Dendrochronologia 32 (2014) 205–209

Contents lists available at ScienceDirect

Dendrochronologia

journal homepage: www.elsevier.com/locate/dendro

TECHNICAL NOTE

Optimizing cell-anatomical chronologies of Scots pine by stepwise increasing the number of radial tracheid rows included—Case study based on three Scandinavian sites

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ARTICLE INFO

Article history:
Received 4 September 2013
Accepted 7 February 2014

Keywords:
Tracheids
Cell anatomy
Time series
Scandinavia

ABSTRACT

Cell-anatomical studies are a fast growing branch of dendrochronology, since they promise additional environmental proxy information with high temporal resolution. It is unclear, however, how many radial files of tracheids have to be considered to establish reliable wood anatomical time series for conifer species. Here, we investigate this question for four common cell-anatomical variables (cell-wall thickness, lumen area, lumen diameter and cell diameter) in Scots pine using examples from three Scandinavian sites. Cell-anatomical time series averaged from six measurement paths can reach the 90% confidence level of ten measurement paths at all sites. While lumen area generally required the most measurement paths in other cell-anatomical variables, cell-wall thickness required the least measurement paths.
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Introduction

Annual tree rings are the result of radial growth of wood cells and wood cells present intra- and inter-annual variability of diameter, wall thickness and length (e.g., Wodzicki, 1971; Panyushkina et al., 2003; Deslauriers and Morin, 2005; Seo et al., 2011, 2012). Despite the increasingly realized potential of cell-anatomical variables, most tree-ring studies still rely on annual tree-ring width, density or stable isotopes to reconstruct past environmental conditions (e.g., Briffa et al., 1990; Grudd, 2008; Dorado Liñán et al., 2011; Esper et al., 2012), mostly because annual resolution is often sufficient and the time needed to establish reliable cell-component chronologies is high.

In deciduous tree species, vessels have been often the primary research focus (e.g., Woodcock, 1989; Sass and Eckstein, 1995; Pumijumnong and Park, 1999; García-González and Eckstein, 2003; Fonti and García-González, 2004), because they are relatively easily detectable and measurable. In coniferous tree species, however, where tracheids form approximately 90–98% of all wood cells, data acquisition is much more time consuming. Studies on cell-anatomical variables of tracheids in conifers to obtain environmental signals other than tree-ring width or density have been recently increasing in number, mainly due to improved equipment and software for sample preparation and measurements (e.g., Vaganov, 1990; Spiecker et al., 2000; Silkin and Kirdyanov, 2003; DeSoto et al., 2011; Seo et al., 2012; Bryukhanova and Fonti, 2013; Liang et al., 2013; von Arx and Carrer, 2014). In these studies, the data acquisition was mainly done along three to five randomly selected radial files (or measurement paths) per individual tree. This number seems somewhat arbitrary, since the number of measurement paths appears to be crucial for the quality of the resulting cell-anatomical chronologies.

The aim of our study is to contribute to this question by testing, how many measurement paths of cell-anatomical variables (i.e., lumen area, cell-wall thickness or cell diameter) are necessary to build statistically reliable cell-anatomical chronologies from Scots pine growing under natural conditions in Scandinavia.

Materials and methods

Study sites

Our analysis has been performed on Scots pine (*Pinus sylvestris* L.) trees from three sites in northern Europe: (1) Virkan (VN)/Norway and (2) Kevo (KF)/Finland are located near the northern treeline of Scots pine where the local climate is subarctic

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http://dx.doi.org/10.1016/j.dendro.2014.02.002
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Table 1
Study sites, meteorological data (1989–2008) and study trees.

Site/country	ID	Lat./long./m a.s.l.	Temp. (°C)			Prec. (mm)	Trees	
			Ave.	Min.	Max.		Age	D^a (cm)
Virkan/Norway	VN	69°39′ N/18°49′E/100	3.5	−2.9	12.2	1110	184	25.5
Kevo/Finland	KF	69°45′ N/27°01′E/101	−0.9	−12.8	13.3	443	123	28.0
Salmisuo/Finland	SF	64°46′ N/30°58′E/150	2.8	−9.0	16.4	666	39	5.0

[a] *D*: diameter at the height of sampling.

(Table 1). Due to the influence of the Gulf Stream, however, it is warmer (average annual temperature 3.5 °C) and wetter (annual sum of precipitation 1110 mm) at VN than at KF (−0.9 °C/443 mm). (3) At Salmisuo (SF)/Finland, Scots pine is growing on top of a peatland in eastern Finland approximately 500 km south from VN and KF (Table 1). SF is characterized by changing ground-water levels.

Scots pine samples were collected in 2009 (KF), 2011 (VN) and 2012 (SF). The wood samples at KF and VN were taken by an increment borer (Ø 5.2 mm) at 1.4 m above the ground and at SF as a disk at ca. 0.9 m above the peatland surface. The number of tree rings at KF, VN and SF are 123, 184 and 39 years, respectively. We used the last 20 tree rings for the wood anatomical analysis.

Sample preparation for light microscopy

The increment cores, one from VN and one from KF, were cut into short pieces (≤2.5 cm in length) with respect to the size of the embedding forms; moreover, these separating cuts were made obliquely to enable a precise re-assembly of the images into a continuous time series after microscopy. The short pieces were embedded in polyethylene-glycol (PEG) 2000 and then cut by a sliding microtome to a thickness of 7–9 μm. The small disk from SF was cut in 1 cm × 2 cm × 1.5 cm blocks from which thin cross-sections were obtained without embedding. All transverse thin sections were stained with safranine (1%, 2 min) and astrablue (0.5%, 3 min) to enhance the contrast between cell walls and lumina. Then, these transverse thin sections were dehydrated using ethanol (40%, 70%, 95% and 100%) in order to fix them on slides with Euparal.

Measuring the cell-anatomical variables

Digital images for VN and SF were captured from the thin sections by a digital camera (DFC450 C, Leica Microsystems AG) (resolution of 2560 × 1920 pixel) installed on a light microscope (DM2500, Leica Microsystems AG) and for KF by a digital camera (AxioCam MRc, Carl Zeiss AG) (resolution of 1388 × 1040 pixel) installed on a light microscope (Axiovert25, Carl Zeiss AG). In both cases, a 20× magnification (objective) was applied. The single digital images covering the complete transverse area of a tree ring were merged into one file by the software Adobe Photoshop CS4. On these composed images, ten tracheid rows (measurement paths) were selected while avoiding resin ducts, ray cells and tracheids abruptly changing their shape (Fig. 1).

Four cell anatomical variables of the tracheids, namely lumen area (LA), lumen diameter (LD), cell-wall thickness (CW) and cell diameter (CD), were measured across each tree ring from the pith to the cambium (WinCELL Pro software (Régent Instruments Inc.)) and averaged for early- and latewood, respectively. Early- and latewood were determined by Mork's formula (1928). Since there were no age-related trends in our data, we performed all analyses on raw data.

Optimizing test

For the analysis of how many measurement paths are necessary to establish statistically reliable chronologies, we calculated the mean time series of each variable by averaging a progressive number, from one to nine, of randomly selected measurement paths with 10,000 replications for each number of measurement paths. These series were then grouped together per number of measurement paths used to build them, with each of the nine groups having 10,000 replicates. The Merssenne-Twister algorithm (Matsumoto and Nishimura, 1998), implemented in the base package of R 2.4.12. (R Development Core Team), with seed set by set.seed function and an initial value of 1000 was used to generate pseudo-random numbers needed for the selection of measurement paths.

Groups of time series were used to estimate the optimal number of measurement paths needed to get a statistically significant signal as well as to determine the inter-row variability of the cell elements. The optimal number of rows was estimated by two methods:

1. Correlating the series with a reference series obtained by averaging all ten measured paths and calculating the median correlation for each number of measurement paths.
2. Calculating the median correlations from the correlation matrix of the series built from a certain number of measurement paths.

Both methods use the Pearson's *r* index of correlation and determine confidence intervals for the calculated correlations at the 90% and 95% levels of significance. For this study, the optimal number of measurement paths was considered to be the minimum number of paths needed to achieve 0.90 median correlations.

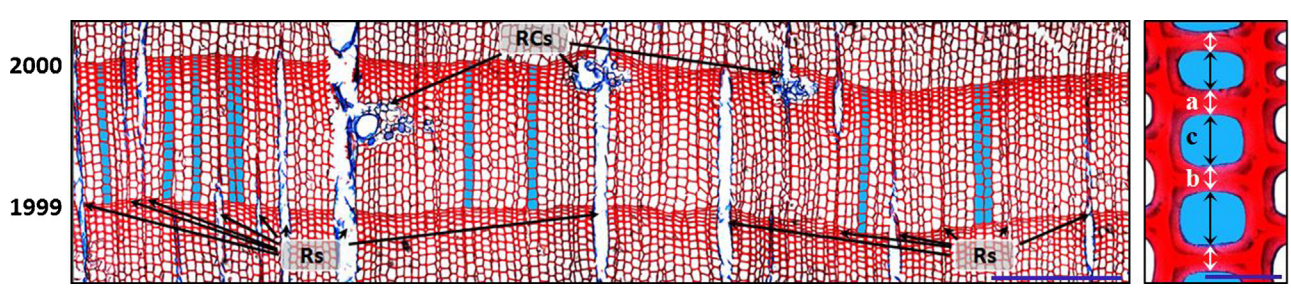

Fig. 1. Left: Transverse thin section from an increment core (*Pinus sylvestris* L.) at Virkan in Norway (VN) with ten selected rows (in blue) of tracheids in radial direction (path); Right: Definition of lumen area (LA = green color), radial lumen diameter (LD = *c*), cell-wall thickness (CW = (*a* + *b*)/2) and cell diameter (CD = LD + CW). RCs: resin canals, Rs: rays. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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