

Suitability of chestnut earlywood vessel chronologies for ecological studies

Patrick Fonti¹ and Ignacio García-González²

¹WSL, Sottostazione Sud delle Alpi, Via Belsoggiorno 22, CH-6504 Bellinzona-Ravecchia, Switzerland; ²Dep. de Botánica – University Santiago de Compostela, Escola Politécnica Superior – Campus de Lugo, E-27002 Lugo, Spain

Summary

Author for correspondence:

Patrick Fonti

Tel: +41 91 8215233

Fax: +41 91 8215239

Email: patrick.fonti@wsl.ch

Received: 10 December 2003

Accepted: 8 March 2004

doi: 10.1111/j.1469-8137.2004.01089.x

- Wood anatomical features measured in dated tree rings have often proven to be of ecological value. However, little is known about the suitability and power of such measurements studied in a year-to-year basis as is done in dendrochronology.
- The present work is based on a comparative analysis of 60 dated time-series of earlywood features of chestnut (*Castanea sativa*) grown in the climatic context of the Southern part of the Swiss Alps.
- It has been shown that the earlywood vessel area is a suitable ecological indicator. This variable, although not very sensitive, contains environmental information that is different from that stored in all other ring-width and earlywood features we considered. The vessel size is mainly related to the temperature during two physiologically crucial periods for vessel growth: the end of the previous vegetation period (during reserve storage) and the onset of cambial activity (during cell division and vessel differentiation).
- Our work shows that the mean vessel size of the ring-porous chestnut contains ecophysiological information that can be used for research in dendrochronology.

Key words: *Castanea sativa* (European chestnut), earlywood, tree rings, dendrochronology, vessel size, ring-porous.

© *New Phytologist* (2004) **163**: 77–86

Introduction

Tree-ring widths are traditionally used as the main feature to describe radial growth. They are easy to measure and have an annual resolution so that time series of this variable can be taken to analyze relationships between tree growth and climate (Fritts, 1976) and to reconstruct past climates. However, dated annual rings contain much more information than merely that integrated in their width. Many anatomical features of the annual rings have proven to be useful for identifying tree responses under a wide variety of environmental events (Schweingruber, 2001; Wimmer, 2002).

One of the main fields in modern dendroecology is the search for additional variables to describe the physiological relationship between tree growth and environmental conditions. Investigations of coniferous wood have mainly concentrated on the intra-annual variation in wood density (e.g. using radiodensitometry; Schweingruber, 1992) or more recently on cell-size characteristics (e.g. the ratio between lumen and cell walls in tracheids; Munro *et al.*, 1996; Vaganov

et al., 1996). Unlike conifers, work on angiosperm trees is rather scarce. Although the separation of earlywood and latewood widths of ring-porous trees presents few methodological difficulties (Blank, 1997), it is not common practice to take earlywood features into account in dendrochronology. Only a few studies have been performed on oak (Eckstein & Schmidt, 1974; Nola, 1996; St George *et al.*, 2002), *Fraxinus nigra* (Tardif, 1996) and *Schefflera delavayi* (Xiong *et al.*, 1998–99).

Anatomical features, especially those related to vessels, often bear ecologically relevant information. Vessel size and length or vessel density usually vary with site conditions (Carquist, 1975). These features can be used to study the adaptations of tree species along climatic gradients (Villar-Salvador *et al.*, 1997). Little work has been done on exploring how such adaptations may reflect annual variations in weather conditions at a site (i.e. vessel features can be analysed as a dendrochronological time series). Sass & Eckstein (1995) showed that the vessel area of European beech could be a good indicator of water availability, while Woodcock (1989) pointed out a similar result for the latewood vessel diameter of *Quercus*

macrocarpa. Nevertheless, there are only a small number of works where the climatic content of the earlywood vessels of ring-porous trees have been analysed. Earlywood vessel features have been studied in oak (St George *et al.*, 2002; García González & Eckstein, 2003) and in teak (*Tectona grandis*) (Pumijumnong & Park, 1999). The results, however, have been contradictory; whereas García González & Eckstein (2003) found a strong signal mainly related to the water availability during the time of vessel formation, results for other oak species or for teak were not consistent.

Modern automatic image analysis systems have made such kinds of investigations much more feasible, in particular when measurement can be performed directly on wood surfaces, as for earlywood vessels in ring-porous species. This avoids the time-consuming procedure of microsection preparation that would be required for latewood vessels or diffuse-porous species. Our study is a new contribution to this discussion, based on a quantitative anatomical analysis of earlywood vessel features in European chestnut (*Castanea sativa*). Chestnut is a ring-porous tree species that has not been used for this kind of dendroecological analyses before. The aims of the project were: to quantify the strength of the environmental signal stored in several earlywood vessel characteristics; to verify whether the resulting information is different from that recorded in ring width; and to determine the climatic content of the signal and its ecophysiological meaning.

Materials and Methods

Study sites and wood material

Stem discs *c.* 5-cm thick of European chestnut (*Castanea sativa*) were gathered at the base (0.5 m above ground) of 60 overstorey chestnut trees from three even-aged 45- to 52-yr-old

coppice plots on the southern side of the Swiss Alps. All sites (Novaggio, Gerra and Bedano) belong to the chestnut belt, an area between 530 m and 710 m above sea level (asl) with an insubric climatic regime on acidophilous soil with an adequate supply of water. The plots have not been silviculturally managed since the last coppicing, with the exception of Gerra, which was thinned once at the end of the 1980s. The discs were polished with a 400-grit sandpaper and then cleaned with a high-pressure water blast to remove tyloses and wood dust inside the earlywood vessel lumina.

Variable survey

A tree-ring measuring device (a Lintab (Rinntech, Heidelberg, Germany) linear stage combined with a binocular microscope) was used to measure ring width (RW), earlywood width (EW) and latewood width (LW) to the nearest 0.01 mm along a radius. A radius was chosen midway between the longest and the shortest radii of each disc in order to reduce the undesired effect of stem-eccentricity.

Earlywood vessels were automatically recognized and performed using the image analysis software IMAGE PRO PLUS (Media Cybernetics, Inc., Silver Spring, MD, USA). Digital images were acquired directly from the polished wood surfaces by means of a color video camera (sony 96D, module 3CCD DC 12 V) mounted on a stereo-microscope (Leica MZ12, Leica Microsystems, <http://www.leica-microsystems.com>) set with a fixed $\times 64$ magnification and connected to a personal computer. Images were captured ring by ring on an 8 mm wide radial strip along the same line used for the tree-ring width measurement. Image resolution was set to 300×300 d.p.i. and 16.7 million colors. Earlywood and latewood were distinguished on the basis of vessel size. The earlywood vessel data relative to each annual ring were then derived from the digitized image (Fig. 1).

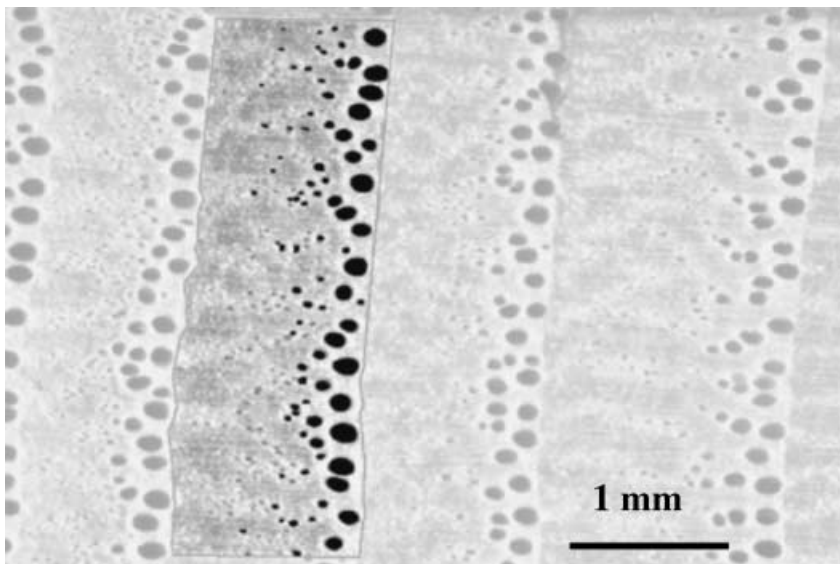


Fig. 1 Digitized black and white image showing the structure of a cross-section of the wood of European chestnut (*Castanea sativa*) transferred from the microscope to the monitor. Black surfaces within the foreground (annual ring) are the earlywood vessels recognized by the image analysis program.

An essential step for the automatic measurement is the discrimination of the vessel lumen from the ground tissue according to color brightness. The image program was set up with filters (morphological 2×2 squares, one pass, which erodes the edges of bright objects and enlarges dark ones) and an image enhancer (equalize, best fit) in order to improve the contrast and better recognize all dark objects (vessel lumen). A selecting filter ensured that only dark objects with a ratio between their horizontal and vertical axes < 2 and with an area $> 0.01 \text{ mm}^2$ were considered to avoid misidentification.

Several earlywood variables, calculated from the data obtained, were considered for this study: the number of vessels (NV), mean vessel lumen area (MVA), total vessel lumen area (TVA) and conductive area (CA). The CA was estimated as the fourth power of the radius of each vessel (Zimmerman, 1983).

Analyses

All data were processed using dendrochronological procedures. First, all tree-ring series within each site were crossdated by assigning each ring to the correct calendar year (Schweingruber, 1988). Tree-ring curves were plotted and compared with the other trees at each site. Crossdating was performed only for the ring-width series, but the other variables were plotted to check for outliers caused by measurement errors. The dating was statistically verified using the program COFECHA (Holmes, 1983; Grissino-Mayer & Fritts, 1997), and nine trees were excluded from further analysis because there was lack of agreement with their tree-ring width series. A total of 51 trees were analysed: 19 at Bedano, 15 at Novaggio and 17 at Gerra. The time series cover the periods 1941–97 at Bedano, 1956–98 at Novaggio, 1956–95 at Gerra.

Growth-related trends had to be removed from the series. Generally, RW, LW and NV showed a descending age-trend, MVA an ascending one, whereas CA, EW and TVA had nearly no age-trend. Nevertheless, owing to variations in individual growth patterns that could lead to a misrepresentation of the climatic signal, all series were filtered by a cubic smoothing spline function (Cook *et al.*, 1992) with 32-yr stiffness and 50% cutoff, using the program ARSTAN (Grissino-Mayer & Fritts, 1997). The same detrending method was applied to all growth variables. After detrending, time-series of growth indices (Fritts, 1976) were obtained and they were averaged into a chronology for each variable and site, using a biweight robust mean (Mosteller & Tukey, 1977) that minimizes the effects of extreme values. The statistical quality of each single chronology was evaluated using three coefficients commonly used in dendrochronology: the mean correlation between trees (Rbt), the expressed population signal (EPS) and the mean sensitivity (MS). Rbt is the mean value of all possible Pearson's cross-correlation coefficients, EPS indicates the extent to which the sample size is representative of a theoretical population with an infinite number of individuals (Wigley *et al.*, 1984) and MS describes the mean

percentage change from each measured annual ring value to the next (Fritts, 1976).

The comparative analyses were performed for the period with data available for all sites, i.e. from 1956 to 1995. The chronologies of sites and variables were compared using simple correlation; for an easier evaluation, the differences between the signals contained in each variable were summarized using principal component analysis.

Finally, for the variables showing a high similarity between sites, a composite chronology was calculated by averaging the single chronologies of the three plots, and then related to monthly meteorological data by performing response function analysis (Fritts, 1976). This is based on a multiple regression using climatic records as predictors (previously transformed into principal components to prevent collinearity) and growth indices as predictants. The model was calibrated and verified through the bootstrap technique (Guiot, 1991), using the program PRECON (Fritts, 1990), that statistically analyses the relationship between climate and tree ring variation, and applied to all site and composite chronologies. Before computing the response functions, climate–growth relationships were initially explored by computing Pearson's correlation coefficients for the same data set. Mean monthly temperature and total monthly precipitation data were taken from the nearby stations of Lugano and Locarno, not more than 20 km distant from the sites. The period for comparison was from the previous August to the current October for RW and LW and from the previous June to the current May with all the earlywood variables, as these are the periods which most probably have some influence upon growth and vessel formation.

Chestnut phenological records for the period 1996–2002, supplied by MeteoSwiss National Weather Service, were used for the interpretation of the climate–growth relationships.

Results

Strength of the signal

The tree-ring width has a stronger common signal than all the earlywood vessel variables (Table 1); it lies within the ranges usually reported for ring width of ring-porous trees at temperate latitudes (Pilcher & Baillie, 1980; Wazny & Eckstein, 1991; Santini *et al.*, 1994; Tardif, 1996). As shown in Fig. 2, RW indices within sites show a synchronous behavior. Among the earlywood vessel variables, both TVA and NV have the strongest signals, whereas the values for CA and MVA are rather small. In most cases the common signal was higher at Gerra (except for the CA). This is probably due to the thinning that frequently resulted in the same kind of reaction in the subsequently formed wood. This could also be responsible for the higher autocorrelation at this site.

Variability from year to year within the series is expressed by the mean sensitivity (MS) (Table 1). Again, values are higher for RW and LW (0.17–0.28) than for the vessel variables and

Table 1 Mean correlation (Rbt), expressed population signal (EPS), mean sensitivity (MS) and first order autocorrelation coefficient (AutoR) for each plot and variable for European chestnut (*Castanea sativa*)

Plot	RW			LW			EW			TVA			NV			CA			MVA		
	N	G	B	N	G	B	N	G	B	N	G	B	N	G	B	N	G	B	N	G	B
Rbt	0.38	0.52	0.39	0.18	0.22	0.20	0.15	0.28	0.19	0.12	0.13	0.15	0.38	0.52	0.43	0.16	0.26	0.17	0.12	0.22	0.07
EPS	0.90	0.95	0.92	0.77	0.83	0.83	0.73	0.87	0.81	0.68	0.72	0.78	0.90	0.95	0.93	0.75	0.86	0.80	0.67	0.83	0.60
MS	0.21	0.18	0.17	0.11	0.10	0.08	0.10	0.10	0.07	0.14	0.12	0.11	0.28	0.24	0.26	0.08	0.08	0.07	0.05	0.05	0.04
AutoR	0.25	0.66	0.28	0.36	0.50	0.63	0.36	0.63	0.67	0.22	0.29	0.53	0.25	0.64	0.17	0.28	0.54	0.33	0.17	0.50	0.12

Variables: RW, ring width; LW, latewood width; EW, earlywood width; TVA, total vessel area; NV, number of vessels; CA, conductive area; MVA, mean vessel area. Plots: N, Novaggio; G, Gerra; B, Bedano.

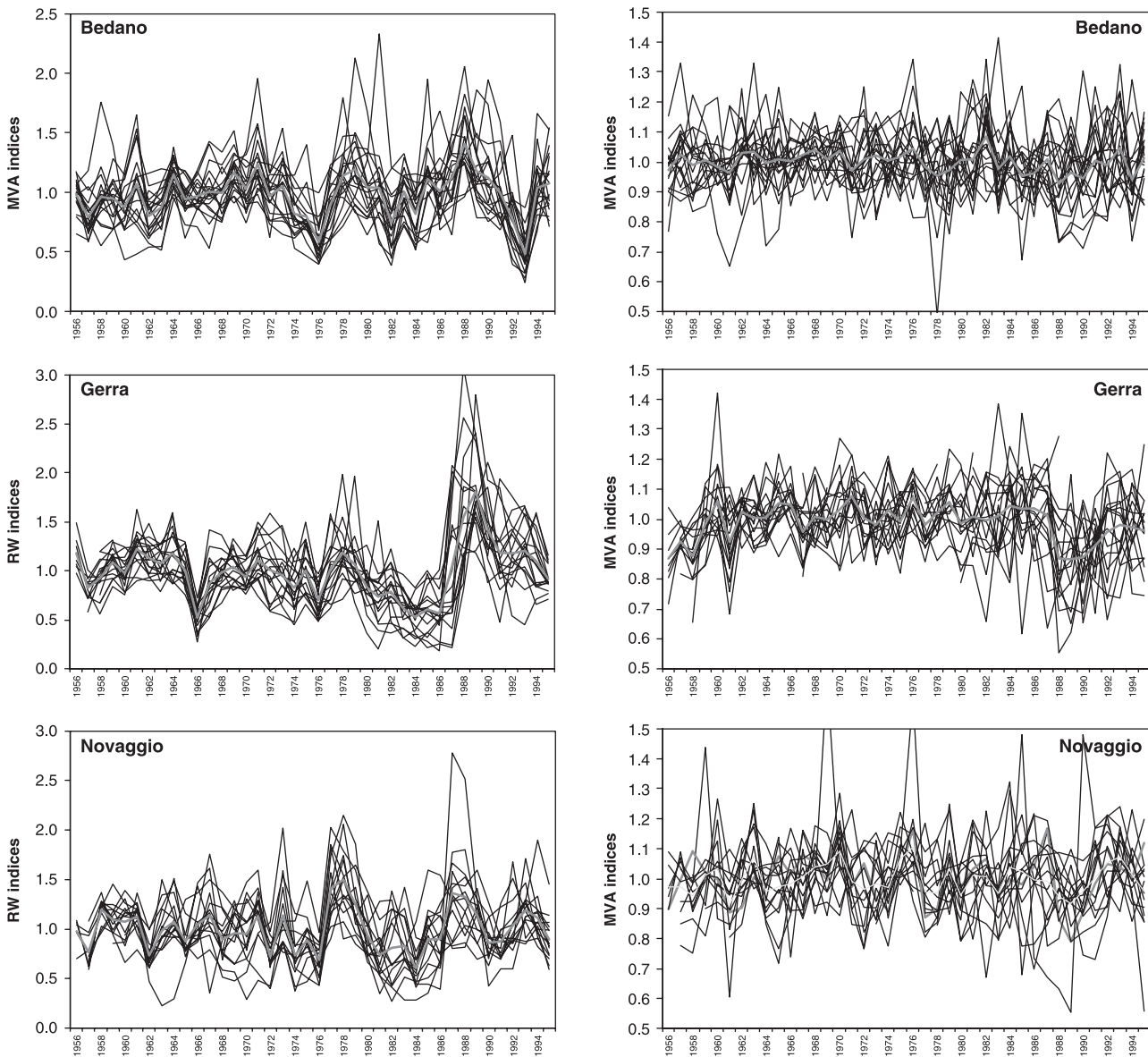
**Fig. 2** Time series of ring width (RW) and mean vessel area (MVA) indices for the period 1956-95. Dark thin lines refer to single trees; grey thick lines correspond to the chronology.

Table 2 Correlations among plot chronologies of seven variables for *Castanea sativa* (European chestnut)

	RW	LW	EW	TVA	NV	CA	MVA
N-G	0.47***	0.50***	0.46**	0.47***	0.38**	0.50***	0.48***
N-B	0.41**	0.42**	0.24	0.22	0.27*	0.20	0.50***
G-B	0.34*	0.29*	0.42**	0.19	0.25	0.18	0.37**

Variables: RW, ring width; LW, latewood width; EW, earlywood width; TVA, total vessel area; NV, number of vessels; CA, conductive area; MVA, mean vessel area. Plots: N, Novaggio; G, Gerra; B, Bedano. *, **, ***, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

lie within the usual range. The lowest value (0.04–0.05) is found in the MVA series; nevertheless, there was enough year-to-year variation to allow the analysis of climate-growth relationships.

The degree of crossdating between sites is expressed as the correlation between their chronologies for each variable (Table 2). With the exception of some specific years as 1966 in Gerra, 1993 in Bedano, when site-specific events likely occurred, RW chronologies are synchronous (Fig. 2): Novaggio was similar to Gerra (0.47, $P < 0.001$) and Bedano (0.41, $P < 0.01$), while the correlation between Bedano and Gerra was lower (0.34, $P < 0.05$). For MVA, although the common signal within each site was low, a similarity between all three sites can be observed (Fig. 2). A synchronous behavior in the site chronologies is visible in specific pointer years (e.g. 1959, 1961, 1962, 1977, 1984, and 1988). Correlation between Novaggio and the other

two sites was higher than 0.48 ($P < 0.001$) and correlation between Gerra and Bedano was still significant ($P < 0.01$). Similarity between sites was very low for TVA, NV, and CA. However, significant correlations were found between Novaggio and Gerra.

Comparison between variables

The chronologies of all variables were compared at each site to determine whether they contained the same kind of information (Table 3). In general, RW and LW were very closely related, displaying a correlation value of 0.99 at all three sites. The correlations are considerably lower between EW and RW, although still significant. However, if EW is compared with LW (and consequently with RW) of the previous

Table 3 Correlations among chronologies of European chestnut (*Castanea sativa*) tree-ring variables

Plot	LW(-1)	RW	LW	EW	TVA	NV	CA
N							
RW	0.263	–	–	–	–	–	–
LW	0.251	0.996***	–	–	–	–	–
EW	0.437*	0.425*	0.371*	–	–	–	–
TVA	0.178	0.561***	0.535**	0.729***	–	–	–
NV	0.310	0.713***	0.699***	0.604***	0.898***	–	–
CA	–0.003	0.354	0.320	0.675***	0.878***	0.620***	–
MVA	–0.314	–0.261	–0.291	0.315	0.311	–0.123	0.629***
G							
RW	0.671***	–	–	–	–	–	–
LW	0.642***	0.997***	–	–	–	–	–
EW	0.734***	0.657***	0.603***	–	–	–	–
TVA	0.707***	0.664***	0.622***	0.882***	–	–	–
NV	0.771***	0.842***	0.816***	0.803***	0.894***	–	–
CA	0.490**	0.409*	0.367*	0.723***	0.885***	0.619***	–
MVA	–0.481**	–0.646***	–0.662***	–0.203	–0.177	–0.591***	0.232
B							
RW	0.246	–	–	–	–	–	–
LW	0.171	0.990***	–	–	–	–	–
EW	0.617***	0.323	0.213	–	–	–	–
TVA	0.444**	0.466*	0.372*	0.837***	–	–	–
NV	0.416**	0.644***	0.563***	0.744***	0.938***	–	–
CA	0.425	0.360	0.278	0.789***	0.934***	0.797***	–
MVA	0.037	–0.593***	–0.602***	0.028	–0.065	–0.393*	0.164

Variables: LW(-1), latewood width of the previous year; RW, ring width; LW, latewood width; EW, earlywood width; TVA, total vessel area; NV, number of vessels; CA, conductive area; MVA, mean vessel area. Plots: N, Novaggio; G, Gerra; B, Bedano. *, **, ***, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively.

year, the correlation is highly significant ($P < 0.0001$ for Gerra and Bedano). Earlywood width, CA, TVA and NV were also highly correlated with each other, with values often above 0.80. The correlations between these four earlywood variables and RW were also positive and significant. On the other hand, MVA tended to negatively correlate with RW, LW and NV, whereas correlations with the other earlywood variables were mostly nonsignificant. This result can be best expressed by principal component analysis at each site (Fig. 3), which sorts the variables according to their correlations. The two first

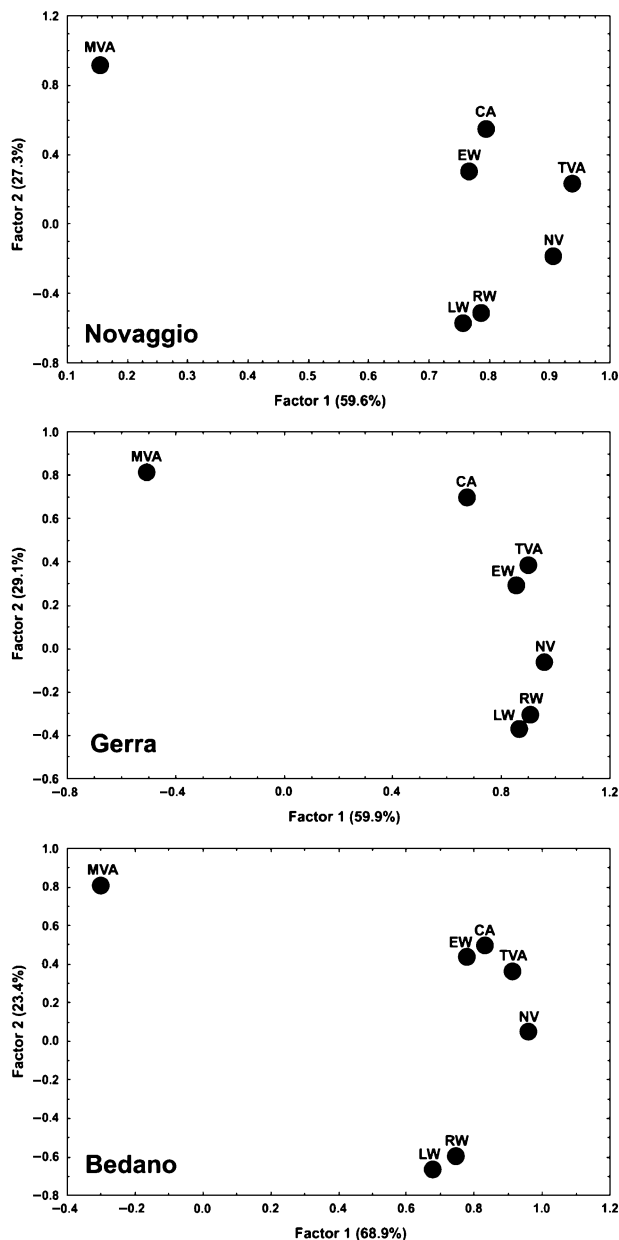


Fig. 3 Ordination of all variables of European chestnut (*Castanea sativa*) along the axes of the two first principal components. RW, ring width; LW, latewood width; EW, earlywood width; MVA, mean vessel area; TVA, total vessel area; NV, number of vessels; CA, conductive area.

principal components retained *c.* 90% of the total variance. The ordination along the first axis (59.9% to 68.9% of the variance) shows a clear distinction between MVA and the other six variables, indicating that this variable contains a different kind of information. The small differences between all the other six variables are expressed by the second axis (23.4–29.1% of explained variance), which, however, separated earlywood variables from the variables involving latewood (RW and LW).

Climate sensitivity

Analysis of climate sensitivity has been focused mainly on the variables that showed a strong agreement between site chronologies (i.e. for RW, LW and MVA). Thus, in order to minimize site local factors and maximize the regional signal, the composite chronologies of these variables were used in the analysis of climate–growth relationships (Fig. 4). These response functions do not substantially differ from the correlation functions neither do the results differ between the two weather stations. Ring width (and LW) responded positively ($P > 0.05$) to both the May temperature and the July rainfall of the same year, and also slightly to the temperature in the current March ($P < 0.1$). For MVA, the response function shows a very clear result, with climate explaining 76% of the total variance. Mean vessel lumen area is mainly negatively related to temperature in the current March ($P < 0.01$), and to some extent, in February ($P < 0.05$) (i.e. just before the beginning of the growing season). The single correlation coefficient between the composite and the March temperature reached a value of -0.62 ($P < 0.001$), being also significant for the three site chronologies. This means that a high temperature in March results in small earlywood vessels and vice versa. Temperature in the prior October is also negatively related to MVA ($P < 0.05$) (i.e. at the end of the previous growing season). Figure 4 shows the quality of the adjustment between site and composite chronologies and the most significant climatic variable (July precipitation for RW and March temperature for MVA).

For those earlywood variables not recording a regional signal (EW, TVA, NV and CA), responses to climate had to be analysed at each site. Although they vary among sites, a slight common pattern in relationship to temperature at the end of the previous growing season can still be observed (Table 4). In particular, for Bedano, the variables are significant and positively correlated to the temperature of the previous October, as opposed to that observed for MVA.

Discussion

Quality and precision of vessel features measurement

Success in using computer-assisted image analysis strongly depends on the quality of the digital images captured

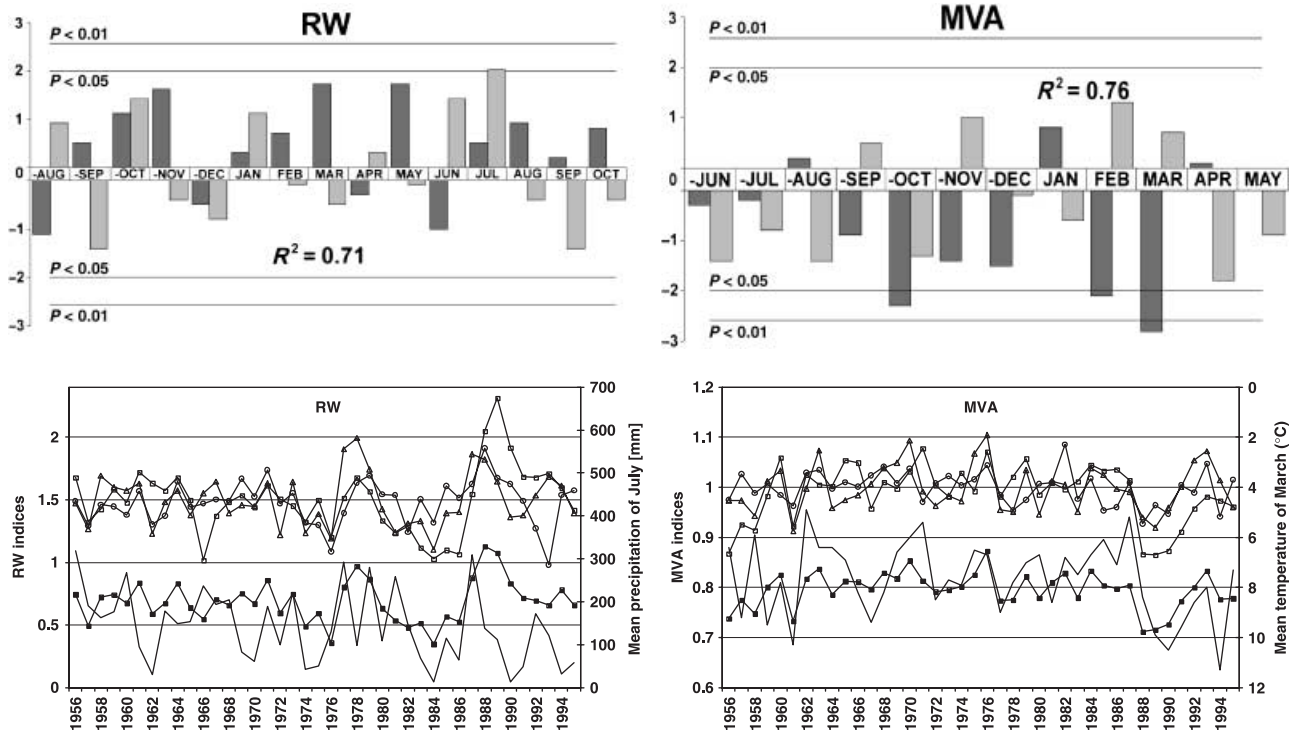


Fig. 4 Upper charts show response functions of European chestnut (*Castanea sativa*) ring width and mean vessel area: tinted bars, temperature; open bars, precipitation. Lower graphs show adjustment between the most significant climatic variable and the site and composite chronologies: triangles, Novaggio; open squares, Gerra; circles, Bedano; closed squares, composite; plain line shows July precipitation (left) and March temperature (right). Climatic data from 1956 to 1995 come from Lugano's meteorological station. RW, ring width; MVA, mean vessel area.

Table 4 Correlation among European chestnut (*Castanea sativa*) earlywood variables displaying little agreement between sites and monthly mean temperatures at the end of the previous growing season

Plot	EW			TVA			NV			CA		
	N	G	B	N	G	B	N	G	B	N	G	B
August	-0.06	0.28*	-0.20	-0.14	0.30*	-0.23	-0.30*	0.27	-0.27	0.03	0.24	-0.20
September	-0.08	0.03	0.22	-0.04	0.21	0.21	-0.09	0.17	0.20	0.07	0.28*	0.16
October	0.18	0.14	0.67***	0.21	0.26	0.65***	0.28*	0.23	0.62***	0.09	0.26	0.61***

Variables: EW, earlywood width; TVA, total vessel area; NV, number of vessels; CA, conductive area. Plots: N, Novaggio; G, Gerra; B, Bedano. *, **, ***, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

(Spiecker *et al.*, 2000). A high-quality preparation of the samples was therefore required. Since we dealt with relatively large cells (100–300 μm) the surface finishing using the 400-grit sandpaper was satisfactory enough for our purposes. A determining factor for vessel recognition was the dark color of the vessel lumina, which was clearly discernible from the wood-brown background. This was accomplished by clearing the lumen content (wood dust and tyloses). Nevertheless, smooth transition between the vessel lumina and the background tissue or the occurrence of micro cracks can lead to misrecognition or imprecise measurement. A visual control was therefore needed and where quality was judged insufficient the preparation procedure was repeated. Once the image was ready and the object correctly recognized, precision in

measurement could only be affected by pixel resolution or by the choice of filtering.

A $\times 64$ magnification permitted capturing a tangential 8-mm wide strip that included 20–250 vessels per annual ring. No repetitions were thus necessary.

Evaluation of signal strength

Ring width is easy to measure, being a priori the most appropriate variable to analyse for dendrochronological research. Ring-porous wood species such as chestnut are distinguishable from other wood species because they have several rows of conspicuous large vessels that form early in the growing season. This wood structure enables the use of additional variables

for the establishment of environment–growth relationships. Both EW and LW can easily be recognized on the wood and measured, whereas more time-consuming techniques may be applied to record other earlywood anatomical features (such as MVA, TVA, NV or CA). However, the study of these earlywood features is only worthwhile if they provide different environmental information from that gained by RW.

When site chronologies are evaluated, width variables such as RW and LW showed a stronger common signal (Rbt and EPS values) and a higher sensitivity (MS) than EW, confirming most of the earlier studies in different ring-porous species, such as *Quercus* spp. (Eckstein & Schmidt, 1974; Nola, 1996; St George *et al.*, 2002; García González & Eckstein, 2003), *Fraxinus nigra* (Tardif, 1996) or *Schefflera delavayi*. (Xiong *et al.*, 1998–99). A low common signal and sensitivity within sites were also found for all earlywood variables analysed (EW, MVA, TVA, NV and CA). Previous studies on other ring-porous trees, such as *Quercus robur* (García González & Eckstein, 2003), *Quercus macrocarpa* (Woodcock, 1989) or *Tectona grandis* (Pumijumngong & Park, 1999) are consistent with these findings. Therefore, earlywood formation of chestnut appears to be much less dependent on external factors than LW or total RW.

When several sites are compared within a well-defined climatic region, homogeneity of chronologies is needed to characterize their ecological responses (Serre-Bachet & Tessier, 1992). Chronologies of most earlywood variables (EW, TVA, NV and CA) did not crossdate well between sites, indicating that they are noticeably influenced by local factors. By contrast, although the signal strength of MVA was low within each site, correlations between chronologies were high – even better than RW and LW. This suggests that there is a meso- or macro-climatic signal that MVA records better than the other variables. If this can be confirmed in further studies, it would be possible to obtain a regional chronology from a small set of trees at different sites within a geographic area. This would increase the consistency of the results and greatly reduce the work needed.

The higher statistical quality (values of Rbt, EPS and MS) of RW and LW would make these variables preferable for dendrochronological studies. However, among the earlywood features, MVA appears to be very attractive because of its capability of recording a regional environmental signal.

Difference in the signal

The common signal discussed in the previous paragraphs indicates the statistical quality of each variable, but does not describe analogies or differences in the ecological information they record. The identification of such information requires a comparison of the chronologies with each other and also with climatic data, as they may not respond independently (Wimmer & Grabner, 2000).

Width variables do not differ much in their signals. Since LW determines most of the year-to-year variability of the RW ($r > 0.99$), there is no additional information that could be

gained by performing the analysis of one instead of the other. This confirms what has been observed in previous studies of oak (Nola, 1996; García González, 2000). By contrast, when EW is related to RW (or LW), correlations, although still significant, are considerably lower. Apparently, this suggests that EW contains ecological information that greatly differs from that yielded by the other ring-width features. However, if EW is compared with LW (and consequently with RW) of the previous year, the correlation is highly significant, which is physiologically understandable. Ring-porous trees like chestnut begin developing the first earlywood vessels just before or at the time of bud break (Suzuki *et al.*, 1996; Schmitt *et al.*, 2000), earlier than the resumption of photosynthetic activity. Thus, the beginning of earlywood formation is supported by the mobilization of reserves stored during the previous growing season (Barbaroux & Bréda, 2002). The additional information that can be gained from EW is consequently more limited than it first appeared.

For all earlywood variables, EW also exhibits a very high positive correlation to TVA, NV and CA. Thus, EW and TVA are determined more by the number of vessels (probably the number of rows of earlywood vessels) than by their size, since MVA usually does not correlate to these variables. There is, however, a significant negative correlation between MVA and NV, indicating that the formation of numerous vessels is associated with a small size, probably as a result of rapid differentiation, and vice versa; this result should be interpreted in terms of density. The inverse relationship between vessel size and density was observed not only when comparing wood anatomy of different species (Carlquist, 1975), but also under different climatic conditions (Villar-Salvador *et al.*, 1997) or even in time series of vessel features (Woodcock, 1989; Pumijumngong & Park, 1999). All earlywood variables are correlated with RW (MVA negatively, the others positively); favorable growth conditions increase wood production, therefore producing more earlywood vessels and wider rings, but also results in faster differentiation that leads to smaller vessels.

It became clear from these results that all the earlywood variables considered, except MVA, contain signals that are very close to each other and to RW (and LW). We can therefore conclude that these variables account for nearly the same kind of information. This was visible in the principal component analysis, which clearly showed that MVA did not group like the others. The different nature of environmental signals recorded by MVA makes this variable worthy of consideration in addition to RW or LW.

Relationships to climate

Although not strong, RW (and LW) showed a clear response to climate. Precipitation during the summer (July) appeared as the main climatic variable controlling growth, which is consistent with other results found for ring-porous trees in a similar climatic context (Tessier *et al.*, 1994; Nola, 1996).

Also, a weak positive effect of temperature in both March and May was observed.

Climatic conditions at the end of the previous summer are in part registered by the earlywood variables, causing a positive response in EW, NV, CA and TVA at some sites. However, the MVA expresses a negative response that can partly explain the negative relationship between MVA and the other variables. Above-average temperature at the end of the vegetation period may affect the storage of assimilates that are required for maintenance during the dormant season and the resumption of growth in the spring (Dickson, 1991). Barbaroux and Bréda (2002) observed that, for oak and beech, this accumulation could extend for at least 2 months after stem growth had ceased. Late in the season photosynthetic activity is low due to leaf aging (Escudero & Mediavilla, 2003), as opposed to respiration rates that can be raised by temperature, causing a degradation of the accumulating carbohydrates. An important mobilization of carbohydrates should be occurring within the tree at the time recorded by the earlywood variables, as can be assumed from phenological observations made by MeteoSwiss for the period 1996–2002. Discoloration of leaves in chestnut can start between mid September to the beginning of October and continue until the first week of November, while fructification occurs between the beginning and the middle of September, continuing until the beginning of October. Climate conditions at this time could also affect vessel ontogeny, as the first conductive elements of ring-porous trees differentiate from overwintering cambial derivatives that divide before the dormant season (Imagawa & Ishida, 1972; Kitin *et al.*, 1999).

Mean vessel lumen area was mostly controlled by the conditions at the moment of vessel expansion, recorded as the negative response to temperature in March. The time-span between the initiation of vessel growth and its lignification constitutes the phase when climate can directly determine its final size. Low temperature should inhibit cell division and differentiation, prolonging the time for vessel expansion, secondary wall formation and lignification. For ring-porous trees like chestnut, the first earlywood vessels are formed a few weeks or just before budbreak (Schmitt *et al.*, 2000). Suzuki *et al.* (1996) established that this time lag can be up to 2–8 wk, depending on the species. In *Castanea crenata*, these authors observed that vessel growth began 3–4 wk before budbreak, and secondary wall deposition took several days to complete after the end of leaf expansion. In the study area, budbreak starts between the end of March and mid April; thus, it can be inferred that vessel differentiation should be occurring during March and the beginning of April. Also, the positive relationship between RW to March temperature might suggest that temperature during this month triggers the onset of cambial activity.

The response of earlywood MVA in ring-porous trees to the conditions at the beginning of the vegetation season has not been described by many authors. García González & Eckstein

(2003), however, found a positive relationship for oak, which mainly depended on the water availability.

In conclusion, the present study showed that the mean vessel area of the ring-porous chestnut contains an ecophysiological signal that can be used as a climate proxy. It appears to be little affected by local factors, which makes it an attractive variable for investigating regional variation. Moreover the signal differs from that contained in other earlywood variables (mainly related to the previous year) and from that in ring width or latewood width (mostly in response to the conditions during the summer). Specifically, this variable is able to record the temperature during two physiologically crucial periods for vessel growth: during reserve storage (in the previous autumn) and during the onset of cambial activity (in the current spring). For these promising reasons, the earlywood vessel area of ring-porous trees should be further investigated, making it possible to assess the potential of this variable for dendroclimatic reconstructions.

Acknowledgements

We are very grateful to Otto Ulrich Bräker for his suggestions, help and encouragement during the early stages of this research. We also thank Prof. Dr Dieter Eckstein and Dr Paolo Cherubini for their valuable and very detailed comments on an earlier version of the manuscript. Anonymous referees also contributed to improving the quality of this paper, thus we gratefully thank them. The English was revised by Dr Silvia Dingwall and Christine Favre.

References

- Barbaroux C, Bréda N. 2002. Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. *Tree Physiology* 22: 1201–1210.
- Blank R. 1997. Ringporigkeit des Holzes und häufige Entlaubung durch Insekten als spezifische Risikofaktoren der Eichen. *Forst und Holz* 52: 235–242.
- Carlquist S. 1975. *Ecological strategies of xylem evolution*. Berkeley, CA, USA: University of California Press.
- Cook E, Briffa K, Shiyatov S, Mazepa V. 1992. Tree-ring standardization and growth trend estimation. In: Cook ER, Kairiukstis LA, eds. *Methods of dendrochronology: applications in the environmental sciences. International Institute for Applied Systems Analysis*. Boston, MA, USA: Kluwer Academic Publishers, 104–123.
- Dickson RE. 1991. Assimilate distribution and store. In: Raghavendra AS, ed. *Physiology of trees*. New York, NY, USA: J Wiley and Sons, 51–85.
- Eckstein D, Schmidt B. 1974. Dendroklimatologische Untersuchungen an Stieleichen aus dem maritimen Klimagebiet Schleswig-Holsteins. *Angewandte Botanik* 48: 371–383.
- Escudero A, Mediavilla S. 2003. Decline in photosynthetic nitrogen use efficiency with leaf age and nitrogen resorption as determinants of life span. *Journal of Ecology* 91: 880–889.
- Fritts HC. 1976. *Tree rings and climate*. New York, NY, USA: Academic Press.
- Fritts HC. 1990. Modeling tree-ring and environmental relationships or dendrochronological analysis. In: Dixon RK, Meldahl RS, Ruark GA, Warren WG, eds. *Forest growth: process modeling of responses to environmental stress*. Portland, OR, USA: Timber Press, 368–382.

- García González I. 2000. Estudio dendroecológico de *Quercus robur*. L. en el norte de Galicia. PhD thesis, University of Santiago de Compostela, Spain.
- García González I, Eckstein D. 2003. Climatic signal of earlywood vessels of oak on a maritime site. *Tree Physiology* 23: 497–504.
- Grissino-Mayer H, Fritts HC. 1997. The international Tree-Ring Data Bank: an enhanced global database serving the global scientific community. *Holocene* 7: 235–238.
- Guiot J. 1991. The bootstrapped response function. *Tree-Ring Bulletin* 51: 39–41.
- Holmes RL. 1983. Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin* 43: 69–78.
- Imagawa H, Ishida S. 1972. Study on the wood formation in trees. III. Occurrence of the overwintering cells in the cambial zone in several ring-porous trees. *Research Bulletin of the Hokkaido University Forests* 29: 207–211.
- Kitin P, Funada R, Sano Y, Beeckman H, Ohtani H. 1999. Variations in the lengths of fusiform cambial cells and vessel elements in *Kalopanax pictus*. *Annals of Botany* 84: 621–632.
- Mosteller F, Tukey JW. 1977. *Data analysis and regression*. Reading, MA, USA: Addison-Wesley.
- Munro MAR, Brown PM, Hughes MK, Garcia EMR. 1996. Image analysis of tracheid dimensions for dendrochronological use. In: Dean JS, Meko DM, Swetnam TW, eds. *Tree rings, environment, and humanity. Proceedings of the International Conference, Tucson, Arizona, 17–21 May 1994. Radiocarbon*. Tucson, AZ, USA: The University of Arizona, 843–851.
- Nola P. 1996. Climatic signal in earlywood and latewood of deciduous oaks from northern Italy. In: Dean JS, Meko DM, Swetnam TW, eds. *Tree rings, environment, and humanity. Proceedings of the International Conference, Tucson, Arizona, 17–21 May 1994. Radiocarbon*. Tucson, AZ, USA: The University of Arizona, 149–258.
- Pilcher JR, Baillie MGL. 1980. Eight modern oak chronologies from England and Scotland. *Tree-Ring Bulletin* 40: 45–58.
- Pumijumnong N, Park WK. 1999. Vessel chronologies from teak in northern Thailand and their climatic signal. *IAWA Journal* 20: 285–294.
- Santini A, Bottacci A, Gellini R. 1994. Preliminary dendroecological survey on pedunculate oak (*Quercus robur* L.) stands in Tuscany (Italy). *Annales des Sciences Forestières* 51: 1–10.
- Sass U, Eckstein D. 1995. The variability of vessel size in beech (*Fagus sylvatica* L.) and its ecophysiological interpretation. *Trees* 9: 247–252.
- Schmitt U, Moeller R, Eckstein D. 2000. Seasonal wood formation of beech (*Fagus sylvatica* L.) and black locust (*Robinia pseudoacacia* L.) as determined by the 'pinning'-technique. *Journal of Applied Botany* 74: 10–16.
- Schweingruber FH. 1988. *Tree rings: basics and applications of dendrochronology*. Dordrecht, The Netherlands: D. Reidel.
- Schweingruber FH. 1992. Radiodensitometry. In: Cook ER, Kairiukstis LA, eds. *Methods of dendrochronology: applications in the environmental sciences. International Institute for Applied Systems Analysis*. Boston, MA, USA: Kluwer Academic Publishers, 55–63.
- Schweingruber FH. 2001. *Dendroökologische Holz Anatomie: Anatomische Grundlagen der Dendrochronologie*. Berne, Switzerland: Paul Haupt.
- Serre-Bachet F, Tessier L. 1992. Response function analysis for ecological study. In: Cook ER, Kairiukstis LA, eds. *Methods of dendrochronology: applications in the environmental sciences. International Institute for Applied Systems Analysis*. Boston, MA, USA: Kluwer Academic Publishers, 247–258.
- Spiecker H, Schinker MG, Hansen J, Park Y, Ebding T, Döll W. 2000. Cell structure in tree rings: novel methods for preparation and image analysis of large cross sections. *IAWA Journal* 21: 361–373.
- St George S, Nielsen E, Conciatori F, Tardif J. 2002. Trends in *Quercus macrocarpa* vessel areas and their implications for tree-ring paleoflood studies. *Tree-Ring Research* 58: 3–10.
- Suzuki M, Yoda K, Suzuki H. 1996. Phenological comparison of the onset of vessel formation between ring-porous and diffuse-porous deciduous trees in a Japanese temperate forest. *IAWA Journal* 17: 131–144.
- Tardif J. 1996. Earlywood, latewood and total ring width of a ring-porous species (*Fraxinus nigra* Marsh.) in relation to climatic and hydrologic factors. In: Dean JS, Meko DM, Swetnam TW, eds. *Tree rings, environment, and humanity. Proceedings of the International Conference, Tucson, Arizona, 17–21 May 1994. Radiocarbon*. Tucson, AZ, USA: The University of Arizona, 315–324.
- Tessier L, Nola P, Serre-Bachet F. 1994. Deciduous *Quercus* in the Mediterranean region: tree-ring/climate relationships. *New Phytologist* 126: 355–367.
- Vaganov EA, Vysotskaya LG, Shashkin AV. 1996. Using cell chronologies in seasonal tree growth analysis and dendroclimatology. In: Dean JS, Meko DM, Swetnam TW, eds. *Tree rings, environment, and humanity. Proceedings of the International Conference, Tucson, Arizona, 17–21 May 1994. Radiocarbon*. Tucson, AZ, USA: The University of Arizona, 95–105.
- Villar-Salvador P, Castro-Díez P, Pérez-Rontomé C, Montserrat-Martí G. 1997. Stem xylem features in three *Quercus* (Fagaceae) species along a climatic gradient in NE Spain. *Trees* 12: 90–96.
- Wazny T, Eckstein D. 1991. The dendrochronological signal of oak (*Quercus* spp.) in Poland. *Dendrochronologia* 9: 35–49.
- Wigley TML, Briffa KR, Jones PD. 1984. On the average value of correlated time series, with applications in dendroclimatology and hydrometeorology. *Journal of Climate and Applied Meteorology* 23: 201–213.
- Wimmer R. 2002. Wood anatomical features in tree-rings as indicators of environmental change. *Dendrochronologia* 20: 21–36.
- Wimmer R, Grabner M. 2000. A comparison of tree-ring features in *Picea abies* as correlated with climate. *IAWA Journal* 12: 403–416.
- Woodcock DW. 1989. Climate sensitivity of wood-anatomical features in a ring-porous oak (*Quercus macrocarpa*). *Canadian Journal of Forest Research* 19: 639–644.
- Xiong L, Okada N, Zhong Z, Fujiwara T. 1998–99. The relationship between *Schefflera delavayi* growth and climate in the Three Gorges Reservoir region of the Yangtze River, China. *Dendrochronologia* 16–17: 99–118.
- Zimmerman MH. 1983. *Xylem structure and ascent of sap*. New York, USA: Springer-Verlag.