ENSURING A REPRESENTATIVE SAMPLE OF EARLYWOOD VESSELS FOR DENDROECOLOGICAL STUDIES: AN EXAMPLE FROM TWO RING-POROUS SPECIES

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Abstract

The analysis of time-series of wood cell anatomical features (such as the earlywood vessels of ring-porous trees) is a successful approach to understand the effect of environmental factors on tree growth and thus constitutes a valuable source of information about past environmental conditions. However, despite the rising interest in analyzing wood anatomical time-series, little or no attention has been paid to establish an adequate sample of cells in order to minimize the risk of missing a valuable environmental signal.

In order to contribute to such methodological bases, this paper is aimed at i) identifying a representative sample of earlywood vessels within a tree which encode the same climatic information and ii) assessing if it is preferable to obtain the sample of vessels along one or two radii. Four individuals of sessile oak (*Quercus petraea* (Mattuschka) Liebl.) and sweet chestnut (*Castanea sativa* Mill.) were harvested and all their earlywood vessel lumina measured along two 40-mm wide radial strips. Measured vessels were stepwise selected while increasing tangential width of the wood section from 1 to 40 mm, analyzing at each step i) the common signal of chronologies and ii) the correlation to the main climatic variables controlling growth. Additionally, both radii in each tree were analyzed together and separately.

The results showed that a total tangential width of 10 mm was enough to stabilize the climatic signal, with improvement if it is distributed along two different radii, but a slightly larger tangential width is required to reach an optimal common signal.We suggest that, at least for the case of these two species growing at this specific climatic context, two 5-mm increment cores ensure a representative vessel selection.

Keywords: Dendrochronology, tree rings, earlywood vessel size, ring-porous, environmental signal, *Castanea sativa* Mill., *Quercus petraea* Mattuschka (Liebl.)**.**

Introduction

Tree growth and consequently wood formation are strongly influenced by the environment (Denn and Dodd 1981), causing variations in wood characteristics that can be analyzed in datable tree rings and provide valuable information about plant functioning and past environmental conditions (e.g. Schweingruber 1996). This information is usually extracted by means of tree-ring proxies such as ring width, maximum density or isotopic content in wood.

Recent studies on water conducting elements of hardwoods show that year-to-year variations in their dimensions are capable of encoding valuable ecological information (Eckstein 2004), which varies among species, climatic regions and anatomical features considered. For example, vessel size of different ring-porous oaks (*Quercus* spp.) recorded temperature signals in a dry inneralpine valley (Eilmann et al. 2006) and at their northern distribution limit in Canada (Tardif and Conciatori 2006), but also precipitation under oceanic climate (García-González and Eckstein 2003). Analogously, earlywood vessel size of sweet chestnut (*Castanea sativa* Mill.) registered spring temperature in the southern Swiss Alps (Fonti and García-González 2004).

The use of time series of vessel features is becoming more popular (e.g. Pumijumnong and Park 1999, 2001; Corcuera et al. 2004, 2006; Schume et al. 2004; Verheyden et al. 2005; Eilmann et al. 2006), but there is no common practice in their sampling procedure. The identification of ecological signals in wood anatomical features also depends on how vessels are sampled within each annual ring. Different studies on water conductive elements differ in the sampling procedures, including the number of radii per tree (one to three), the tangential width of the radii (from 2.4 to 8 mm) and the criteria applied to select the vessels (visual criteria, fixed number, size threshold or *n*-largest vessels; e.g., see methods in Fonti and García-González (2004), Tardif and

Conciatori (2006), St. George et al. (2002)). This diversity of procedures causes large differences in which and how many vessels are considered for the analysis and can influence the final results of the studies considerably.

St. George et al. (2002) first dealt with the problematic of vessel sampling for *Quercus macrocarpa* Michx., arguing that 20 vessels per ring (threshold for vessel detection > 1,200 μm^2) were enough to provide a good estimate of mean vessel size (i.e. the standard error of mean stabilized at a low level). However, they did not discuss if this selection was representative for the expression of the environmental signal. More recently, a methodological study on earlywood vessels of chestnut (threshold for vessel detection > 10,000 μm^2) showed that climate-growth relationships varied considerably depending on which earlywood vessels were considered for the analysis (García-González and Fonti 2006). In this particular study, correlations to March temperature changed from highly significant to non-significant along with the size criteria used to select the vessels within an 8 mm wide tangential strip. The best result was achieved when only vessels bearing the same information, e.g., only the earlywood vessels in the first row, were considered. However, it has not been verified if previous approaches to select earlywood vessels with the same signal result in a representative sample size.

The current study is specifically aimed at identifying the optimal sample of earlywood vessels to be considered representative to build a time series for each tree. We assume that vessel sampling is representative when an additional increase of vessels bearing the same information does not further improve the quality of the chronology (common signal) or the relationships to climatic variables (climatic signal). In particular, we progressively increased the tangential width on one or two radii for four individuals of chestnut and oak, computed chronologies of mean vessel area and compared them to meteorological data, following the changes in i) the common and ii) climatic signal.

Material and methods

Wood material and earlywood vessel survey

The work was carried out on circular stem discs (with a well-centred pith) of 50-year-old dominant stools of chestnut (*Castanea sativa* Mill.) and sessile oak (*Quercus petraea* (Mattuschka) Liebl.). Four discs for each species (8 in total) were selected from an oak-chestnut coppice forest from the southern Swiss Alps (Bedigliora, lat. 46°00’ N, long. 8°50’ E). Stem discs were cut at a height of 50 cm above ground. Cross-sections were sanded to 400-grit, ring widths measured and rings accurately dated. Crossdating was validated using COFECHA (Holmes 1983).

The measurement of earlywood vessels was performed on digital images of wood surface along two 40 mm-wide radial strips on each disc (A and B) that were captured with a 4800-dpi resolution scanner (Epson Perfection 4990 Photo, Seiko Epson Corporation, Japan) (Fig. 1). To improve vessel recognition the earlywood vessel lumina were first cleaned with a high-pressure water blast. Afterwards, the strips were coloured black using a marker pen and vessel lumina filled with white chalk powder. Images were semi-automatically analysed using the “Image Pro Plus” digital analysis software (version 1.3 for windows; Media Cybernetics, Inc., Silverspring, MD, USA). The program was set up with filters (morphological 2x2 squares, 1 pass) and an image enhancer (equalize, best fit) in order to optimize vessel outlines.

Earlywood vessels were measured ring by ring from 1975 to 1999 (25 years); rings prior to 1975 were not used to avoid the presence of juvenile wood, which has a strong growth trend with smaller and more numerous vessels (Gasson, 1987; Helinska-Raczkowska, 1994). Earlywood vessels which were misrecognized by the software had to be manually corrected. The lumen cross-sectional area and the position (coordinates) of each vessel were recorded. Only vessels larger than 10,000 μm^2 were considered, so that small vessels produced later in the season, which probably encode a different signal, were not considered into the calculation. This lower limit proved to be successful in previous works (Fonti & García-González, 2004; Fonti et al., 2007), whereby climatic signals in the early season were maximized by the largest vessels (García González & Fonti, 2006).

Chronology computation and climate-growth relationships

For each ring, we considered two values of mean vessel area (MVA): one for all earlywood vessels and one for only those belonging to the first row. The latter integrate a smaller data set but have surely the same ontogeny, and can sometimes lead to more successful results when comparing to climate (e.g., Fonti et al., 2007; García-González & Fonti, 2006). Average chronologies were built from MVA time series and served to establish climate-growth relationships. The analyses were performed separately for radius A or B (at least 90 degree apart), as well as combining A and B (A+B); in the latter case, both series were averaged into a curve per tree.

For chronology computation, non-desired growth trends (related to age and forest dynamics) were removed from the MVA individual series by fitting a cubic smoothing spline (32-year stiffness, 50% frequency cuttoff) and dividing each value by the function (Cook et al. 1992). The detrended series were then averaged into chronology's growth indices (Fritts 1976). The quality of the chronology, i.e., the quantification of the common signal, was assessed through the mean correlation between trees (Rbt), mean correlation within trees (Rwt), and the standard error of the chronology (SE) (for a more detailed explanation see Briffa and Jones (1992), Fritts (1976) and Wigley et al. (1984)). When combining radii A and B, chronology error was calculated considering their average (A+B) and both separately (A&B), and using the correction for sample size suggested by Briffa and Jones (1992) for more than one radius per tree.

Climate-growth relationships were established by means of correlation functions, i.e., computing Pearson's correlation coefficient between the MVA chronologies and monthly values of mean temperature and total precipitation from previous May to current June. Climatic data came from the nearby weather station of Lugano (MeteoSwiss, Locarno-Monti), located 10 km from the study plot, which has a complete record for the study period.

Progressive selection of vessels

The first step was to verify if the signal within the earlywood vessels of the four selected trees per species was in agreement with similar studies in the same region (Fonti & García-González, 2004; García-González & Fonti, 2006; Fonti et al., 2007). Then we assessed the influence of sample size on chronology quality and climate-growth relationships by progressively selecting the earlywood vessels according to their tangential position within each radial strip of 40 mm. Computations started with a tangential width of 1 mm, adding progressively the vessels belonging to the following 0.5 mm, until the maximum width (40 mm) had been reached (Figure 1); vessels were considered when their centroid fitted within the width frame. After each selection step, new vessel chronologies were computed, assessed for their quality and correlated to climatic data. When combining both radii, the tangential width represents the added width of them (e.g., 5 mm corresponds to 2.5 mm for each radius).

Variations in chronology common signal, standard error and correlations to climatic data were tracked along with the tangential width considered. Thus, the assessment of an adequate sample width was evaluated according to how fast the results changed or when they stabilized.

Results

Results using the maximal strip width

On a tangential ring width of 10 mm we counted an average of 49.1 earlywood vessels for chestnut and 41.0 for oak, with about half of them in the first row (24.2 for chestnut, 20.1 for oak). Oak vessels are larger than those of chestnut if all earlywood vessels are considered (49,772 μm^2 vs. 45,963 μm^2) but are nearly equal in size for the first row (around 62,000 μm^2).

The main results for the MVA chronologies based on the maximal amount of earlywood vessels considered (i.e., on two 40-mm wide radial strips for each tree) are shown in Figure 2. For chestnut, the signal is better for the first row than for all vessels (higher chronology quality and correlations to climate), whereas the use of all vessels is slightly better for oak. Except for the case "chestnut, all earlywood vessels", values of Rbt are over 0.50 indicating that the four trees have a strong common signal. This common variation in MVA is highly correlated with climatic records for the period 1975-1999. In particular, the first row of earlywood vessels of chestnut responds negatively to March temperature (r=-0.40, P<0.05) and is also positively related to precipitation during the previous late summer (July to September, r=0.54, P<0.01). For oak, all earlywood vessels clearly respond to spring precipitation (March to May, r=-0.69, P<0.001) and also to previous July temperatures (r=-0.61, P<0.01).

Variation in the quality of the chronology

The variations of common signal along with the considered tangential width of the wood section are shown in Figure 3. In all cases, the pattern is initially characterized by a clear ascending trend which stabilizes at 10-15 mm, becoming very close to the value observed when both 40-mm strips are entirely used; at that point, an additional increase in tangential width results in an irrelevant improvement of the correlations (Rbt and

Rwt). For example, if both radii are used (A+B), 80% of the maximum value of Rbt is attained at 7 mm (all vessels) and 9 mm (first row) for oak, and at 12 mm (all vessels) and 7 mm (first row) for chestnut. The trend is similar regardless of considering a single radius (A or B) or both (A+B), but values are higher and stabilize slightly earlier (at a lower tangential width) for the latter. There is an important intra-tree variability of earlywood vessel size as indicated by values of mean correlation within trees (Rwt) that are not clearly higher than those of Rbt.

Likewise common signal, the reduction of the standard error of the chronology (Figure 4) is more efficient when increasing a small tangential width, but a sample size over 10-15 mm hardly contributes to decrease the error. When using only one radius (A or B), chronology error is considerably higher but, for the case of two radii, there is no improvement if processing them separately to compute the error (after Briffa and Jones (1992)) instead of combining them, despite the increase in the number of time series (8 vs. 4). This result confirms the important variation within trees and the considerable reduction of error by averaging two different radii.

Variation in the climatic signal

The correlations between MVA chronologies and climatic factors have a similar trend to the common signal, but a smaller tangential width of only a few millimeters is sufficient to stabilize the results in this case. Figure 5 shows three examples for the variation of significant ($P<0.05$ to $P<0.01$) climatic factors in spring as tangential width increases. In general, the main factors controlling growth are significant from the early beginning (i.e., for only 2-3 mm). For a radial strip around 5 mm (the width of a standard increment core), correlations are very similar to those with the whole 40-mm strip. In general two radii provide a better result than a single one (especially at small tangential widths), but differences between A and B are negligible and either of them is able to identify climate-growth relationships unequivocally.

Discussion

The assessment of an adequate sample size is a very important aspect in tree-ring research. For this, several statistic parameters were proposed and are widely accepted by the dendrochronological community (e.g. Wigley et al., 1984 or Briffa and Jones, 1992). Upon these bases, some attempts are occasionally made to optimize sample size for some specific objectives (e.g., Leavitt and Long, 1984; Mäkinen and Vanninen, 1999). Such methodological works, which usually include only a few trees at specific areas, can provide useful procedures for other researches working on the same field. In the present study, although it had to be limited to only four trees per species and short chronologies of 25 years, the results were highly consistent with similar works for the same species and within the same climatic context (Fonti and García-González, 2004, García-González and Fonti, 2006, Fonti et al. 2007), confirming that the material was representative for the prevailing environmental conditions. Therefore, some indications can be drawn to help with other studies on the earlywood vessels of ring-porous trees, since it demonstrates the importance of an adequate vessel sample.

It has been shown that when enlarging tangential width, both common and climatic signals initially increase rapidly while standard error decreases and they become stable afterwards, though at a different sample size. Values of common signal stabilize at earliest for a total tangential width of 7-12 mm over two radii. This fact explains why common signal in the present study (based on the whole 40 mm strip at two radii) is higher than previously observed for MVA (e.g., García-González and Eckstein (2003) or Tardif and Conciatori (2006)) reported values of Rbt ranging from 0.2 to 0.4 for longer time periods); but similar when tangential width is reduced to that of 1-2 increment cores. Contrary to the common signal, the main climatic signals can be established at a narrower tangential width. A unique core of 5 mm is sufficient to detect a relationship between vessel size and climate, but does not correctly characterize the common signal. This is why very low common signals in previous works (Fonti and García-González, 2004; Fonti et al., 2007) led to satisfactory results when chronologies were compared to climate. The required tangential width to stabilize the signal when only the first row of earlywood is considered is similar to that for all vessels, despite its lower vessel number. These results show how a vessel sample based on a minimum number disregarding for their radial position into the ring can result in an under-expression of the signal (García-González and Fonti 2006). Therefore, a 'minimum tangential width' should be considered rather than a 'minimum number of vessels' for a representative sampling.

Although common and climatic signals are reliable when both radii are considered, the use of a single radius can affect common signal, mainly for narrow tangential width (< 5 mm), but has less impact on the identification of climate-growth relationships. Consequently, within-tree variability needs to be minimized to improve chronology quality, done in this work by averaging the MVA series of both radii. If otherwise all their vessels are pooled for each ring and MVA calculated afterwards, differences in common signal are irrelevant (data not shown). On the other hand, correlations to climate do not differ significantly if vessels are measured on radius A or B, nor do they deviate from the average of both radii (A+B).

The results of this paper showed that an adequate sample size can be established to optimize the amount of earlywood vessels to be measured. However, this procedure depends on the type of material available. The highest confidence would be gained for cross sections or 12 mm increment cores, from which two strips of about 10 mm would be optimal. Choosing a single strip would be expected to lead also to satisfactory results, but common signal would not be maximized. Unfortunately, cross sections are usually not available, and 12 mm cores cannot be extracted in very hard woods, but it is still possible to succeed with 5-mm cores provided that at least two cores are used per tree. Although one single core might be enough for climate-growth relationships, this is required to correctly assess the quality of the chronology. Finally, these working recommendations should be verified for other species and climatic contexts, since the establishment of a representative sample size or earlywood vessels would facilitate their use in tree-ring research and thus yield more ecological information.

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Figures

Fig. 1: Example of *Quercus petraea* cross-section image used for calculations. Size and position of earlywood vessels are surveyed year by year along a radial strip. Time series of mean vessel size are repeatedly computed by progressively enlarging the width of the radial strip so that an increasing number of vessels are included into the calculations. Vessels within the frame correspond to first row vessels of 1995 annual ring; vessel numbers indicate object labels during analysis.

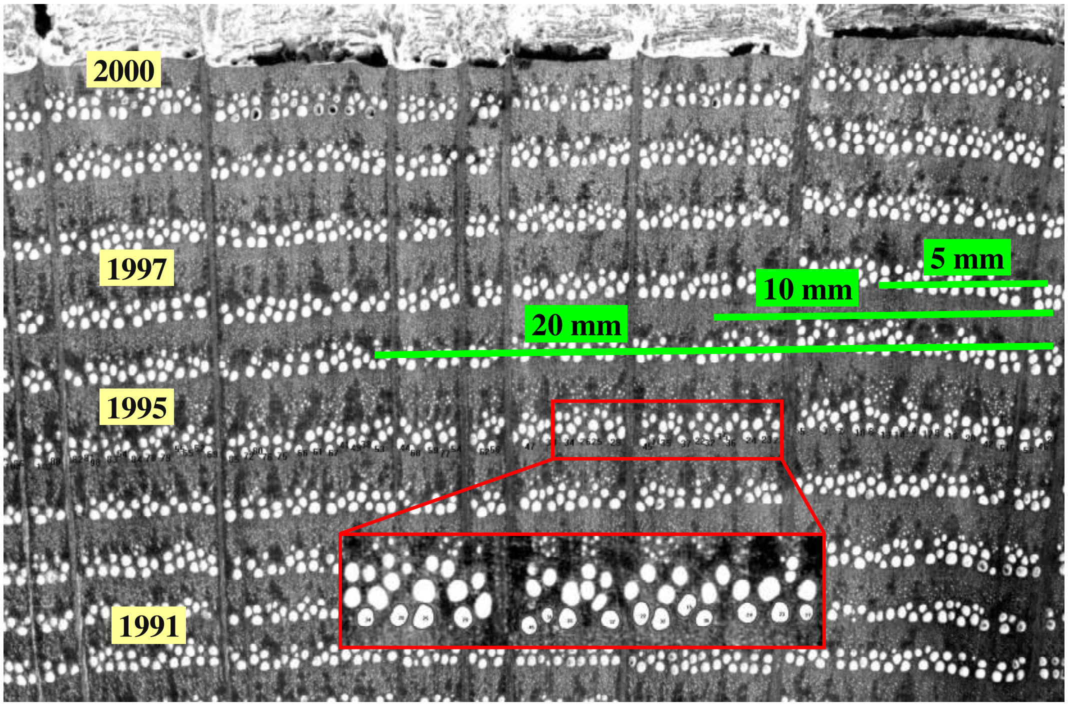
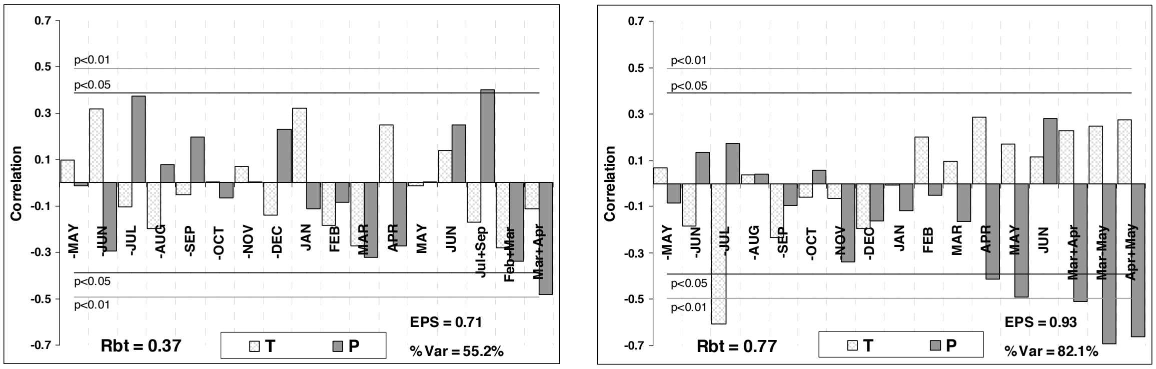


Fig. 2: Simple correlations between earlywood mean vessel area and monthly climatic factors for the period 1975-1999. Data refer to vessel measurements performed on two radial strips of 40 mm in each tree. Graphs are presented separately for all earlywood vessels and for vessels belonging to the first row only. Horizontal lines indicate the significance level (*P*<0.01 and *P*<0.05). Chronology quality is also shown (Rbt = correlation between trees; EPS = expressed population signal; %Var = variance in the first eigenvector of a principal component analysis on the individual series). EPS and %Var are provided only for comparison with other chronologies.

a) Chestnut, all vessels

b) Oak, all vessels



c) Chestnut, first row

d) Oak, first row

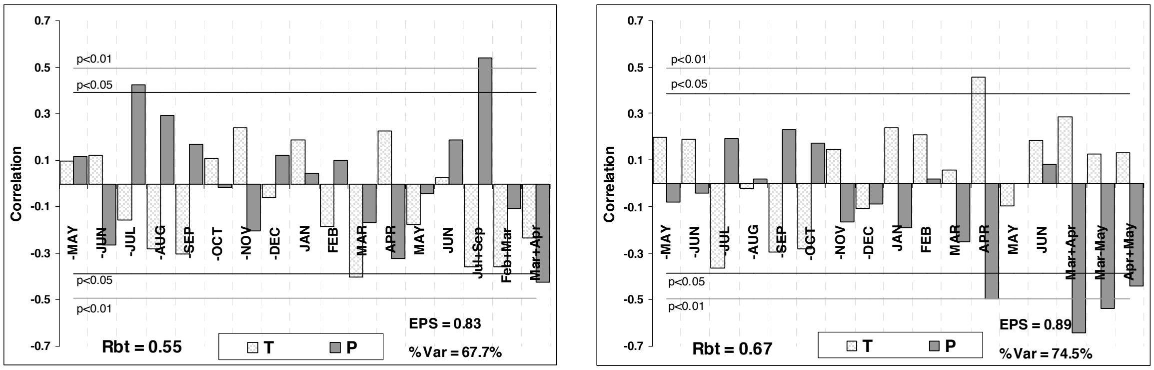
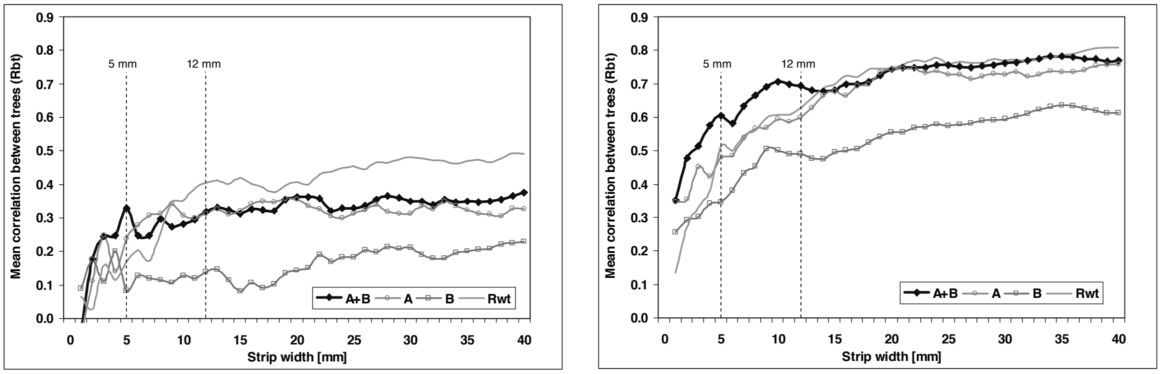
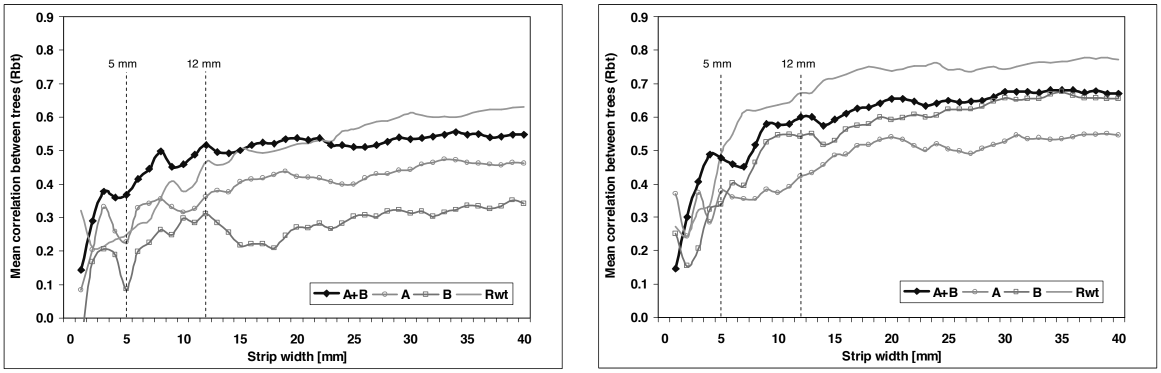


Fig. 3: Variation of common signal (mean correlation between trees) with gradually increasing tangential width. Data refer to measurements performed for both all vessels and the first row of earlywood vessels. Results are presented for both species and for radius A and B as well as for the composite of both radii (A+B). Plain line refers to mean correlation within trees (Rwt).


a) Chestnut, all vessels

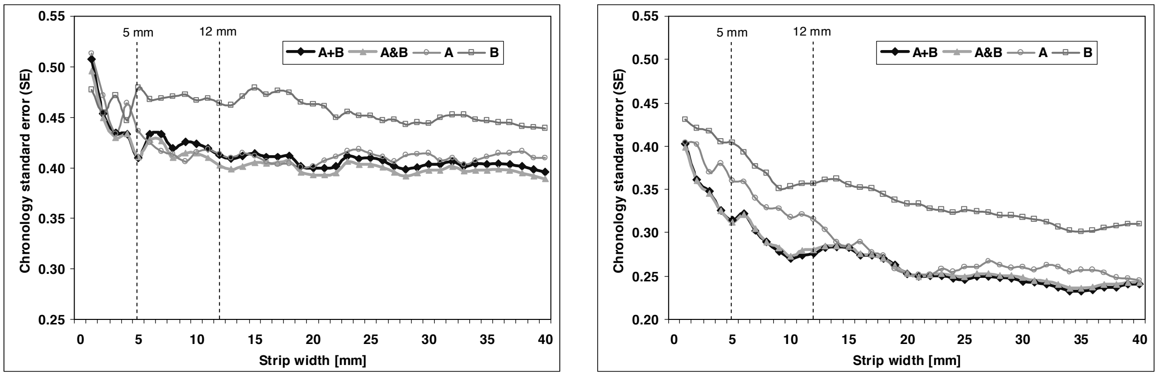
b) Oak, all vessels


c) Chestnut, first row

d) Oak, first row

Fig. 4: Evolution of the standard error of chronology with gradually increasing tangential width. Data refer to measurements performed for both all vessels and the first row of earlywood vessels. Results are presented for both species and when using one (A or B) radius or both; for two radii, standard error is presented for the average (A+B) and when processing both radii separately (A&B).

a) Chestnut, all vessels b) Oak, all vessels



c) Chestnut, first row d) Oak, first row

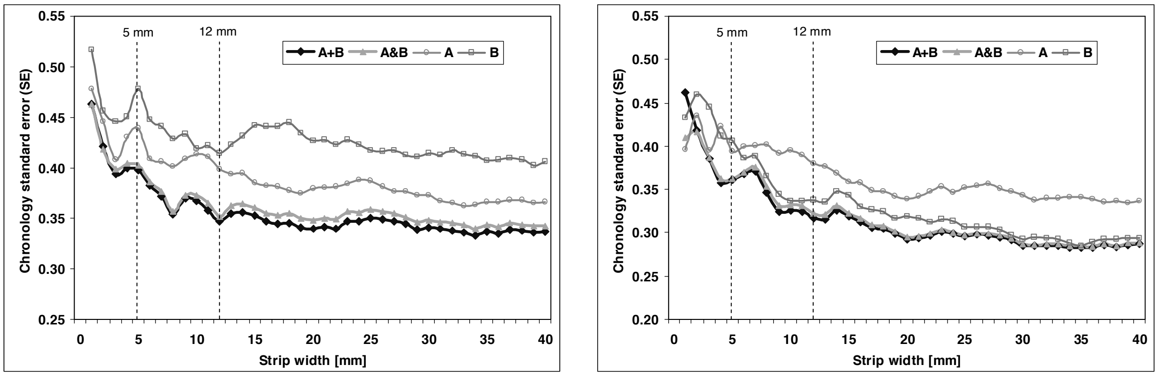
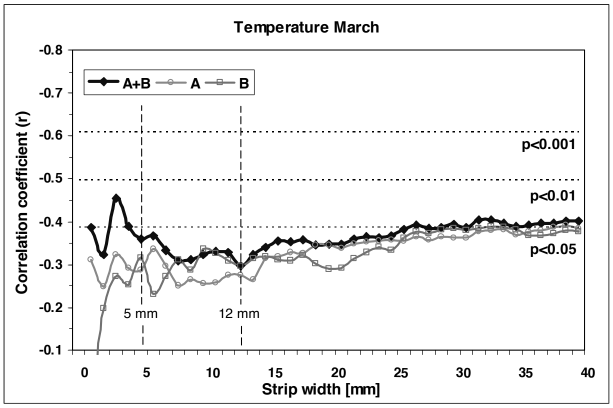
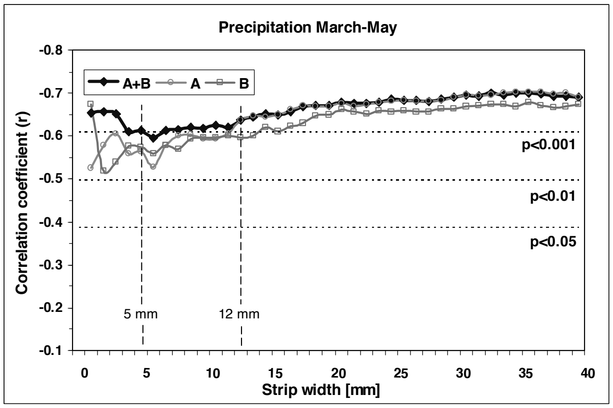


Fig. 5: Examples of variation in growth-climate relationships with gradually increasing tangential width. Results are presented for radius A and B as well as for the composite of both radii (A+B).

a) Chestnut, first row and March temperature



b) Oak, all vessels and March to May precipitation



c) Oak, first row and March to April precipitation