A referential radial file to access to the xylogenesis of temperate angiosperm species.

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Context

- **Wood formation dynamic** in **angiosperms** is still not explained partly due to the complex wood anatomy.
- Current studies focused on the xylem and developmental **zone increments of fibers** elements (Čufar et *al.* 2008, Giagli et *al.* 2015, Prislan et *al.* 2018).
- Comparatively **wider diameter of vessels** to other xylem cell types in angiosperms induce **spatial shifts** of xylem cell arrangement enhancing difficulties to follow radial line produced by one cambial cell.
- To understand the impact of the structure changes (vessel characteristics) induced by climate on the hydraulic performance, as the conduction of the sap, over the growing season, a **reliable estimation** of the **timing and kinetics (**duration and rates of formation) of **vessel formation** is needed.

Objective

To develop an **inclusive methodology** to all temperate angiosperms species, i.e. diffuse porous and ring porous wood structure with potential **adjustable steps** in regard to the studied wood structure.

Material

- Selection de 3 deciduous species with different wood structure:

Species	Site	Wood structure
European beech (*Fagus sylvatica* L.)	Štitna nad Vláří 49°02'01''N, 17°58'12''E; 550 m a.s.l.	Diffuse porous
Pedunculate oak (*Quercus robur*)	Lanžhot 48°40'54''N, 16°56'47''E; 155 m a.s.l.	Ring porous
Narrow-leafed ash (*Fraxinus angustifolia*)		Ring porous

- Wood formation monitored by weekly microcore sampling from April to November in 2018.
- Counting of all xylem cells in the different developmental stages each week by manual clicking on the center of each cell (Y and X coordinates). Number of radial file of the analyzed zone by clicking on cambial cells.
- Vessel area measured of vessel in lignification and mature phases (LM group).

Assumptions and Principles

1. Fibers and axial parenchyma cells display similar **final cell size**. Thus, a fiber present at the same xylem increment value as an axial parenchyma cell were produced at the same time.
2. A vessel was produced the same day as the fiber or the axial parenchyma cells present at the base of this vessel.
3. All vessels in xylem are not spatially and so, temporally, connected enhancing "**temporal gap**" in vessel dynamic reconstitution. At the contrary, by selecting a wide zone, it is possible to reconstruct a radial line constituted only by fiber and axial parenchyma cells and to use it as temporal reference.

Construction of the RRF and vessel indexation

Procedure in 4 main steps

Sectoring: gird creation

- Temporal layers *i* = defined width of xylem increment
- Variable radial width of the radial files r_j

Labelling

- Type of cells within polygons: assessment of the first layer when appears the vessel

Referential radial file (RRF) calculation

- Weighted mean of Axial Parenchyma and Fiber (APF) cell number per layer in regard to polygon labelling = number of APF produced for a defined width of xylem increment

Vessel indexation: Index$_v$ of the vessel in the polygon $ij = \sum_{l=1}^{i} \mathrm{APF}_{RFF} + APF_{pol\,ij} + 1$

- Cumulated number of APF of the RRF of the previous layers *l-i*
- Number of APF within the first polygon where the vessel is detected

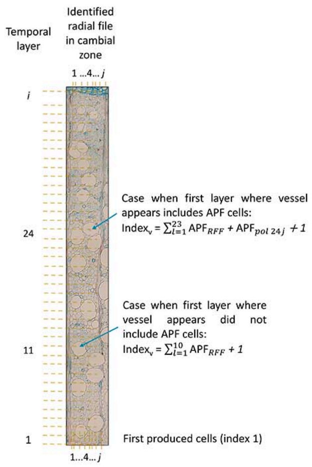

Figure 1: Principle of image sectoring (radial line and temporal layer) and vessel indexation in *Fagus sylvatica* wood sample.

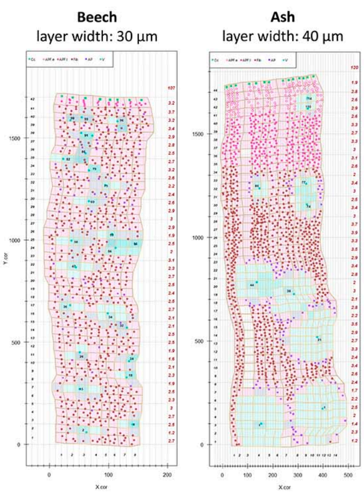

Figure 2: Outputs of the image analysis to estimate the referential radial file. Left: slide of *Fagus sylvatica*. Right : slide of *Fraxinus angustifolia*. Pink polygons are APF polygons, blue polygons are vessel polygons, light purple polygons are APF + vessel polygons Red numbers represent the APF number of the RFF for each layer *i* and the total number (top). Grey number are layer *i* and radial line *j* identifiers. Number within vessel indicate their estimated index.

Critical dates estimation

Case of study of the European Beech over the 2018 growing season

Fitting and data manipulation

- Adjusting GAMs on RRF to estimate the number of APF per DOY and the date of occurrence of each APF
- Vessel reindexation within to the fitting RRF values
- Vessel index *v* appeared the same day as the APF displaying the same index

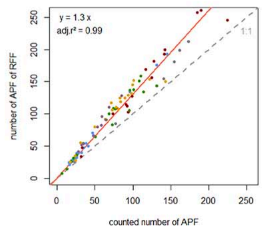

Figure 3: Comparison between the number of APF of the referential radial file and the mean APF counted per radial file. (one color per tree, n= 94)

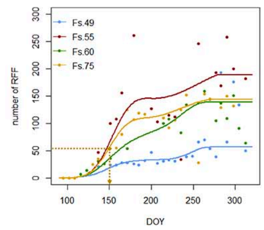

Figure 4: Adjusted GAM on the RRF number to obtain the date of occurrence of cell of the RRF. Yellow dashed line showed that the cell of index 51 of the tree 75 was produced the DOY 150,

Onset of enlargement and lignification phases

- Attribution of developmental phase of each fitted RRF in regard to vessel indexation and there developmental phase
(ex: if vessel indexes 1 to 25 are in lignification phase, APF 1 to 25 of RRF will be labelled as lignified cells)
- Adjusting GAMs following Cuny et *al.* (2013) procedure

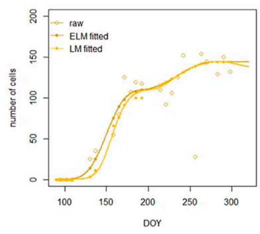

Figure 5: Example of adjusted GAM on the ELM (cells in enlarging, lignification and mature zones) and LM (cells in lignification and mature zones) cell groups of the RRF on the Beech tree number 75.

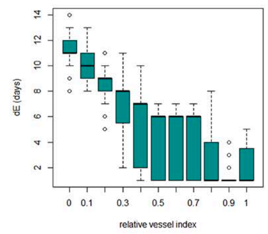

Figure 6: Boxplot of duration of enlargement phase (dE) in days in function of the within tree-ring position of the vessel (earlywood = 0, cambial zone = 1). Number of trees = 5.

To sum up

- The proposed methodology displays reliable results for diffuse porous wood structure which can be used as input on the current developed models at cellular level.
- Improvements will be integrated in vessel polygon identification step for ring porous wood structure (case of 2 wide adjacent vessels). This includes future tests on oak wood samples.
- Preliminary results on *Fagus sylvatica* showed a dynamic of cell production in two times. Despite the RFF values variability and non-normalization of the data, the trends between tree appears synchronous.
- Large differences in dE for *Fagus sylvatica* vessels despite similar vessel size.

This work was supported by Global Change Research Institute of the Czech Academy of Sciences

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