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Technical note

tracheideR—An R package to standardize tracheidograms

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ABSTRACT

Here we present the package tracheideR to standardize profiles of tracheid features, using the R computing environment. This package contains a collection of functions to transform the raw data obtained from image analysis into a tracheidogram to better visualize the radial intra-ring variation of histometric parameters. This procedure is crucial when estimating past weather conditions with a sub-annual resolution, since tracheidograms reflect the influence of fluctuations in weather conditions throughout the growing season (such as temperature and soil water content). The main function of this package is `tracheider`, which takes as input raw tracheidograms and standardizes them using three different methods. The first method standardizes the number of tracheids from different radial files to the mean number of cells, allowing that different annual rings have different number of cells. The second method normalizes the number of cells of different annual rings to the same number. Finally we present a new method to standardize histometric parameters considering the relative position of the cells within the tree ring. This package was tested using two rings of *Pinus pinaster* to demonstrate variations between the three methods. According to our results species with high intra- and inter-annual variability, as shown by conifers species growing under Mediterranean climate, should be standardized using the "relative position" method. Finally, we suggest that this new method should be applied to other species to check its potential to detect intra-ring fluctuations in tracheid features and to improve our capacity to detect intra-annual climatic signals.

1. Introduction

Tree rings are one of the most reliable natural archive of past climate, being traditionally used as a proxy for climate reconstructions (Briffa et al., 2004; Büntgen et al., 2009; Esper et al., 2002). Tree-ring width has an annual resolution although xylem formation occurs continuously during the growing season (Vieira et al., 2014a). Thus most of the climatic information stored in xylem cells is lost when tree-ring analysis is restricted to ring-width variables. Recently, a growing interest in intra-ring anatomical features has emerged due to the technological and methodological advances in quantitative wood anatomy (Rossi et al., 2006; Gärtner et al., 2014, 2015; von Arx and Carrer, 2014). Studies of intra-ring anatomical features allow a finer resolution of the climatic signal present in tree rings, improving our understanding of the relationship between climate and tree growth (Campelo et al., 2015; De Micco et al., 2014; Vieira et al., 2010). Such studies include monitoring the formation of tree-rings by following the xylogenesis throughout the growing season and the study of vessels and tracheids properties retrospectively (Carvalho et al., 2015; Vieira et al., 2015). Studies on vessel features, such as lumen area or vessel number, have proven to improve the extraction of climatic signals from tree rings of hardwood species (Campelo et al., 2010; García-González and Fonti, 2006). In conifers, the study of tracheids properties may be achieved by studying the frequency of intra-ring features such as intra-annual density fluctuations (IADFs) or false rings (Campelo et al., 2013, 2007; De Luis et al., 2007; Rozas et al., 2011; Vieira et al., 2010) or by tracheidogram studies (Carvalho et al., 2015; DeSoto et al., 2011; Fonti et al., 2013; Olano et al., 2012), which consists in analyzing the histometric parameters of tracheids over the entire ring (Vaganov, 1990). In the last years studies using tracheidograms have thrive due to the development of new image and data analysis methods (Fonti et al., 2013; von Arx and Carrer, 2014). However there are still several problems regarding the correct standardization of tracheidograms to make them comparable among different trees and years (De Micco et al., 2012; Gartner et al., 2002).

One of the most recognized methods to standardize tracheidograms is the one proposed by Vaganov (1990). This method involves normalizing the number of cells of different years to a constant number. Although this method is reliable for environ-

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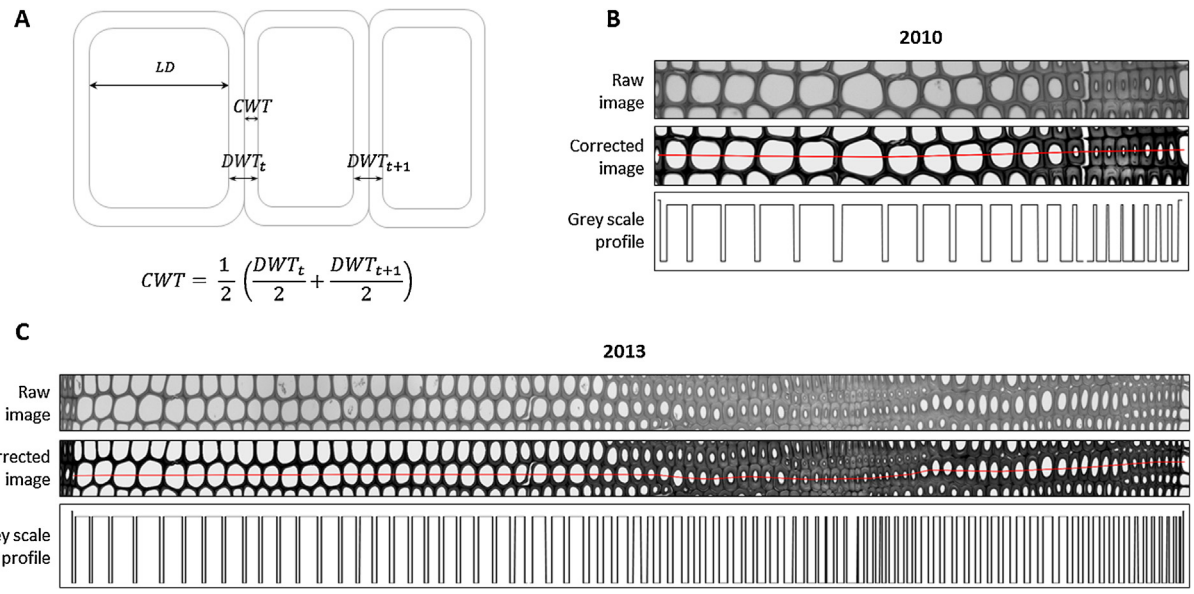

Fig. 1. Procedure of the anatomical measurements. (A) Scheme of the measured anatomical variables: LD, radial lumen diameter; DWT, double cell wall thickness; CWT, cell wall thickness; (B) and (C) raw image of rays from the 2010 and 2013 rings, respectively; corrected image with ray in red and correspondent grayscale profile. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ments with little variation in tracheid production between years, in other environments where there is high year-to-year variability, such as the Mediterranean, this method underestimates the variability observed and significant climatic signals might be lost during the standardization process. For example, DeSoto et al. (2011) standardized tracheidograms of *Juniperus thurifera* L. using an adapted version of the Vaganov method where they selected the average number of earlywood and latewood tracheids of the studied tree rings, which was 20 and 3 respectively. However, in species with high variation in the number of tracheids, such as *Pinus pinaster* Ait., where there can be differences higher than 20 tracheids between consecutive years (Carvalho et al., 2015; Vieira et al., 2015), this approach cannot be applied. Moreover, this method is unable to determine the relative proportion of IADFs, which are common in conifer species growing in the Mediterranean region (Campelo et al., 2015; De Luis et al., 2011; De Micco et al., 2007). Thus, a new method of standardization is necessary. Here we present a new method where tracheidograms are standardized based on the relative position (*relPos*) of each tracheid within the ring and compare it with the two previous methods, in order to demonstrate the importance of the standardization method. In the first method the average number of tracheids observed in each year is used to standardize the tracheidograms (*nCells*), and in the second one a constant number of cells is used (*kCells*).

2. Material and methods

Two rings of *P. pinaster* with distinct growth patterns were selected to illustrate the differences between the methods (Fig. 1). One ring is small without density fluctuations, whereas the other ring is wider and shows a latewood IADF. Microsections of the two rings were analyzed under a light microscope under 200× magnification and images captured using a digital camera fixed on a microscope (image resolution: 0.169 μm/pixel). The freeware program ImageJ was used to analyze tracheid features on digital images (Rasband 1997–2015). First, for each ring three rays were selected and the "plot profile" function applied to obtain a vector of gray values (0–255) along each radial tracheid file (Fig. 1). For each ray, the radial lumen diameter (LD), the radial cell wall thickness (CWT), the ratio of radial lumen diameter to radial cell wall thickness (LD/CWT) and the number of tracheids (*n*) (Fig. 1A) were calculated using the `getTrac` function, an adapted version of the function `tgram` from the R package "tgram" (DeSoto et al., 2011). The function `tracheider` in the R package "tracheideR" was used to obtain standardize tracheidograms using three different methods:

- variable number of cells (*nCells*): this method standardizes different rays of a given ring using the mean number of cells, allowing that different rings show different number of cells;

Table 1
Comparison of the proportion of earlywood (EW), latewood (LW) and intra-annual density fluctuation (IADFs) using the three standardization methods *nCells*, *kCells* and *relPos*. *n* and *k* are the number of tracheids used in each standardization method.

		Standardization method										
		nCells				*kCells*				*relPos*		
		n	EW (%)	LW (%)	IADF (% of LW)	*k*	EW (%)	LW (%)	IADF (% of LW)	EW (%)	LW (%)	IADF (% of LW)
2010	ray1	22	12 (54.55)	10 (45.45)	–	53	30 (56.60)	23 (43.40)	–	72	28	–
	ray2	20	12 (60.00)	8 (40.00)	–	53	31 (58.49)	22 (41.51)	–	77	23	–
	ray3	19	12 (63.16)	7 (36.84)	1 (14.29)	53	33 (62.26)	20 (37.74)	2 (10.00)	77	23	3 (13.04)
	mean	20	12 (60.00)	8 (40.00)	–	53	32 (60.38)	21 (39.62)	–	76	24	–
2013	ray1	89	37 (41.57)	52 (58.43)	17 (32.69)	53	22 (41.51)	31 (58.49)	10 (32.26)	53	47	19 (40.43)
	ray2	82	35 (42.68)	47 (57.32)	18 (38.30)	53	23 (43.40)	30 (56.60)	11 (36.67)	56	44	19 (43.18)
	ray3	85	38 (44.71)	47 (55.29)	24 (51.06)	53	27 (50.94)	26 (49.06)	13 (50.00)	63	37	19 (51.35)
	mean	85	37 (43.53)	48 (56.47)	19 (39.58)	53	23 (43.40)	30 (56.60)	12 (40.00)	55	45	19 (42.22)