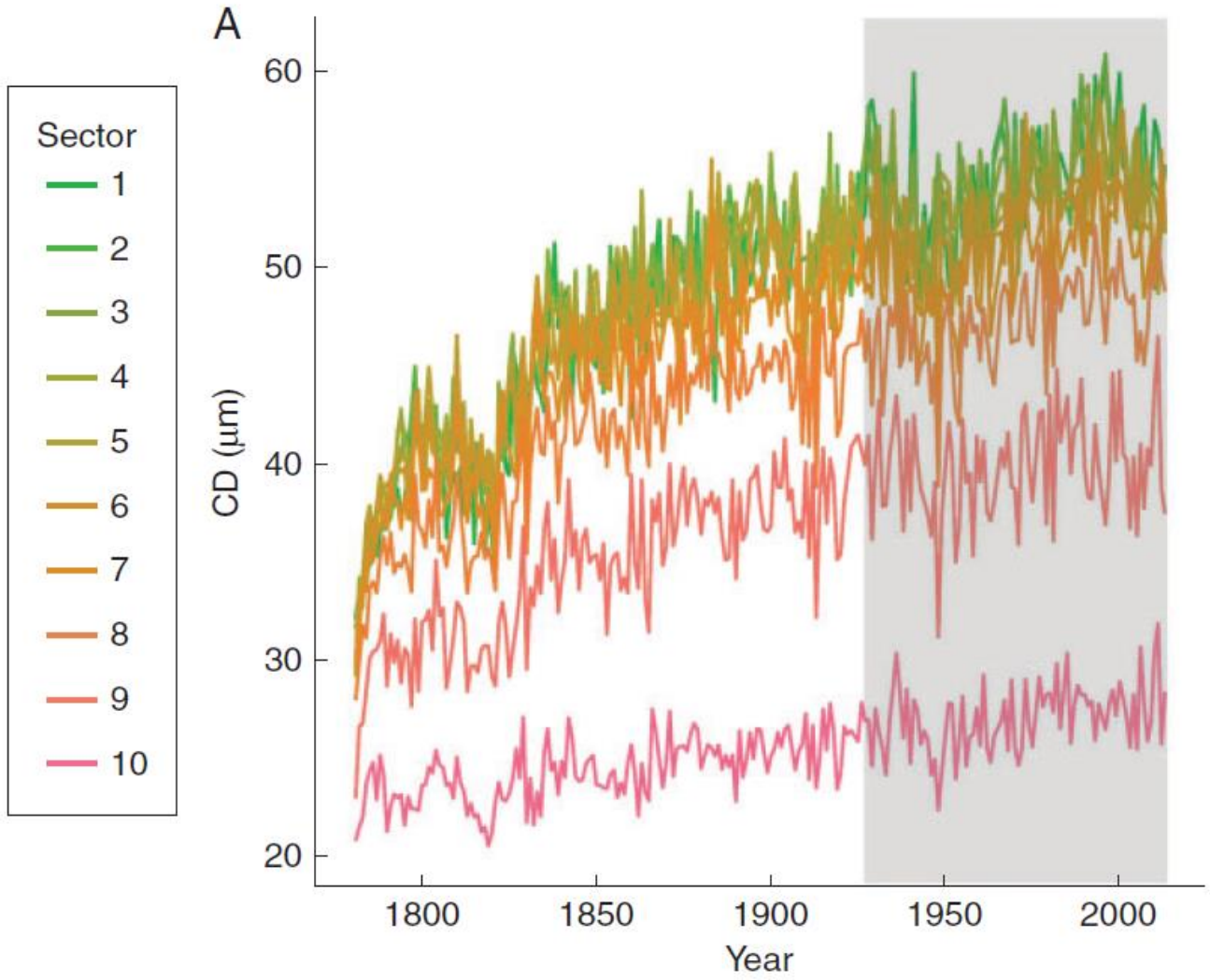
Fig. 1. Explanatory figure of measured parameters and measurement technicalities. a) and b) Visualization of the algorithm used to create intra-annual profiles of anatomical parameters based on cell position in the ring. Cells are here assigned to on average 10 unit wide bands (in the analysis we use 10 µm bands) (red lines) parallel to the ring borders (black lines) based on their center coordinates. Anatomical parameters are then calculated as the 75th percentile in each band. Bands are not allowed to cross ring borders and thus never include cells from two adjacent rings. To avoid a truncated narrower last band towards the ring borders (7 units in the b) example), the terminal band (blue dashed line) is defined as the 10 units adjacent to the terminal ring border. This means that cells in the overlapping part of the last and second-last band are included in both bands. c) Illustration of the basic tracheid dimensions used in this study: rCWT – radial cell wall thickness (i.e., the walls running in radial direction in a cross-sectional view), tCWT – tangential cell wall thickness (walls running in tangential direction in a cross-sectional view), lumen area and wall area. Anatomical density (aD) of each cell is defined as the ratio of wall area to overall cell area (sum of wall and lumen area). d) Exemplary 10-µm-resolution intra-annual profiles for rCWT (blue curve) and aD (black curve). Maximum and minimum values for each parameter (circles) are extracted for each ring, whereas means for earlywood (EW) and latewood (LW) are obtained by averaging the values of the bands assigned to EW and LW, respectively. Scale bar in (d): 100 µm (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Bjorklund, 2020

Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms :
Conduit diameter Age trend

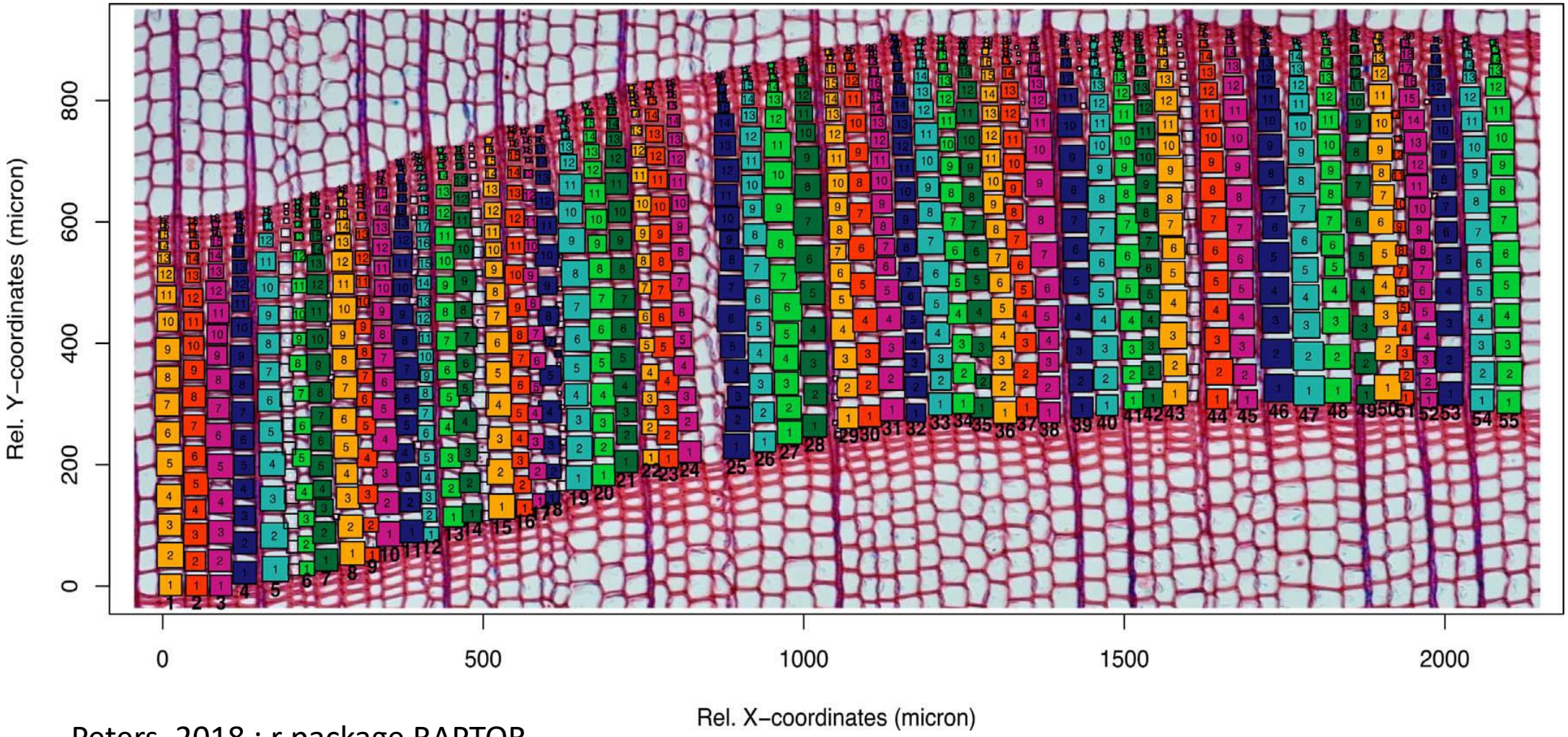


Castagneri, 2017

Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms



Peters, 2018 : r package RAPTOR

Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms

Vaganov standardization :
→ All radial files are standardized to a fixed number of tracheids

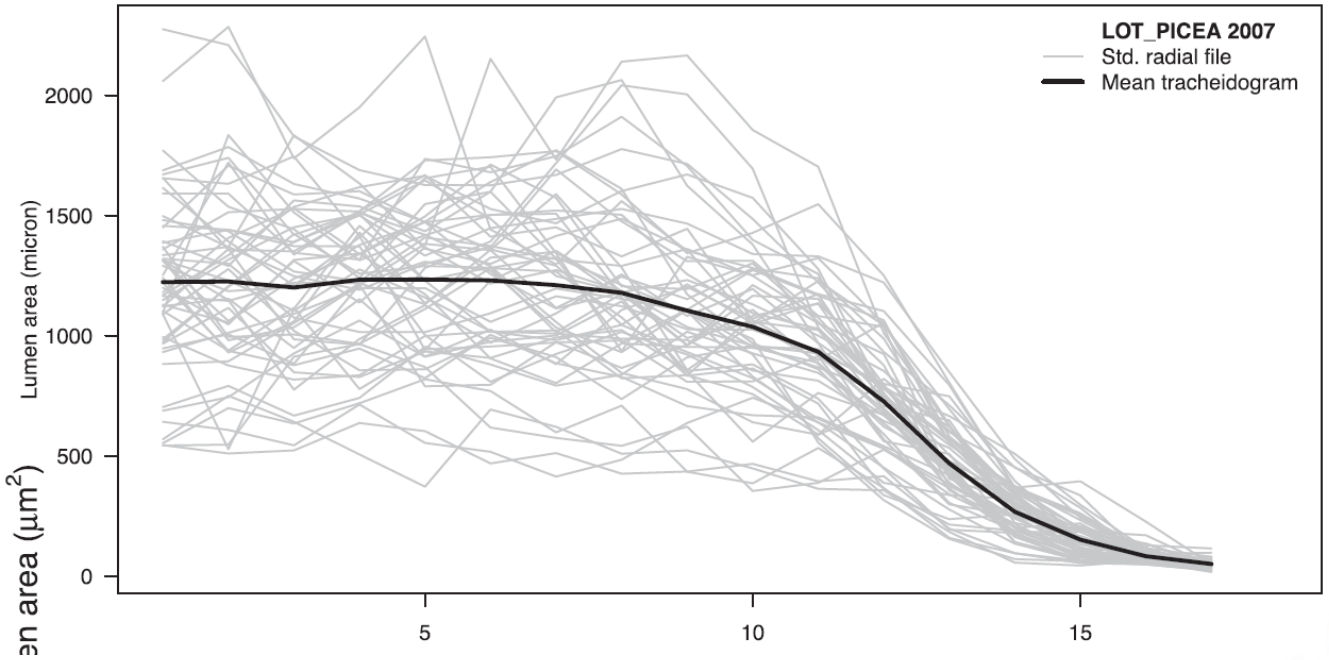
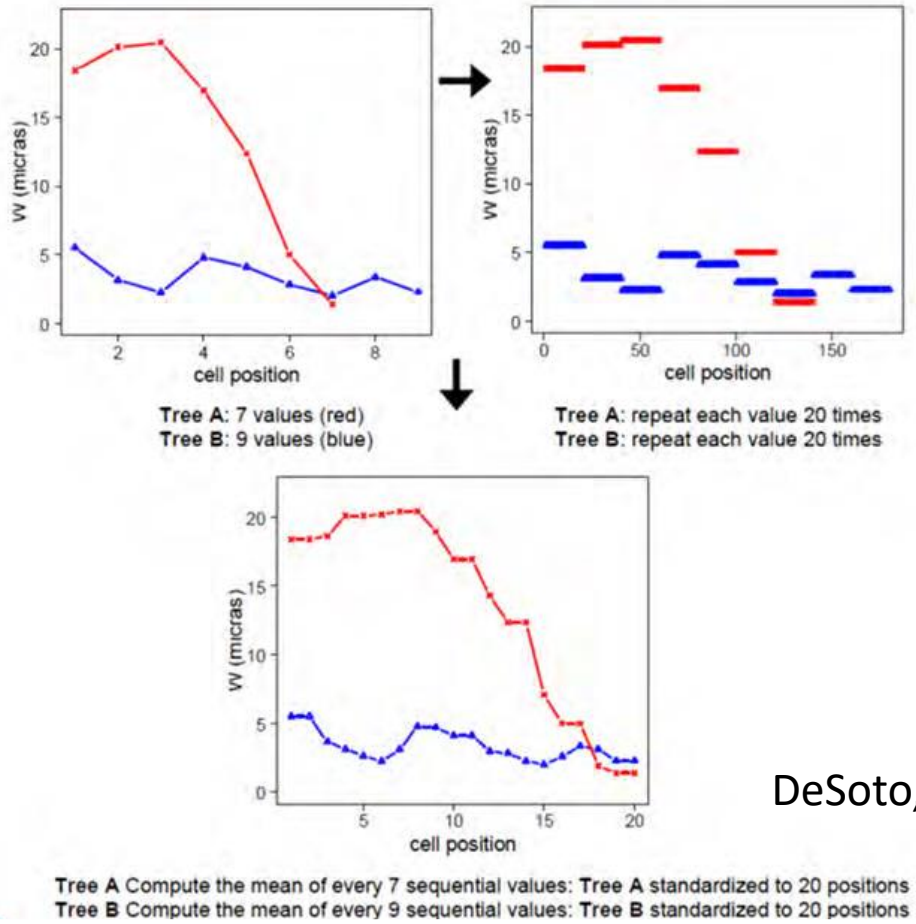


Figure 5. Data normalization procedure as described by Vaganov (1990) y Vaganov et al. (2006) to get the same number of cells in radial files (normalized tracheidogram, i.e. a curve showing variations in cell parameters as a function of the cell position within an annual ring, for instance, 20 cells per radial file in the figures).

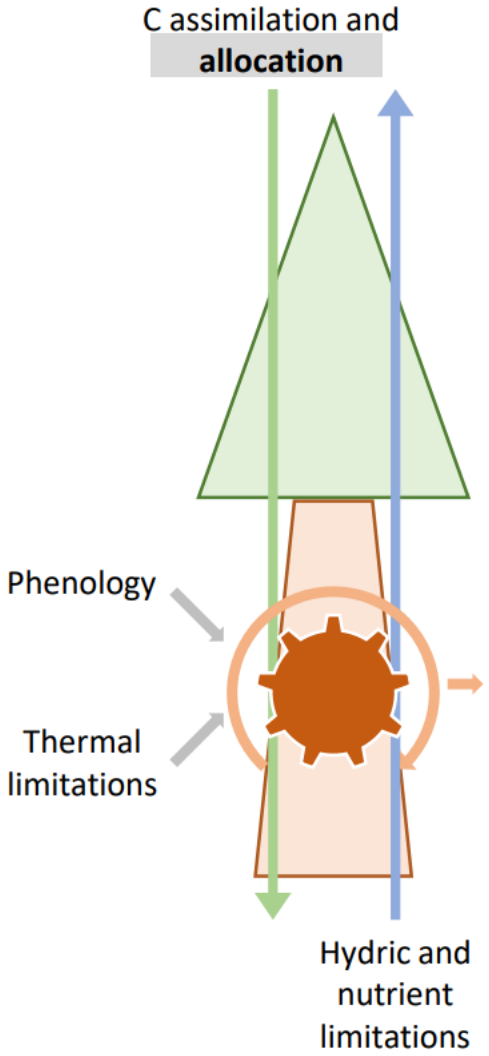


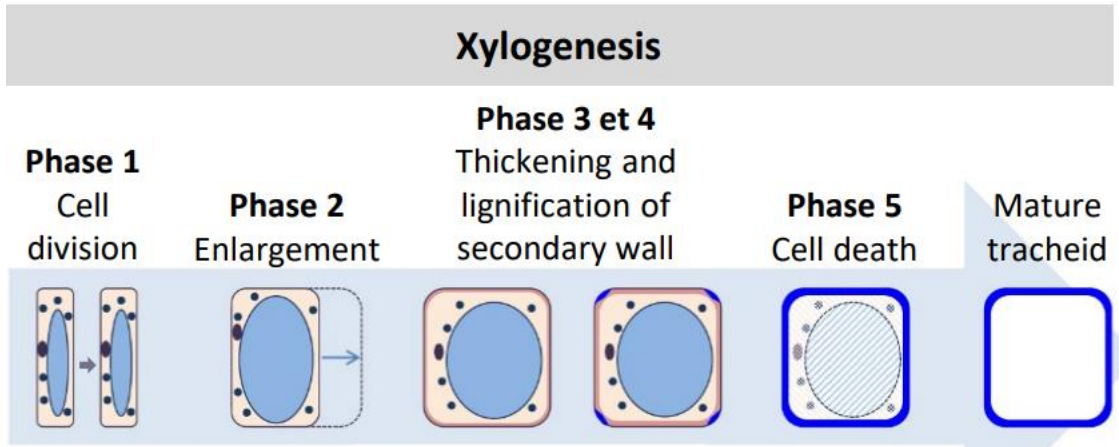
DeSoto, 2011

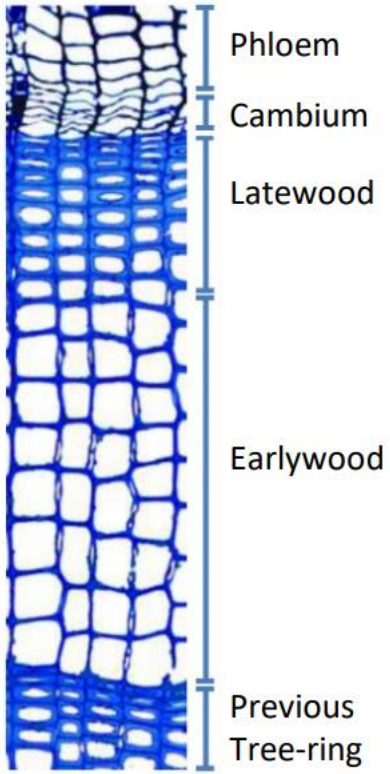
Quantitative Wood Anatomy

Data analysis methods

Retrospective analysis & xylogenesis







Cell Number, Cell Size and Cell Wall Thickness can be seen as proxies for the successive phases of xylogenesis

F.Gennaretti, modified from Cuny, 2014

Quantitative Wood Anatomy

Data analysis methods

Retrospective analysis & xylogenesis

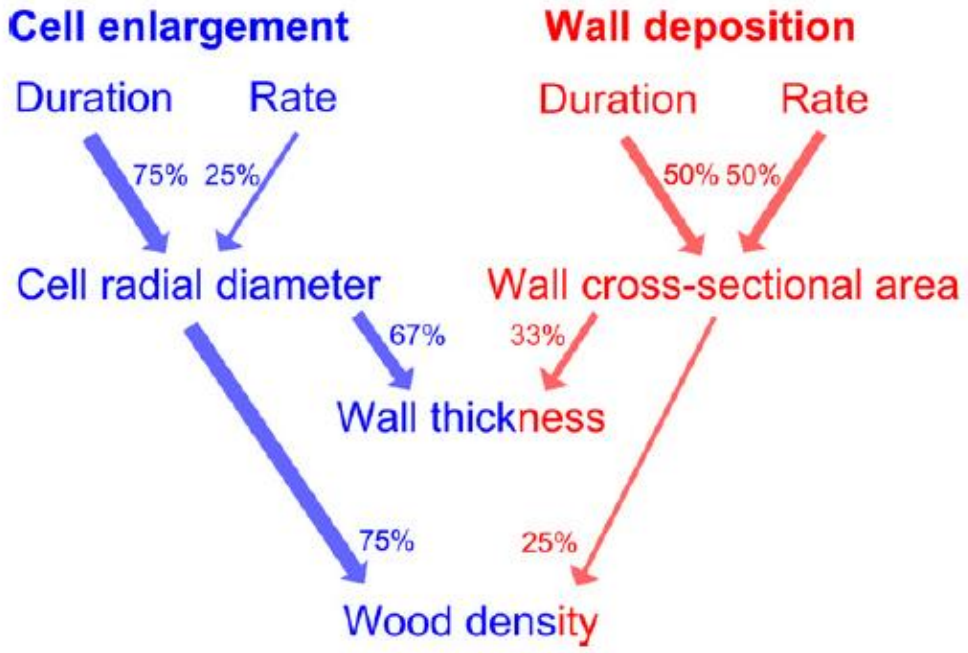
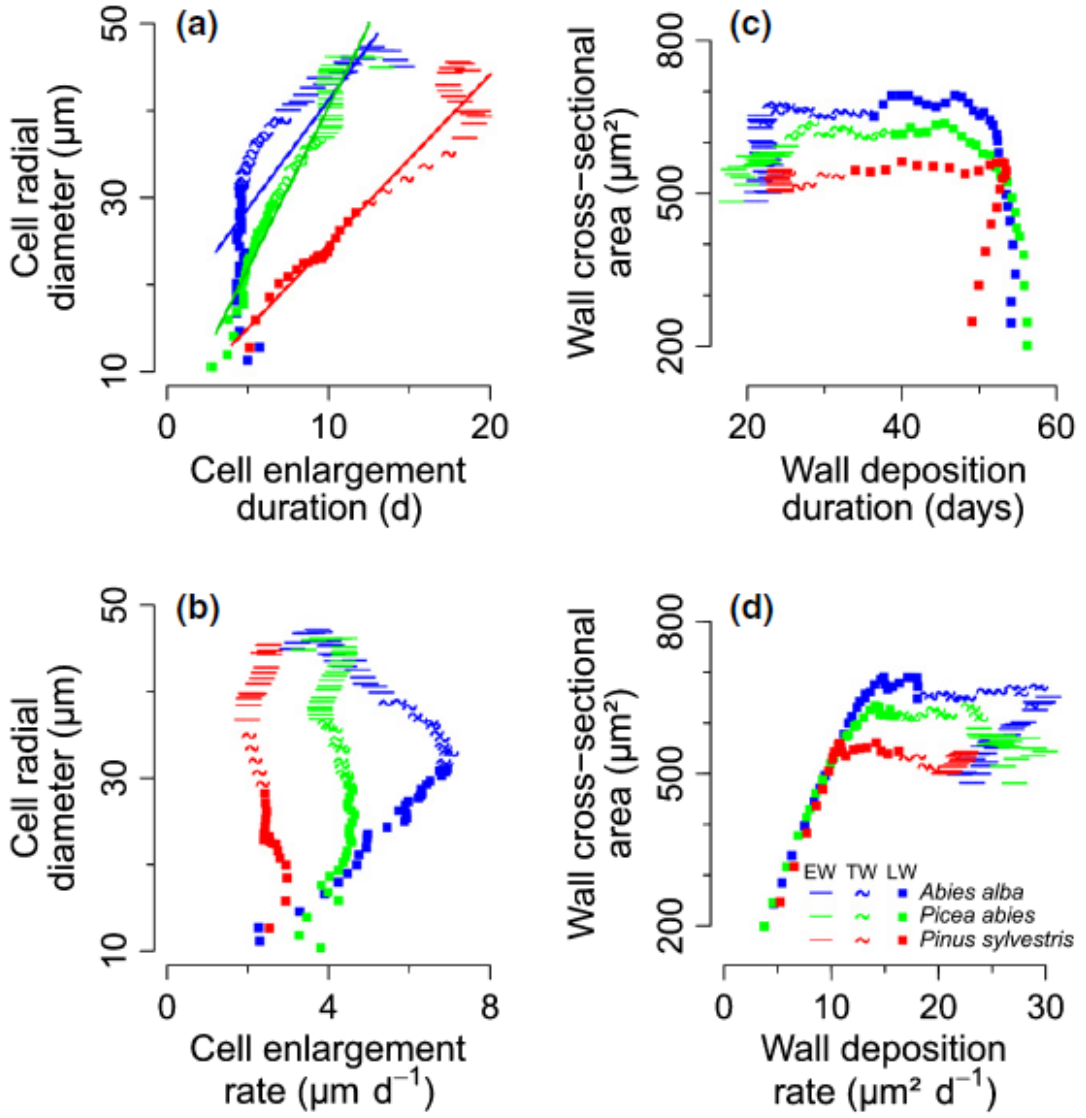


Fig. 8 Relative contributions of the different components of the kinetics of tracheid development to the changes in tracheid dimensions and wood density along the ring. The percentages provided were calculated on the basis of sensitivity analyses.

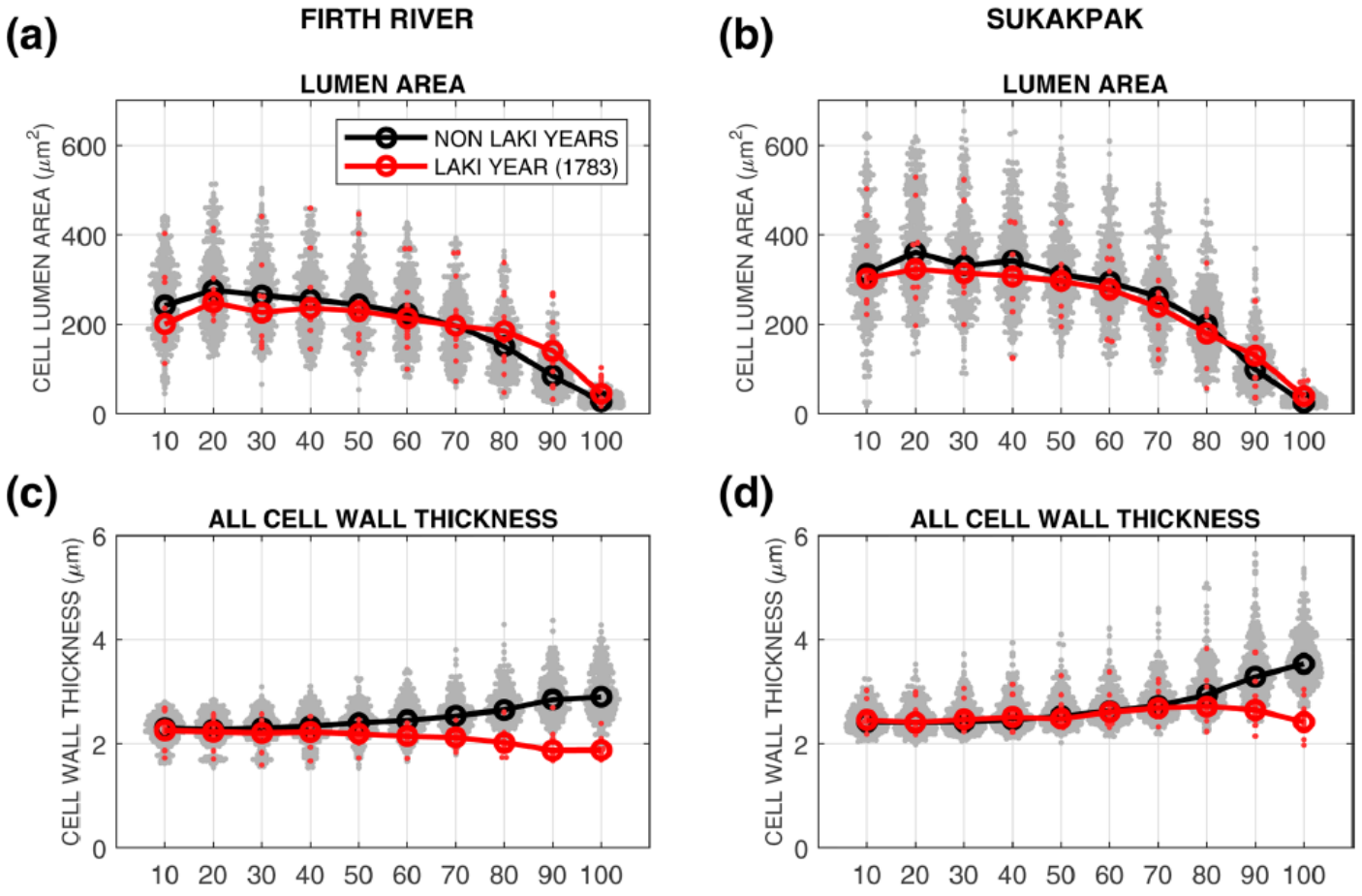
Cuny, 2014

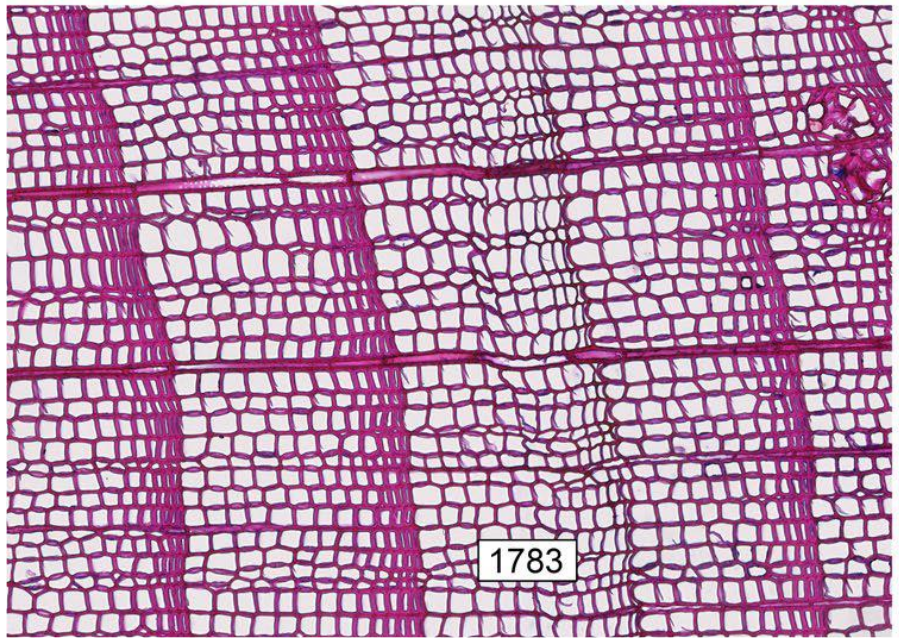


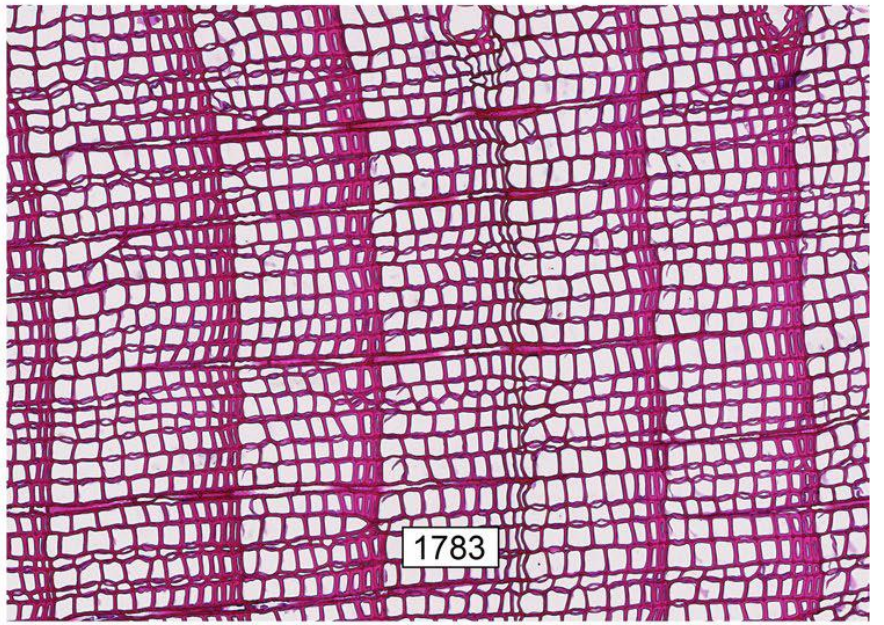
Quantitative Wood Anatomy

Data analysis methods

Edwards, 2020 : Cell division & enlargement haven't been impacted
But abrupt and premature cessation of cell wall thickening
→ rapid temperature decrease toward the end of the growing season following the Laki eruption







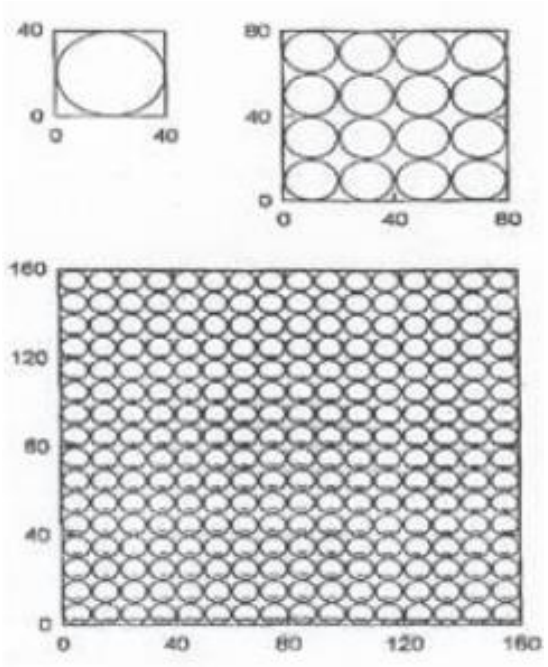
Quantitative Wood Anatomy

Wood anatomical traits as indicators of functional properties

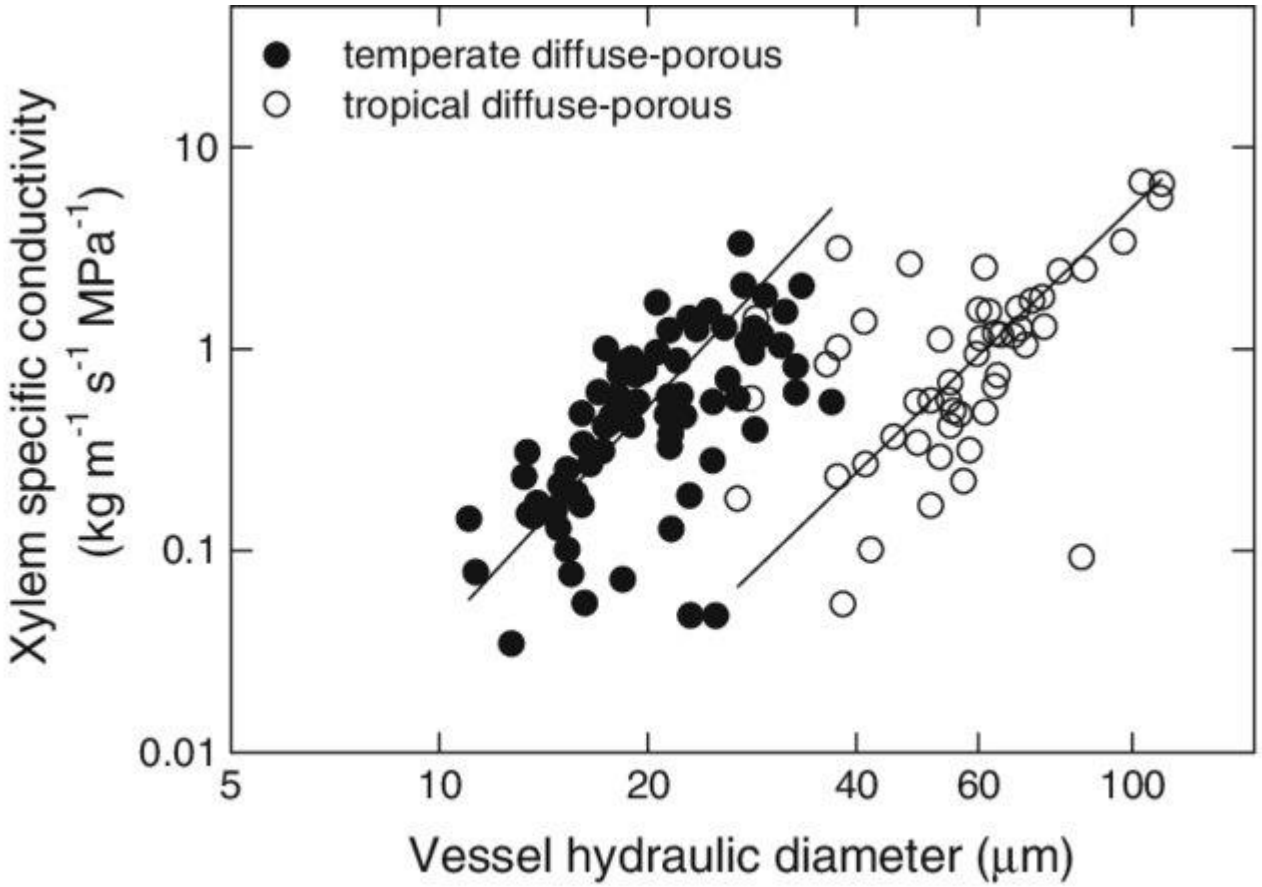
Relationships between anatomy & hydraulic properties

Safety vs. **Efficiency**

Loi de Hagen-Poiseuille :
Le flux traversant un élément conducteur de diamètre d est identique à celui parcourant 16 éléments de diamètre égal à d/2 ou 256 éléments de diamètre égal à d/4. (D'après Tyree *et al.* 1994.)



Meinzer & McCullough, 2010



Quantitative Wood Anatomy

Wood anatomical traits as indicators of functional properties

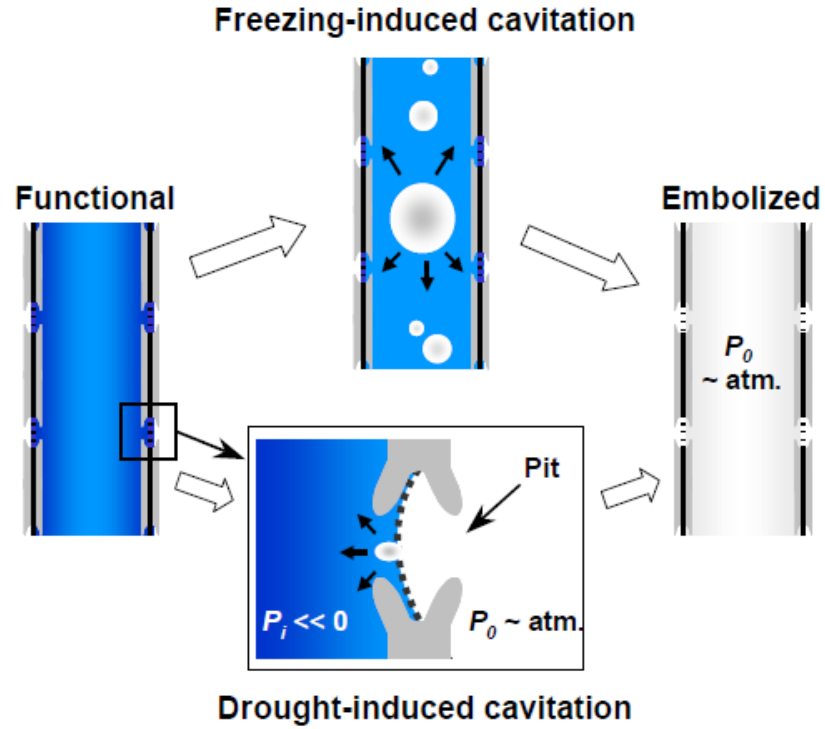
Relationships between anatomy & hydraulic properties

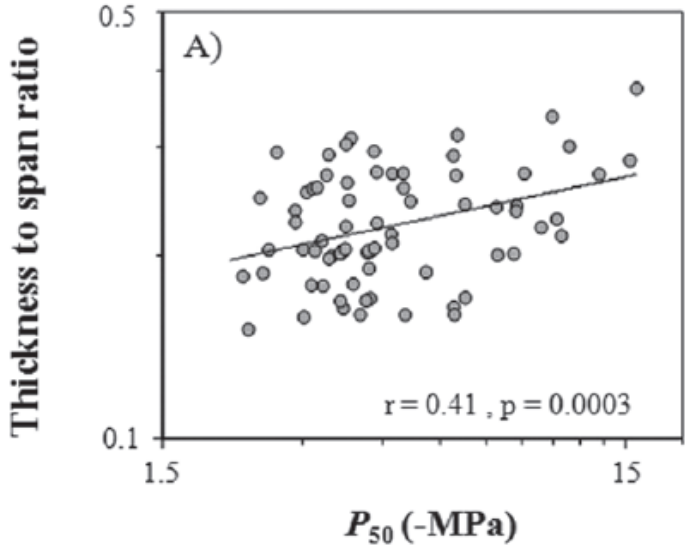
Safety vs. Efficiency

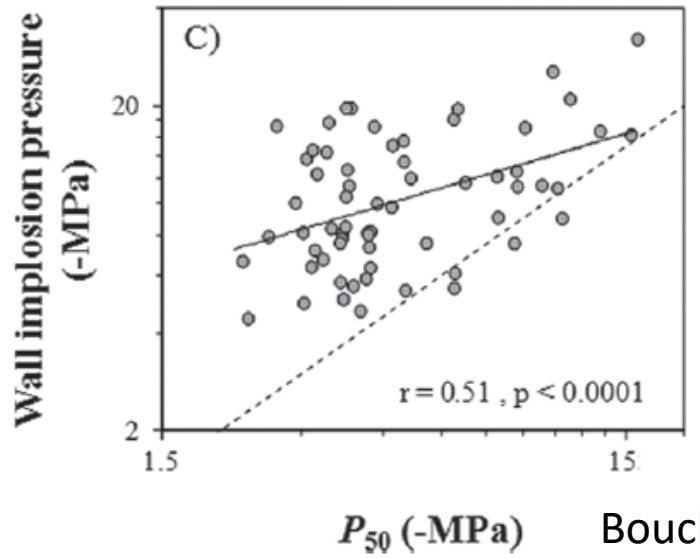
Hydraulic safety traits :

→ Larger conduits are more prone to embolism

→ Thicker walls seems to increase resistance to implosion







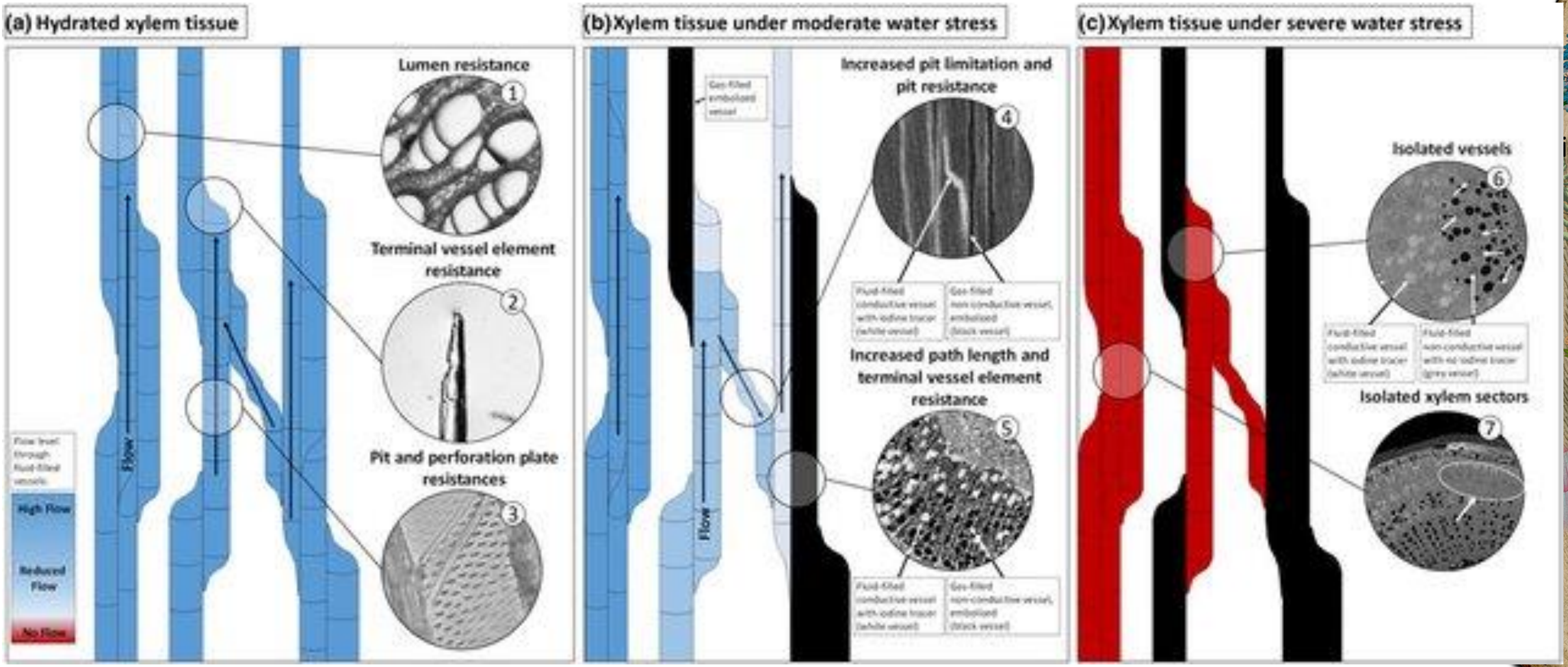
Bouche, 2014

Quantitative Wood Anatomy

Wood anatomical traits as indicators of functional properties

Relationships between anatomy & hydraulic properties

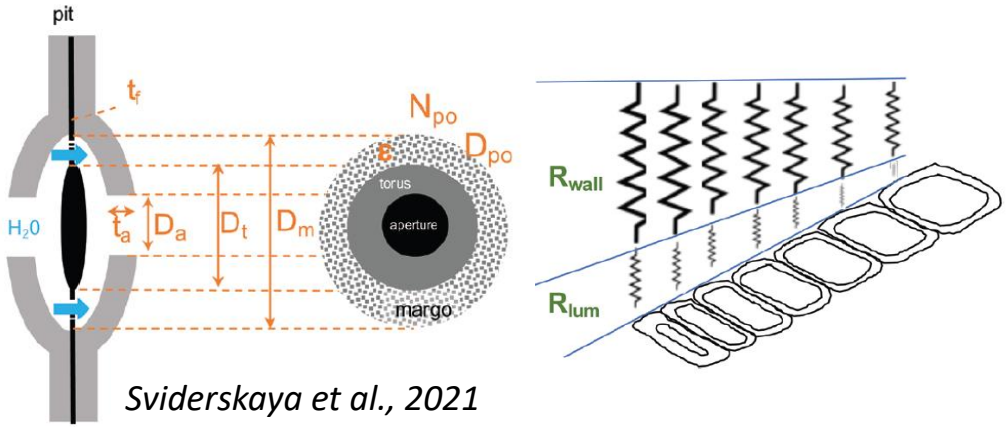
Efficiency vs. Security



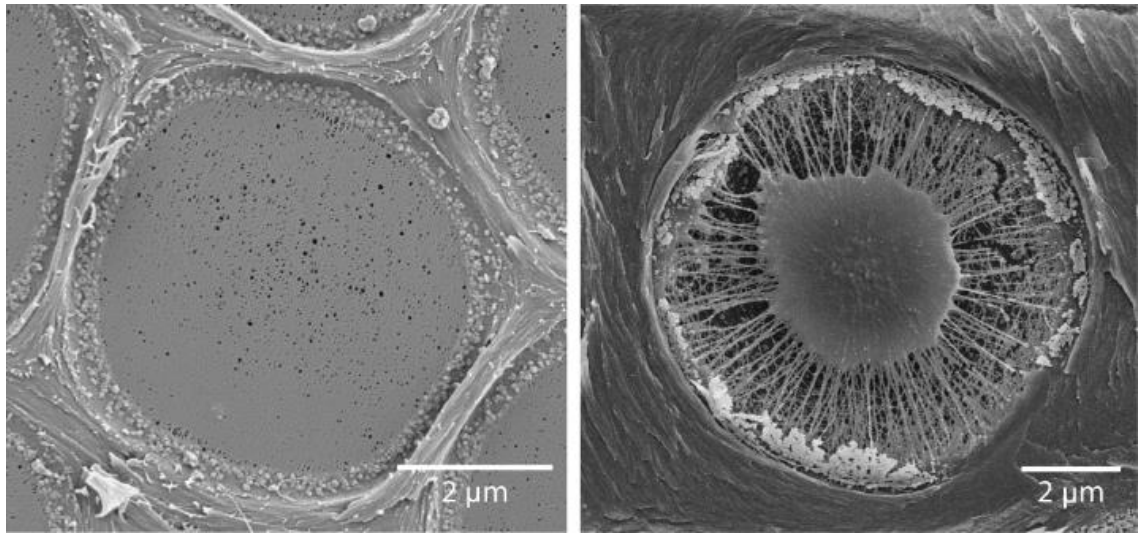
Quantitative Wood Anatomy

Pit influence on hydraulic efficiency and safety

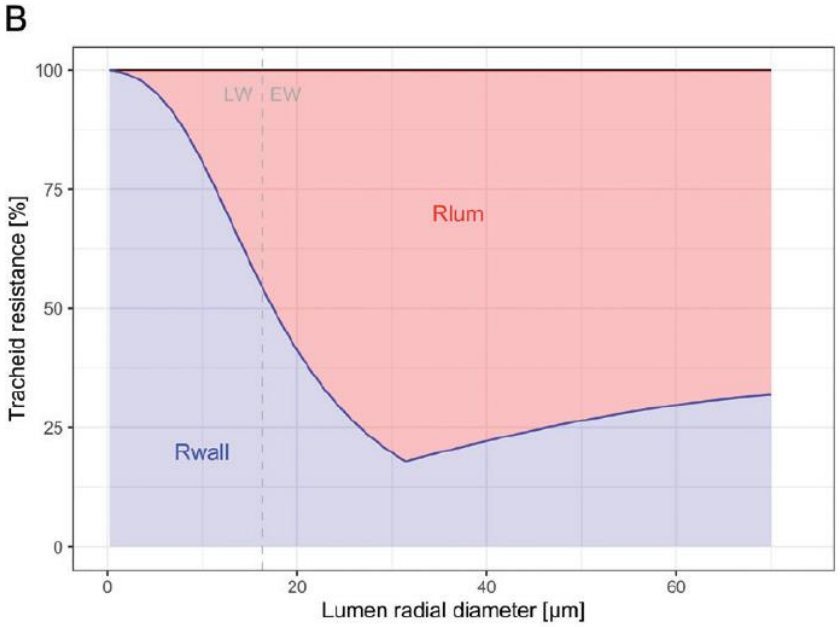
Pitterman et al., 2006 ; Hacke et al., 2004, 2006 ; Domec et al., 2006 ; Schulte et al., 2015 ; Bouche et al., 2014 ; Song et al., 2022 ; Sperry et al., 2006 ; Lens et al., 2011



Sviderskaya et al., 2021



Choat et al., 2009



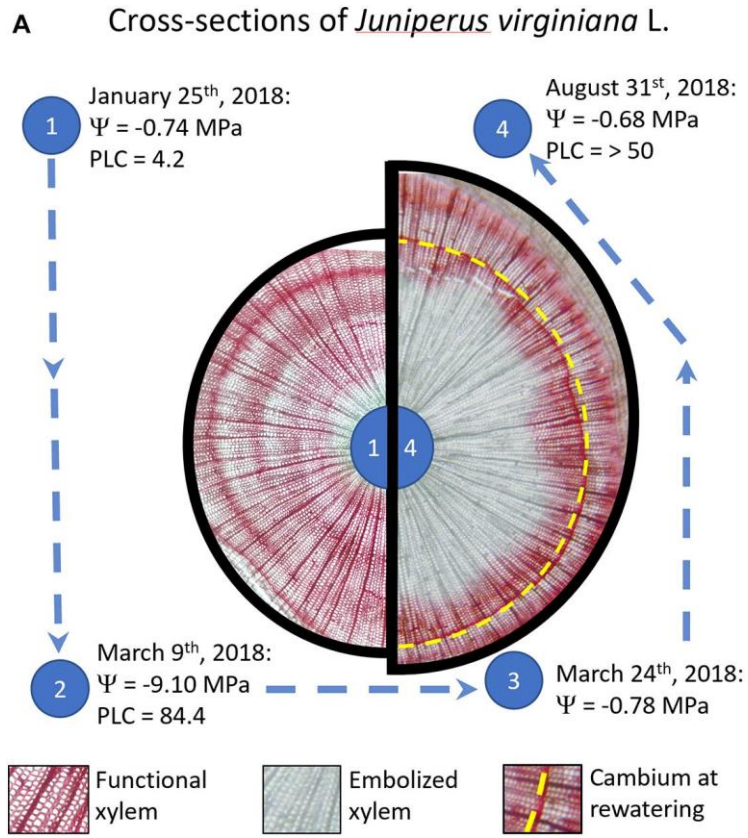
- Relative contributions of Tracheid dimensions & Pit structure to hydraulic conductance & vulnerability to cavitation are not resolved
- Pit scale adjustements can be an efficient strategy to cope with drought
- Possible isometric scaling to take into account pit variability in estimations of hydraulic efficiency & safety ?

Xylem Strategies to Recover hydraulic capacities

Ruehr et al., 2019 ; Brodersen & McElrone, 2013 ; Klein et al., 2018

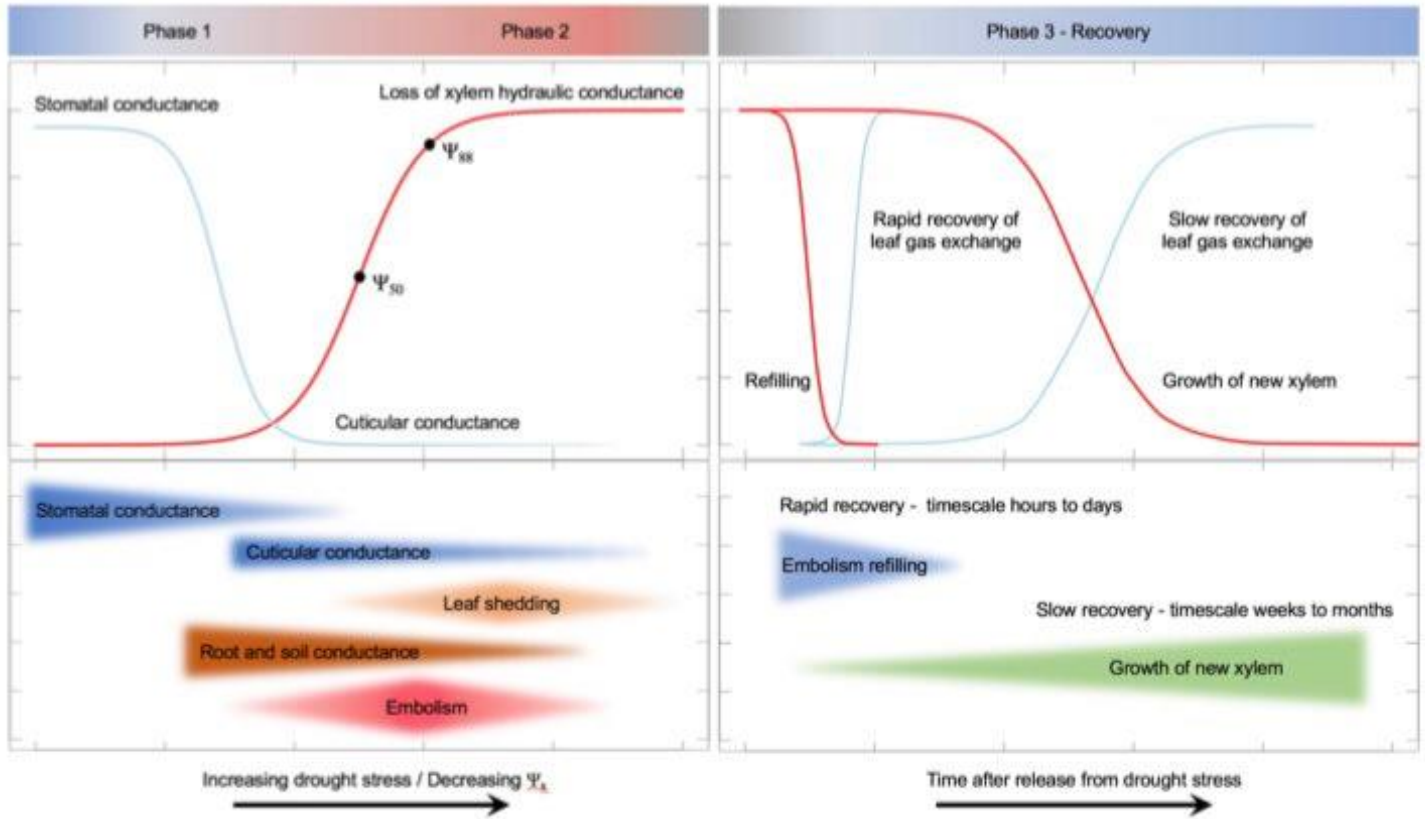
Recovery of hydraulic performance

Growth of new xylem



Hammond, 2020

Refilling of embolized conduits



Potential role of axial & radial parenchyma ?

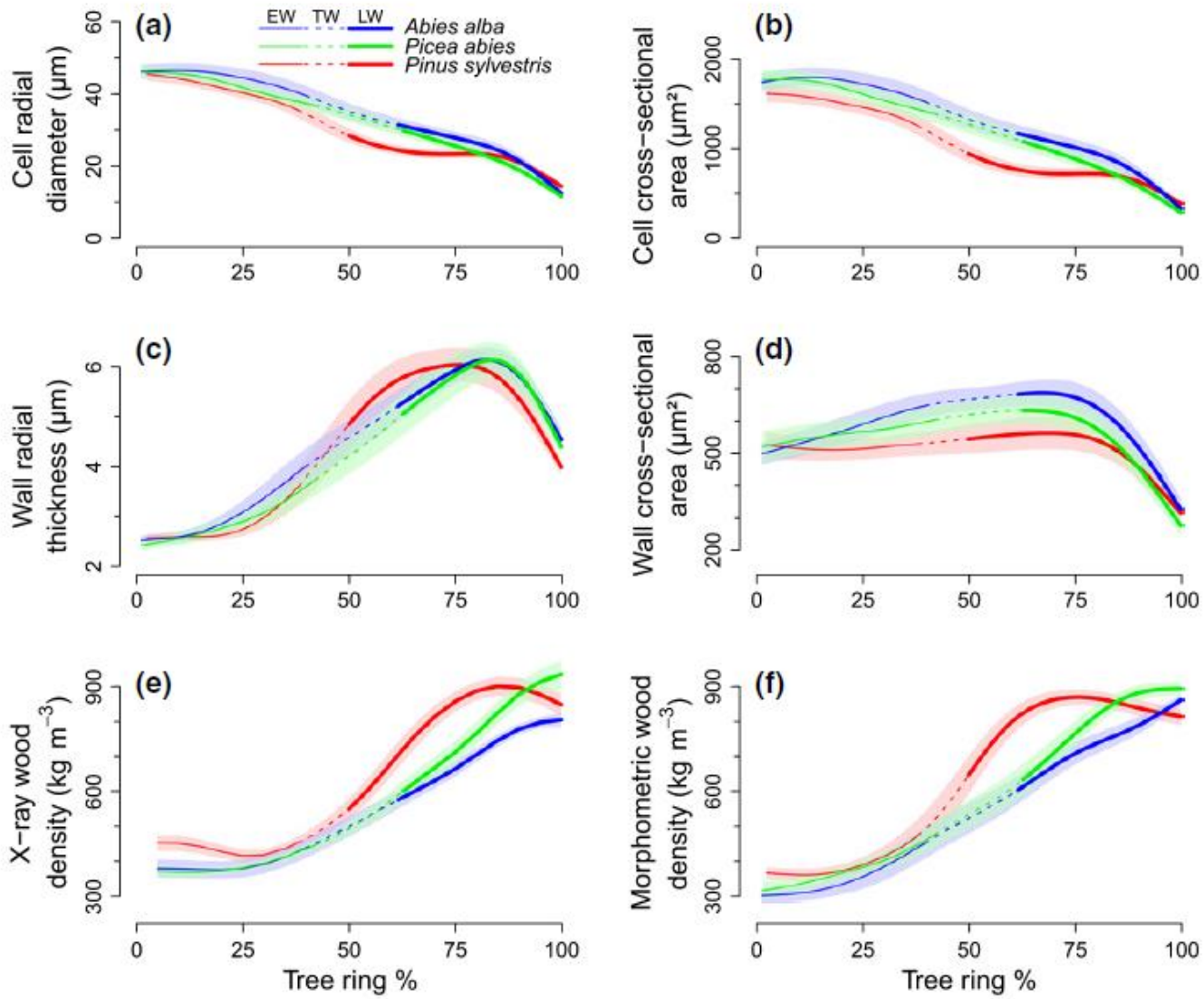
Quantitative Wood Anatomy

Wood anatomical traits as indicators of functional properties

Cuny, 2014

Relationships between Anatomy & Carbon Sequestration

Proxies of Carbon contained in tree-ring :
→ Morphometric density,
→ Anatomical density,
→ Tissue percentages



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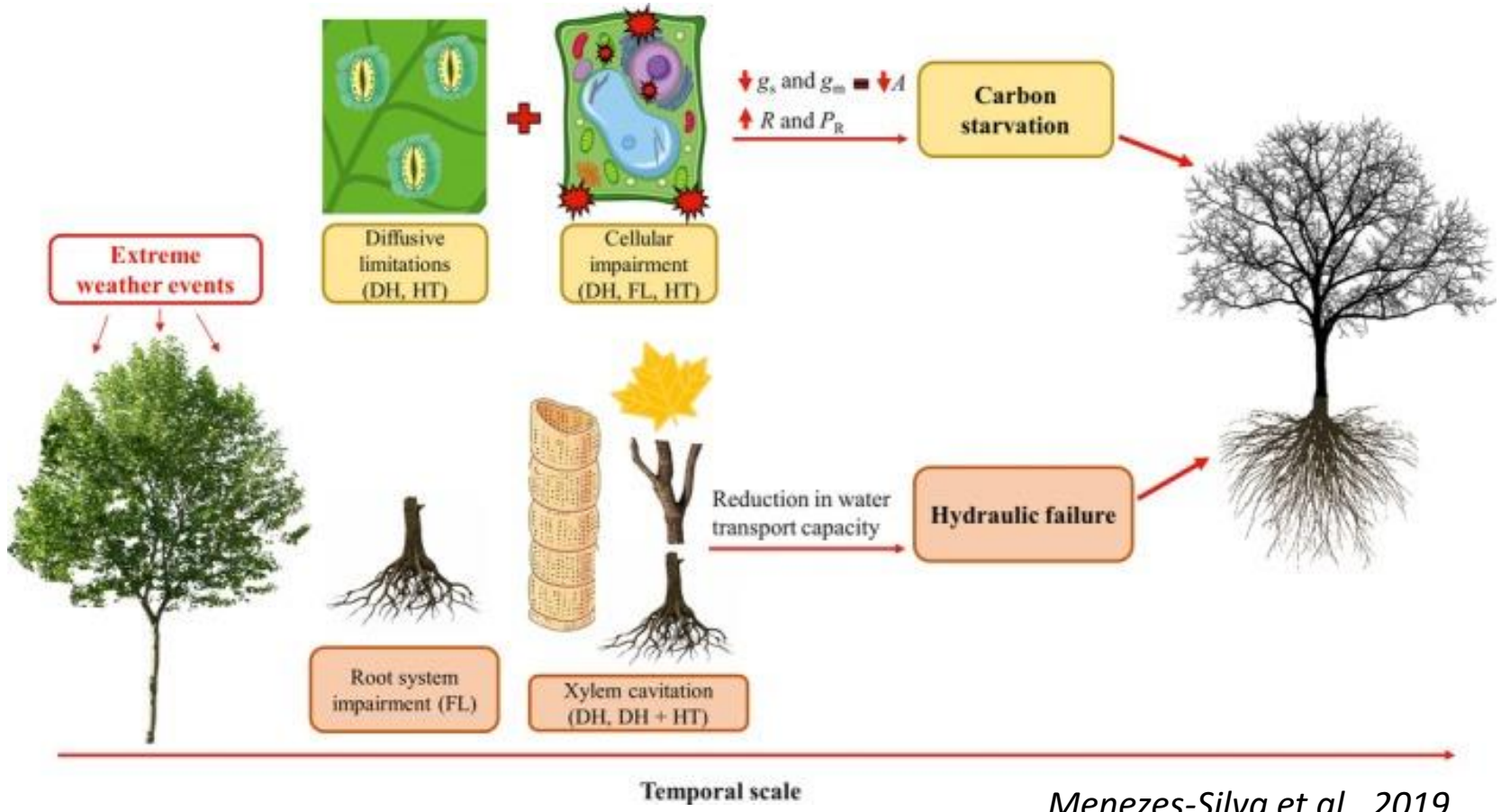
Wood anatomical traits as indicators of functional properties

Mechanisms of tree mortality



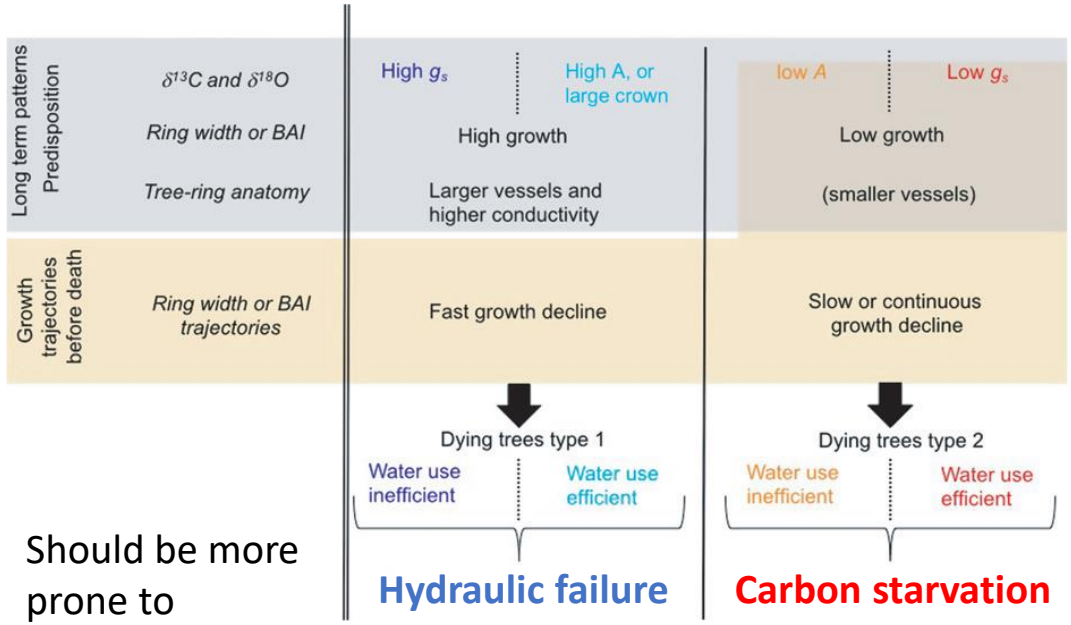
Menezes-Silva et al., 2019

Tree response to disturbances. Is it possible to identify typical patterns in tree-ring traits and what are the potential contributions of quantitative wood anatomy ?

Role of xylem in strategies

Heres et al., 2014 ; Levanic et al., 2011 ; Cailleret et al., 2017

Prevention of hydraulic failure & predispositions



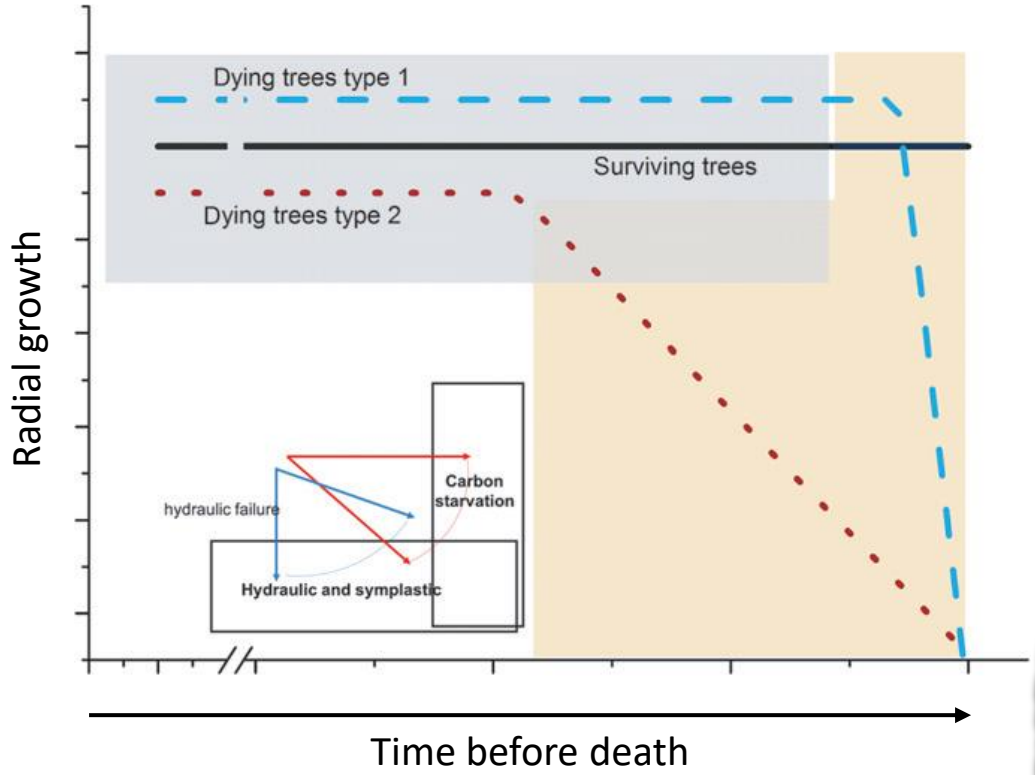
Should be more prone to

Hydraulic failure

Carbon starvation

Or long term-term hydraulic deterioration ?

Gessler et al., 2018



Expected growth patterns for dying trees with different strategies

Gärtner, H., & Schweingruber, F. H. (2013). *Microscopic preparation techniques for plant stem analysis*. Remagen-Oberwinter: Verlag Dr. Kessel

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https://insidewood.lib.ncsu.edu/search;jsessionid=RCRVYdzLg_22xnyOt3w0wAzc_TXaMq17vGMl2Vfa?0

IAWA list of microscopic features for hardwood identification

IAWA list of microscopic features for softwood identification

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