

QUANTITATIVE TRACHEID ANATOMY REVEALS A COMPLEX ENVIRONMENTAL CONTROL OF WOOD STRUCTURE IN CONTINENTAL MEDITERRANEAN CLIMATE

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A detailed understanding of how the climate models the hydraulic system of trees is still lacking, in spite of the need to understand tree response to the ongoing process of climatic change. A systematic correlation of tracheid anatomical features with climate has not been implemented in Mediterranean ecosystems, where climatic change is expected to be particularly intense. We measured ring width and cell number—in addition to intraring position, lumen size, and wall thickness of tracheids—in 10 *Juniperus thurifera* individuals from north-central Spain. We used this information to perform an exploratory analysis of how these parameters correlated with climatic variables in 1965–2004. Cell number and ring width shared a relatively similar climatic signal, whereas the anatomical variables provided differentiated and diverse signals about climatic conditions during their formation. Earlywood and latewood tracheids differed in controlling factors, with earlywood tracheid size and wall thickness being positively related to rainfall during early summer and latewood tracheid size being positively related to August temperature. Tracheid anatomical variables improved our understanding of climate effects on tree growth and wood formation under harsh environmental conditions, as those experienced in continental Mediterranean climates, where limiting factors show multiple shifts across the year.

Keywords: climate-growth relationship, *Juniperus thurifera*, Mediterranean climate, tracheidogram method, xylem anatomy.

Introduction

The potential of crossdating to unequivocally relate annual tree rings to calendar years confers high value to tree rings as an accurate proxy of the environmental conditions that affected tree vigor and performance previous to and during their formation (Fonti et al. 2010). Dendrochronology was initially founded using tree ring width, which is a variable that constitutes a good measure of plant investment in secondary growth with annual resolution (Schweingruber 1996). Although ring width is widely used in dendrochronological research, the range of parameters characterizing annual rings—and their use as environmental proxies—has notably increased during recent decades. Examples include the measurement of intra- and interannual variation of wood density (Rigling et al. 2002; Bouriaud et al. 2005), content of chemical elements (Novak et al. 2010), stable isotope composition (McCarroll and Loader 2004), and anatomical features (Panyushkina et al. 2003). Consequently, the amount of information retrieved from annual tree rings has progressively increased and conferred dendrochronological techniques a prominent role in the reconstruction of past environmental conditions (Hughes et al. 2011), in addition to providing an

understanding of plant-environment relationships from a historical perspective (Fritts 1976; Schweingruber 1996).

Xylem anatomical features are the result of a compromise between competing demands for support, storage, and transport of water and metabolites under changing environmental conditions, all of them primarily subject to anatomical constraints (Zweifel et al. 2006; Chave et al. 2009). Interest in the quantitative analysis of wood anatomy—that is, the discipline that deals quantitatively with xylem anatomic features along series of annual rings—has mostly been focused on the hydraulic architecture. In part, this is because of the major role of xylem as a conductive system and because the relationship between anatomical and functional characteristics of conductive elements is relatively well known (Sperry 2003). Lumen size determines hydraulic conductivity in such a way that small increases in the diameter of conductive elements lead to major gains in hydraulic conductivity, as described by the Hagen-Poiseuille law (Eilmann et al. 2011). However, larger lumen size also implies higher vulnerability to cavitation (Tyree and Zimmerman 2002); consequently, lumen diameter is the result of a trade-off between increasing efficiency and decreasing safety in water conductivity (see the review by Hacke and Sperry 2001). In addition, vulnerability to cavitation relies on the mechanical strength of conduits; hence, conductive elements showing a thicker cell wall provide the hydraulic transport system with higher safety but at

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the expense of higher carbon investments (Hacke and Sperry 2001; Pittermann et al. 2006).

Quantitative xylem anatomy provides large amounts of information per annual ring, including cell position, cell number, cell size, lumen size, cell wall thickness, and wall-to-lumen ratio (Fonti et al. 2010). Temporal variation of xylogenetic phases determines xylem anatomic characteristics, which consequently provide sequential information on environmental conditions controlling cambial activity and cell growth throughout the growing season (Zweifel et al. 2006; Eilmann et al. 2011). Additionally, anatomical features of conductive elements are fixed at different phases of the differentiation process from cambial to xylematic cells, with cell size being fixed during the cell expansion phase and wall thickness being fixed during the cell maturation phase (Rossi et al. 2006). However, it is difficult to establish how specific environmental signals at different times along the active period influence cell size and wall thickness. Our knowledge about xylogenetic processes is currently expanding (Samuels et al. 2006; Rossi et al. 2008; Vaganov et al. 2011), thus improving our ability to estimate the time at which cell features are fixed on the basis of intraring position (García-González and Fonti 2006).

The continental Mediterranean climate is doubly restrictive to plant growth, since growth is limited by cold in winter and drought in summer, which leads to two split optimal growth periods in spring and autumn, when a favorable combination of mild temperatures and water availability occurs (Mitrakos 1980; García-Plazaola et al. 1997). Xylogenetic studies on conifers under Mediterranean conditions corroborate these expectations, with a temperature-linked onset of cambial activity in spring and a reduction or even cessation of xylogenesis in summer, followed by the resumption of cambial activity in autumn (De Luis et al. 2007; Camarero et al. 2010). Such double stress results in xylem growth shifting in response to temperature—from positive in spring to negative in summer—and a generalized positive response to summer rainfall (Rozas et al. 2009; Vieira et al. 2009). Nevertheless, our understanding of how Mediterranean trees adjust their xylem anatomy to inter- and intra-annual changes in climatic conditions remains scarce. Existing information suggests that tracheid size is positively related to water availability during the phase of cell formation (Vieira et al. 2009; DeSoto et al. 2011). However, to the best of our knowledge, the systematic correlation of the anatomical features of tracheids to climatic conditions has not been established in Mediterranean environments. Such information is relevant, since it contributes to our understanding of physiological adaptations of Mediterranean trees to changing climatic conditions. Furthermore, anatomical features may host climatic signals that are complementary to those encoded by tree ring widths (Kiryanov et al. 2003; Panyushkina et al. 2003), thus being potentially useful for climatic reconstructions.

To improve our knowledge of how xylem anatomical traits are controlled by climatic variations in a Mediterranean environment and to evaluate the potential usefulness of these traits as climatic proxies, we performed an exploratory study in which we measured ring widths—in addition to the intraring position, lumen size, and wall thickness of tracheids—in 10 *Juniperus thurifera* individuals. The measurements were then correlated with climatic variables for the period 1965–2004. Our specific

aims were (1) to assess whether there is a climatic signal in tracheid anatomical traits, (2) to ascertain whether anatomical variables contain climatic signals similar to those of ring width, and (3) to evaluate the potential of anatomical traits toward providing additional environmental information to aid the reconstruction of past climatic continental Mediterranean conditions.

Material and Methods

Study Species

Juniperus thurifera L. is a long-lived tree species that is endemic to the western Mediterranean basin, with its most important populations growing under continental Mediterranean climatic conditions in Spain and Morocco. Species-based dendroclimatic studies indicate that radial growth is mainly affected by winter rainfall, spring temperature, and summer water stress (Bertaudière et al. 1999; Rozas et al. 2009; DeSoto et al. 2012). In our study area, the xylogenetic cycle of *J. thurifera* is known to start in early May and extend until late October, while latewood formation is known to initiate in early August (Camarero et al. 2010).

Study Area

The study area is a 3300-ha woodland located at Sierra de Cabrejas, 30 km west of Soria city, in north-central Spain (41°46'N, 02°49'W; 1100–1300 m altitude). The parent rock is Cretaceous limestone, and the soils are calcium rich and shallow. *Juniperus thurifera* forms open woodlands characterized by a mean density of more than 300 trees ha⁻¹, intermingled with pines (*Pinus sylvestris* and *Pinus pinaster*) and holm oaks (*Quercus ilex*). The climate is continental Mediterranean, which is characteristic of the supra-Mediterranean belt in central Spain (Rivas-Martínez and Loidi 1999). Climatic data on mean monthly temperature and total monthly precipitation for the period 1964–2004 were obtained from Soria meteorological station (41°46'N, 02°28'W; 1082 m altitude). The coldest month is January, showing a mean minimum temperature of -1.8°C, and the warmest month is July, showing a mean maximum temperature of 28.1°C (fig. 1). The mean monthly rainfall ranges from 29.2 mm in August to 58.9 mm in May.

Dating Tree Rings, Measuring Anatomical Features, and Computing Chronologies

From 10 trees that had been felled during the 2004–2005 winter, we obtained stem discs at 1.3 m aboveground (for details, see Olano et al. 2008). Stem discs were mechanically surfaced and then manually polished with a series of successively finer grades of sandpaper until the xylem cellular structure was clearly visible under a binocular lens. On each stem disc, two radii were dated by assigning calendar years to rings on the basis of the identification of characteristic ring sequences (Yamaguchi 1991) and with the help of a previous robust chronology for the same site (Rozas et al. 2009). Total ring widths were measured to the nearest 0.001 mm by using a Velmex sliding-stage micrometer interfaced with a computer. The software COFECHA (Grissino-Mayer 2001) was used to quantitatively check crossdating errors.

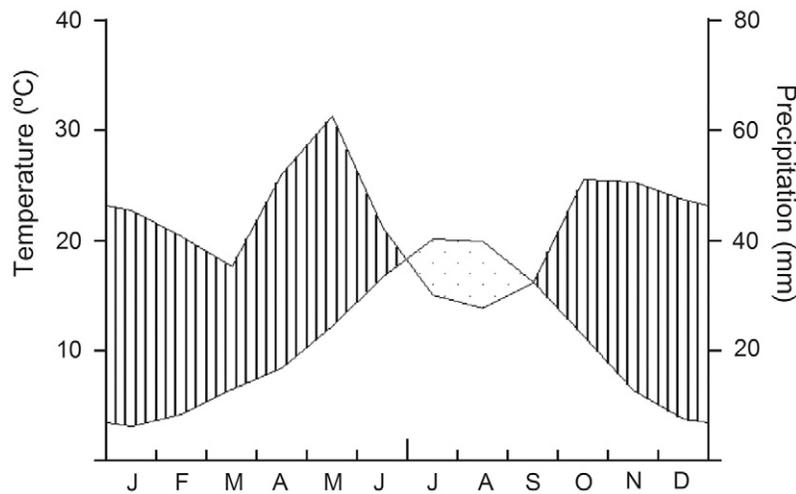


Fig. 1 Climatic diagram obtained from Soria meteorological station for the period 1965–2010.

A 5-mm-wide piece was cut out of every disc along the best crossdated radius to obtain permanent histological preparations, following the method of Schweingruber and Poschod (2005). Sections thinner than 15 μm were cut using a sledge microtome (H. Gärtner/F. H. Schweingruber) and were then placed on a slide and stained with alcian blue (alcian blue 1% solution in 0.7 N HCl) and safranin (safranin 1%, water soluble), which resulted in unlignified cells appearing blue and lignified cells appearing red. Afterward, sections were dehydrated using a series of ethanol solutions of increasing concentrations, washed with xylol, and then permanently preserved by embedding them into Eukitt glue. Images of each annual ring were captured with a Nikon D90 digital camera mounted on a Nikon Eclipse 50i optical microscope with $\times 200$ magnification. Pixel size was 0.272 μm . When an annual ring could not be captured in a single photograph, sequential images were merged (PTGUI, ver. 8.3.10 pro). Image treatment was performed with ImageJ (ver. 1.44; <http://rsb.info.nih.gov/ij/>; developed by W. Rasband, National Institutes of Health, Bethesda, MD), including blue channel selection for contrast enhancement and median filter.

Lumen radial diameter (LUM), cell wall thickness (WAL), tracheid radial diameter (CEL), and wall-to-cell radial diameter (WC) were measured for each tracheid along three radii per annual ring (fig. 2A). Cells were assigned to earlywood or latewood growth according to Mork's index (Denne 1988). When latewood-like cells occurred in earlywood or vice versa, the earlywood-latewood boundary was defined after a detailed analysis of the ring; this procedure was necessary in only 0.2% of the cells. Measurements were normalized to standard numbers of cells (three for earlywood and one for latewood) by using the tracheidogram method (Vaganov 1990), with the tgram package (DeSoto et al. 2011) in R (R Development Core Team 2011). Earlywood was standardized to just 3 cells as a compromise between keeping maximal temporal homogeneity in cell formation within each standardized cell class and obtaining intra-annual information among classes. As a result, we obtained tracheid-size-related parameters that corresponded to the av-

erage tracheid of each earlywood fraction. Earlywood and latewood growth occurs in split periods (Camarero et al. 2010), with latewood comprising a much lower number of cells than earlywood. Consequently, latewood was standardized to one cell. The cell number for earlywood (CNEW), latewood (CNLW), and total ring (CNR) for each year and tree was obtained after averaging the values calculated for each radius. Ring width of earlywood (RWEW), latewood (RWLW), and total ring width (RWT) were measured on the same radii used for previously mentioned measurements.

Each raw series of anatomical features and widths was standardized with ARSTAN (Cook and Holmes 1996). Earlywood and whole-ring related series were fitted to spline functions characterized by a 50% frequency response of 20 yr, which was flexible enough to reduce the nonclimatic variance by preserving high-frequency climatic information (Cook and Peters 1981). Latewood related series showed a very slow declining trend in low-frequency variation and were standardized by simply dividing each series by its mean value. Residuals were prewhitened by autoregressive modeling, thus obtaining dimensionless indices that represent independent annual records for each series. The year-by-year arithmetic mean for the time series of standardized indices was calculated to obtain a chronology for each measured variable.

Data Analysis

To evaluate whether the 22 obtained chronologies (for chronology listing and acronyms, see table 1) shared the same information, principal component analysis (PCA; Legendre and Legendre 1998) was performed with the vegan package (Oksanen et al. 2010) in R (R Development Core Team 2011). PCA aimed to extract a series of orthogonal components expressing the variance contained in our set of growth- and anatomy-related variables (Legendre and Legendre 1998). A scatter plot of the weighting coefficients for the first two PCs displayed clusters of variables with similar information. Pearson's correlations between pairs of chronologies were calculated.

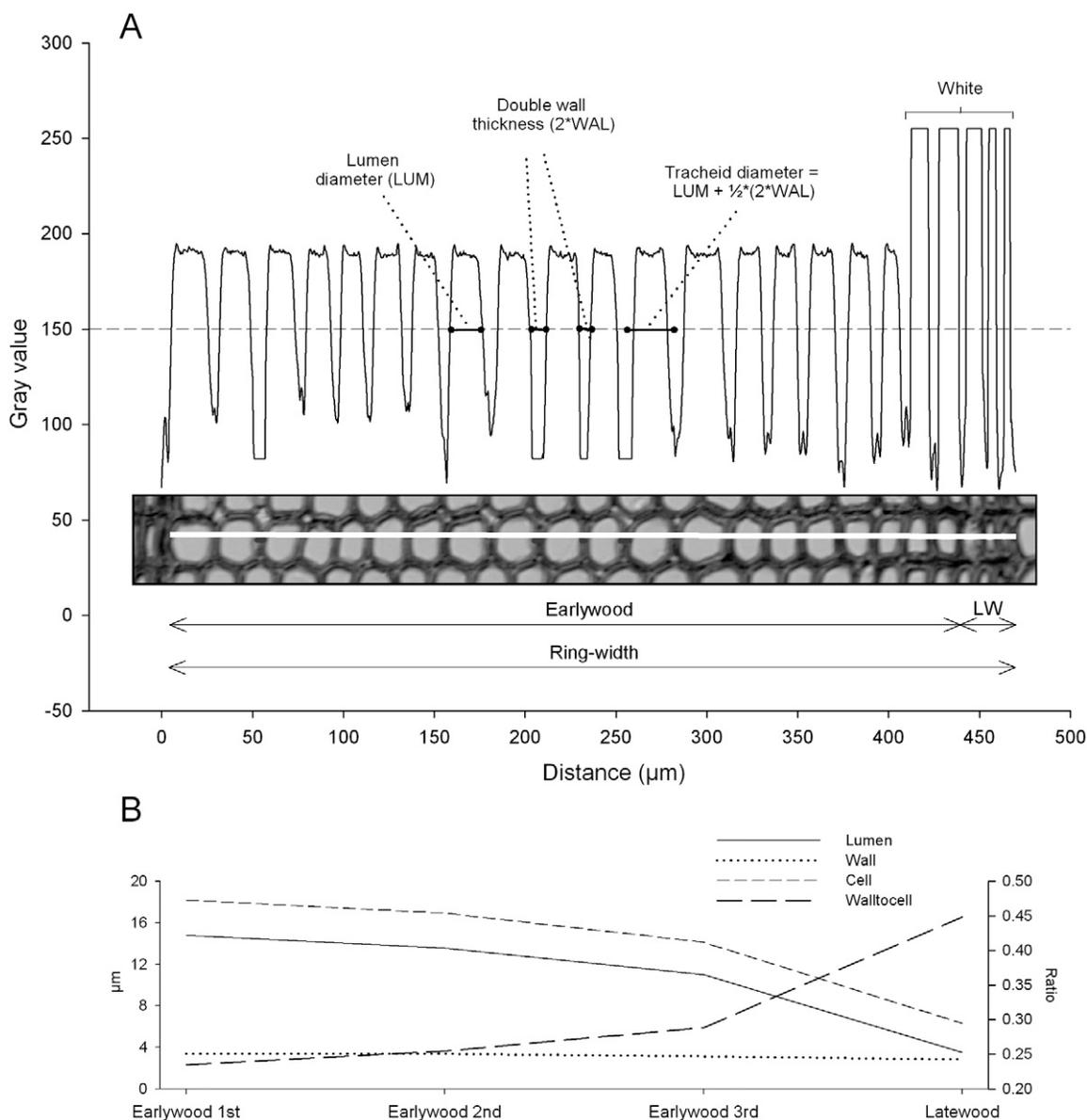


Fig. 2 A, Measurement procedure of the anatomical parameters. The image represents a complete tree ring. Pixel gray values (0 black, 256 white) are plotted along the white line; values below 150 correspond to the wall, whereas values above 150 correspond to the lumen. Whenever images did not allow clear differentiation between the lumen and the wall, the lumen was manually painted with white color (last 5 tracheids in this case). The formulas used to obtain anatomic values are presented at the top. B, Variation in the size of anatomical parameters according to the tracheidogram method (Vaganov 1990) to standardize earlywoods to three tracheids and latewood to one tracheid.

To identify the climatic factors that are related to the indexed chronologies for the period 1965–2004, Pearson's correlations were conducted, with monthly temperature and precipitation records from the previous November to current August for earlywood parameters and from the previous November to current October for latewood and whole-ring parameters. Afterward, multiple regression was performed between chronologies corresponding to each measured parameter and monthly temperature and precipitation records; variables were included in the model according to the Wald's forward stepwise procedure. The cutoff value for inclusion in

the model was 0.05, and it was 0.1 for excluding a previously included variable. Analyses were performed with PASW Statistic (ver. 18; SPSS, Chicago).

Finally, we used structural equation models (SEMs) to globally model climatic factors determining earlywood tracheid size, including the sequential effects of previous tracheid size. Despite the strong potential of SEMs, the application of this technique—in the context of dendrochronology—remains scarce (but see Lupi et al. 2010). These models assess how well data support a set of hypothesized relationships among climatic and anatomical parameters by including both direct

Table 1
Summary of the Statistics Calculated for Ring Width, Cell Number, and Anatomical Variables in the Mean Chronologies Calculated from 10 Trees in the Common Period 1965–2004

Parameter	Abbreviation	Mean \pm SD	SNR	ms _x	r _{bt}	R ² climate
Ring width (μm):						
Total	RWT	632.70 \pm 14.67	2.946	.187	.228	.424
Earlywood	RWEW	617.87 \pm 14.68	3.405	.231	.254	.450
Latewood	RWLW	24.89 \pm 10.41	−.103	.177	−.017	.299
Cell number:						
Total	CNR	40.95 \pm 8.58	3.873	.174	.279	.377
Earlywood	CNEW	37.20 \pm 8.59	3.728	.185	.272	.376
Latewood	CNLW	3.75 \pm .12	.162	.167	.016	.186
Tracheid diameter (μm):						
Earlywood:						
First part	CEL1	18.19 \pm 2.87	1.323	.037	.117	.124
Second part	CEL2	16.93 \pm 2.69	1.738	.043	.148	.410
Third part	CEL3	14.13 \pm 2.33	2.167	.043	.178	.249
Latewood	CELW	6.31 \pm 1.38	−.220	.052	−.023	.140
Lumen diameter (μm):						
Earlywood:						
First part	LUM1	14.82 \pm 2.74	1.123	.044	.101	.115
Second part	LUM2	13.56 \pm 2.49	1.393	.049	.122	.275
Third part	LUM3	11.01 \pm 2.01	1.620	.050	.139	.134
Latewood	LULW	3.49 \pm .92	−.396	.058	−.041	.266
Wall thickness (μm):						
Earlywood:						
First part	WAL1	3.38 \pm .66	.001	.043	.000	.230
Second part	WAL2	3.37 \pm .66	−.447	.028	−.047	.204
Third part	WAL3	3.12 \pm .59	.547	.046	.052	.383
Latewood	LWW	2.82 \pm .63	.376	.052	.036	.219
Wall-to-cell ratio:						
Earlywood:						
First part	WC1	.24 \pm .06	.302	.051	.029	.000
Second part	WC2	.25 \pm .06	−.248	.040	−.025	.126
Third part	WC3	.29 \pm .05	−.036	.034	−.004	.103
Latewood	LWWC	.45 \pm .05	−.198	.028	−.020	.276

Note. SNR, signal-to-noise ratio; ms_x, mean sensitivity; r_{bt}, mean between trees correlation; R² climate, proportion of variance explained by climate after a multiple regression.

and indirect relationships (Iriando et al. 2003; Grace et al. 2010). According to the multiple regression analyses, five climatic predictors were included in the model: (1) February temperature, (2) April temperature, (3) March rainfall, (4) June rainfall, and (5) July rainfall. Endogenous variables met multi-normality, and estimation was based on maximum likelihood estimates. The validity of the model was tested by the goodness-of-fit index (GFI) and root mean square error of approximation (RMSEA). GFI is independent of estimation methods and ranges between 0 and 1, with values above 0.90 indicating good fit (Tanaka 1987). RMSEA is based on predicted versus observed covariance, as a result of it being less affected by sample size, and includes a correction for model complexity. RMSEA is less than 0.05 for very good models (close fit), less than 0.1 for models that fit adequately, and greater than 0.1 for poorly fitted models. Analyses were conducted with AMOS 5.0 (Arbuckle 2003).

Following the previously described methodology, a multiple regression analysis was performed to assess whether climatic signals recorded from different anatomical parameters may be used in combination to improve our ability to reconstruct climate indices. More specifically, June rainfall was modeled

against the indexed chronologies of WAL1, WAL2, and CEL2.

Results

Tree ring widths were crossdated satisfactorily with the average correlation of RWT chronology from the previously available master chronology of 0.503 ($P < 0.001$). Tree age ranged from 71 to 111 yr (mean \pm SD, 86 \pm 4 yr), while cambial age at 1.3 m ranged from 40 to 68 yr (55 \pm 3 yr). Chronologies for tree ring widths and cell numbers in the total ring and the earlywood showed moderately high values of signal-to-noise ratio, r_{bt}, and climatic R² (table 1). In contrast, chronologies for latewood showed very low statistics and a weak climatic signal. The mean sensitivity of the series, however, is similar among tree ring compartments, being higher for earlywood and lower for latewood. Chronology statistics for tracheid and lumen diameters, wall thickness, and wall-to-cell ratios were considerably lower than for ring widths and cell numbers. Noticeably, the statistical quality of anatomical variables was higher in the second and third parts

of the earlywood than in the first part of the earlywood and the latewood.

A total of 44,131 cells from 1191 cell rows were measured. An average of 101.16 cells for earlywood and 11.26 cells for latewood were measured per ring and tree. Latewood comprised 9.2% of the total number of tracheids within a ring but only 3.9% of total tree ring width. The average tracheid size decreased along the ring from 18.19 μm for the first third of earlywood to 6.31 μm for latewood (table 1; fig. 2B). The radial diameter of the lumen followed a similar pattern, also showing an abrupt reduction in the transition from earlywood to latewood. Wall thickness showed small changes across the earlywood growth stages and a small decrease (16.6%) from earlywood to latewood. The contribution of wall-to-tracheid size almost doubled from the third part of earlywood to the latewood.

Chronologies Ordination

The first PCA axis explained 58.10% of the variance of anatomical and width variables and showed a clear distinction between earlywood and total tree rings from the other anatomical parameters, for both width and cell number (fig. 3A). The second PCA axis explained 29.16% of the variance and differentiated latewood cell number and width from the rest of variables. Interestingly, cell number and width chronologies shared almost the same information, as shown by the high correlations between both chronologies for earlywood ($r^2 = 0.92$), latewood ($r^2 = 0.84$), and total ring ($r^2 = 0.95$), all of which were significant at the 0.001 level. The close position of earlywood and total ring width in cell number chronology is probably a consequence of earlywood representing 96.1% of total ring width, that is, 90.8% of the total number of cells. Anatomical parameters related to tracheid size, wall thickness, and wall-to-cell ratio showed a clumped distribution in the lowest values of both PCA axes,

suggesting that their chronologies encode a very different climatic signal from cell number and width chronologies (fig. 3A). In order to assess the relationships between the anatomical parameters within a tree ring, a separate PCA was performed, with the first two PCA axes comprising 61.88% of the variance (fig. 3B). Wall-to-cell ratio showed a separate position at the top of the diagram, while earlywood lumen and cell size parameters were close to each other at the bottom of the diagram. This pattern is consistent with the high correlations between earlywood tracheid lumen and size ($r^2 = 0.95$, $P < 0.001$), suggesting that both parameters shared a common environmental signal. Wall thickness, however, showed an intermediate position between tracheid size and wall-to-cell ratio. Latewood tracheid size, lumen size, and wall thickness were isolated at the right of the diagram, also indicating a particular environmental signal that was differentiated from the other anatomical variables.

Climatic Control of Wood Anatomy and Radial Growth

The cell number in both the complete ring and the earlywood were positively correlated to April temperature and July rainfall and negatively correlated to winter rainfall from the previous December to current March (fig. 4). Regression models reduced the number of significant variables to precipitation in July (positive), December (negative), and February (negative), explaining nearly 38% of cell number variance (table 1). Climate-growth relationships for earlywood and total tree ring widths were relatively similar to their corresponding cell numbers. Earlywood and total ring widths showed negative correlations with winter rainfall and positive correlations with summer rainfall and April temperature. Regression models for earlywood and total ring widths reduced the number of significant variables to April temperature (positive, earlywood) and precipitation in June (positive, total), previous December (negative, total), February (nega-

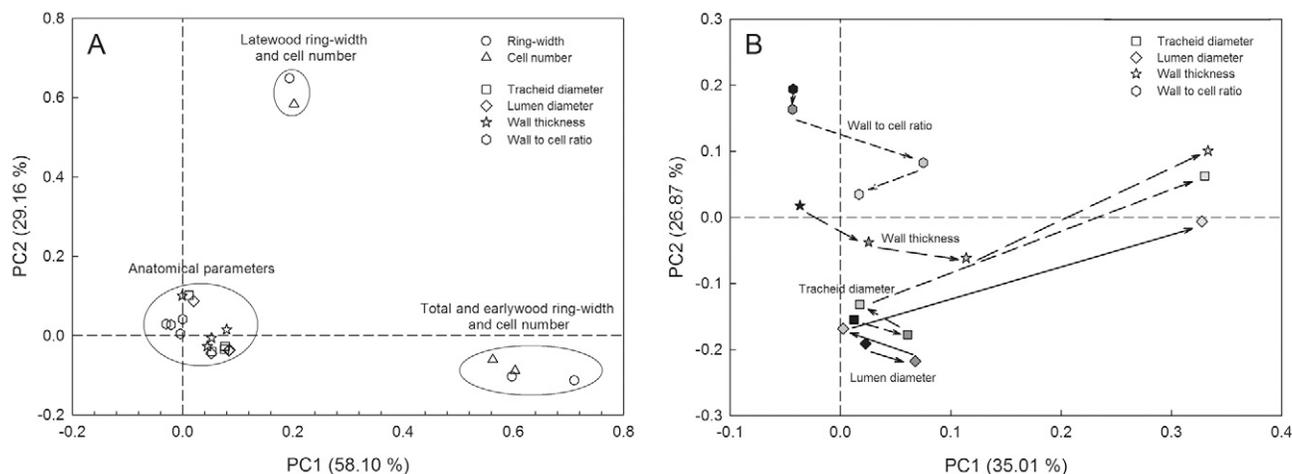


Fig. 3 Biplots of the first two principal component (PC) axes for anatomical, cell number, and ring width chronologies (A) and anatomical chronologies only (B). Arrows and shades in B indicate the temporal sequence from the first part of the earlywood (black) to the latewood (light gray) stage. Values in parentheses indicate the percentage of variance accounted for by each axis.

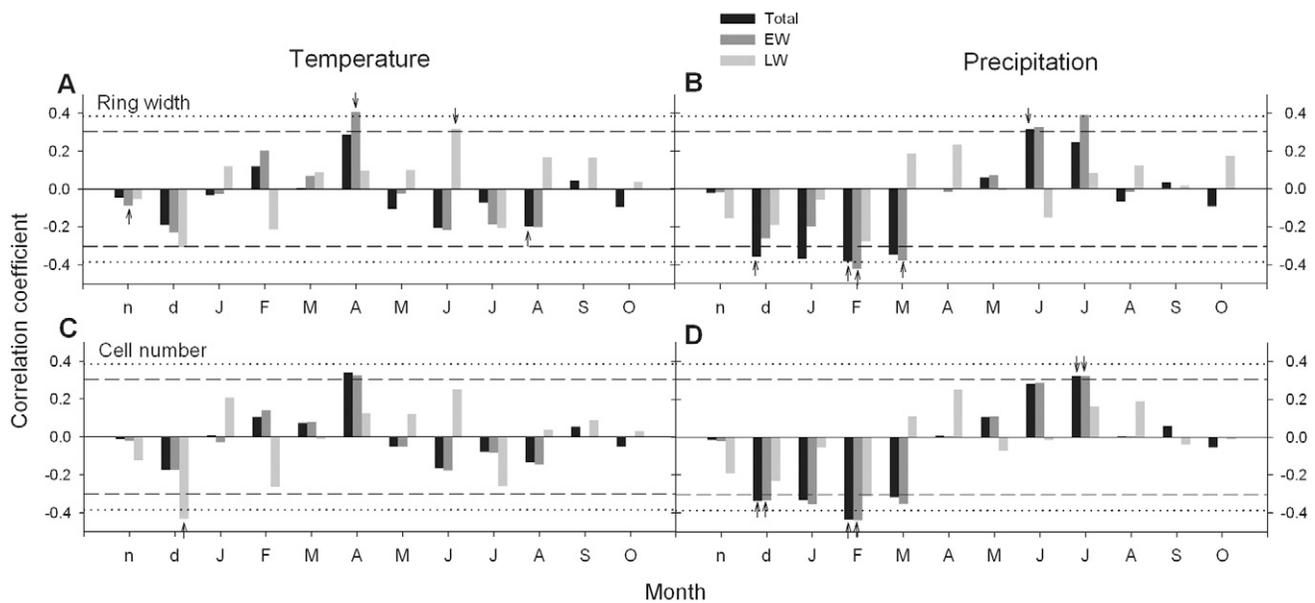


Fig. 4 Correlations between ring widths, cell numbers (for earlywood [EW], latewood [LW], and total ring), and monthly climatic variables (mean temperature and accumulated precipitation) for the period 1965–2004. Dashed lines indicate $P < 0.05$, and dotted lines indicate $P < 0.01$. Lowercase and uppercase letters correspond to the months of the previous and current years, respectively. Arrows indicate the parameters with significant values in multiple regression models.

tive, both), and March (negative, earlywood). Overall, these variables explained higher variance for ring widths than cell number, with 42.4% of explained variance for earlywood width and 45% for total ring width. Latewood cell number was inversely correlated to the previous December temperature, whereas latewood ring width was directly correlated to June temperature. Regression models for latewood-related parameters were poorer than for earlywood and total ring width parameters. This included a negative effect of previous December temperature on cell number and a positive effect of June temperature on width. These models explained 18.6% of total variance in the case of latewood cell number and 29.9% in the case of latewood width (table 1).

Earlywood tracheid diameters were positively correlated to February temperature and negatively correlated to March rainfall (fig. 5). CEL2 was positively correlated to June rainfall and April temperature and negatively correlated to June temperature. July temperature showed a negative effect on CEL3, whereas June and July precipitation showed a positive effect, and August temperature exerted a positive effect on CELLW. The percentage of total variance explained by multiple regression models was low for CEL1 (12.4%), high for CEL2 (41%) and CEL3 (24.9%), and again low for CELLW (14%; table 1). Climate-growth models for earlywood lumen diameter were almost identical to tracheid diameter models, but the explained variance was lower, except for LUMLW, which showed an additional negative effect of November temperature, with a total explained variance of 26.6% (fig. 5; table 1).

Earlywood wall thickness was positively correlated to rainfall during its formation, shifting from June for WAL1 and WAL2 to July for WAL3. The final part of earlywood

growth also had a negative signal from previous November–December temperature and precipitation. Latewood wall thickness (WALLW) was positively related to October rainfall and to August temperature. Regression models revealed significant effects of the same variables that were previously mentioned (table 1; fig. 5).

Wall-to-cell ratios showed no significant correlations for WC1, whereas WC2 was positively correlated to February rainfall ($r = 0.35$, $P < 0.025$), while WC3 was correlated to previous November temperature ($r = -0.32$, $P < 0.043$). Finally, WCLW was positively correlated to July rainfall ($r = 0.38$, $P = 0.016$). The parameters included in the regression models differed only for WCLW, since an additional negative effect of February temperature was observed, with a total explained variance of 27.6%.

The SEM model provided a good overall fit for our data set (fig. 6). Probabilities of χ^2 estimates were greater than 0.05 ($df = 16$, $\chi^2 = 17.930$, $P = 0.328$), with GFI being greater than 0.90 (0.909) and RMSEA being lower than 0.1 (0.056). The power of the model was mainly due to the robust sequential relationship between earlywood tracheid diameters. The second (CEL2) and third (CEL3) tracheid diameters were strongly dependent on the first (CEL1) tracheid diameter. Tracheid diameter was affected by different climatic factors throughout the ring. CEL1 increased after a warm February, whereas CEL2 and CEL3 were positively affected by June and July rainfall, respectively. The effect of March rainfall on CEL2 and CEL3 was nonsignificant, whereas April temperature showed a significant positive effect on CEL2.

The multiple regression model for June rainfall was highly significant ($df = 37$, $F = 11.879$, $P < 0.001$) and included

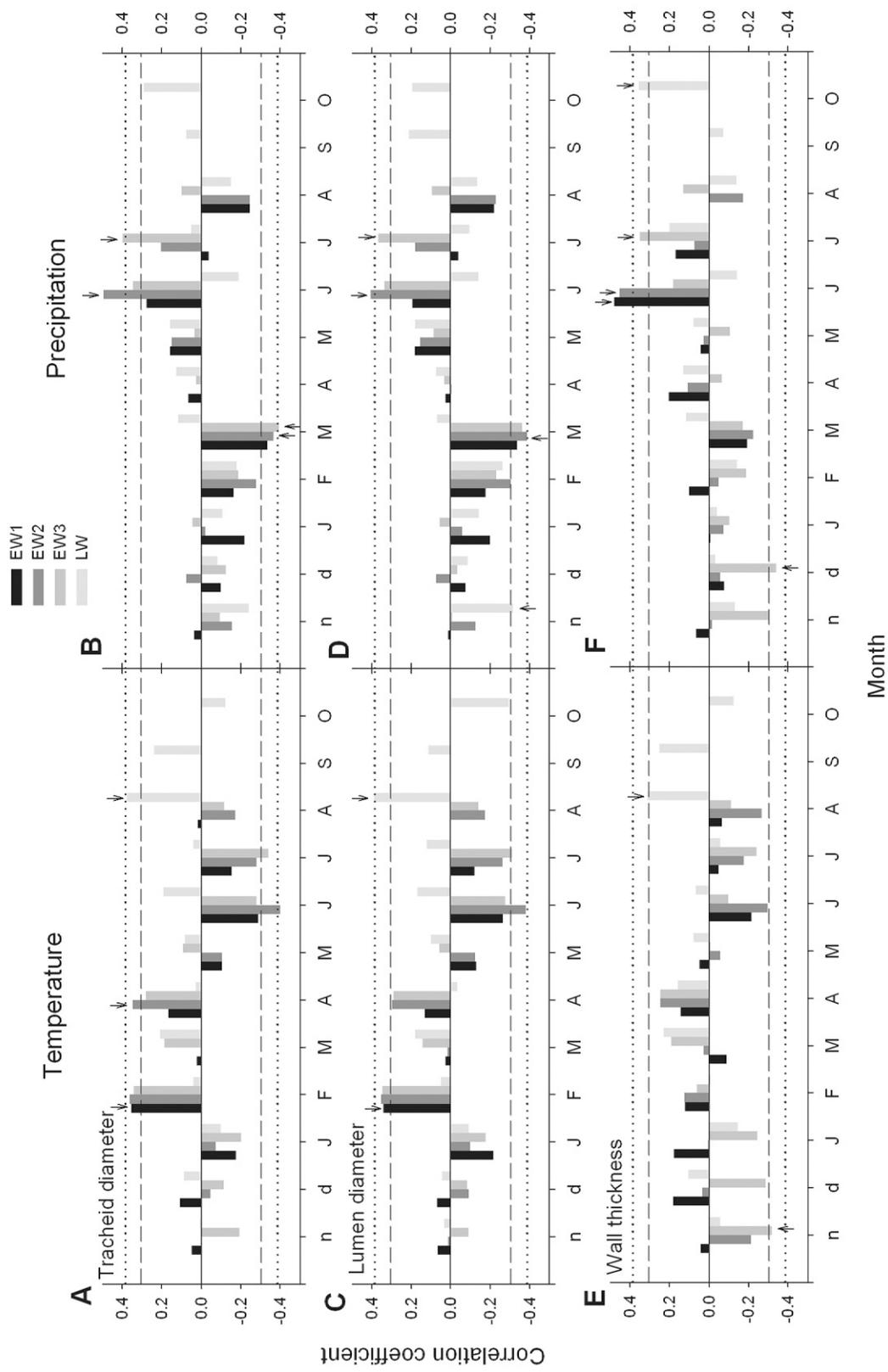


Fig. 5 Correlations between anatomical variables (tracheid size, lumen size, and wall thickness) for different parts of the ring (earlywood first [EW1], second [EW2], and third [EW3] cells; latewood [LW]) and monthly climatic variables (mean temperature and accumulated precipitation) for the period 1965–2004. Dashed lines indicate $P < 0.05$, and dotted lines indicate $P < 0.01$. Lowercase and uppercase letters correspond to the months of the previous and current years, respectively. Arrows indicate the parameters with significant values in multiple regression models.

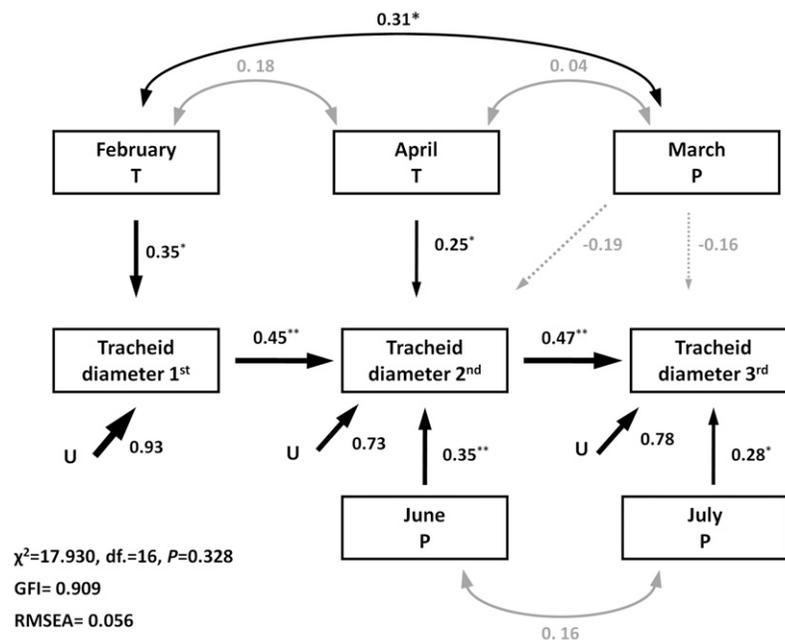


Fig. 6 Structural equation model for the climatic effects on earlywood tracheid diameters. Single-headed arrows depict causal relationships, whereas bidirectional arrows depict correlations. Positive effects are indicated by solid lines and negative effects by dashed lines. Arrow widths are proportional to path coefficients. The numbers in the paths indicate standardized path coefficients. Asterisks indicate significance levels: one asterisk, $P < 0.05$; two asterisks, $P < 0.01$. U, unexplained portion of variance.

CEL2 ($t = 3.124$, $P = 0.003$) and WAL1 ($t = 3.002$, $P = 0.005$) but not WAL2, since its information was redundant with that of CEL2 (partial correlation with June P; $r = 0.23$, $P = 0.15$). The simultaneous use of two proxies resulted in an important gain in explained variance after using this regression model, since r_{adj}^2 increased from 22.3% to 35.8%, with a relative increase of 60.5% from the best single estimator.

Discussion

Tracheid anatomical features provide information that reflects environmental conditions during xylogenesis, particularly during cell expansion and cell maturation phases. As a result, the shift in the climatic signal throughout the complete period of formation of a tree ring is expressed with high temporal resolution. In this study, information retrieved from anatomical features was unrelated to that present in ring width chronology. In contrast, cell number-based chronologies were strongly related to ring width chronologies. Thus, the combined use of cell numbers and tracheid anatomical features in *Juniperus thurifera* significantly improved our capacity to assess and model the effects of limiting climatic conditions in continental Mediterranean environments.

Climatic Control of Anatomical Features

The range in variation of the measured anatomical features is strongly constrained by their functionality. Therefore, the observed low sensitivity of anatomical variables, in compar-

ison to ring widths, was expected. However, small variations in wood anatomy may show both a strong impact on xylem properties and a significant dependence on climatic variability. Nevertheless, in this study, quality control statistics of the anatomical chronologies were also reduced in comparison with ring width-based chronologies, particularly for the wall thickness and wall-to-cell ratio. This result might suggest that tracheid-based chronologies should use larger sample depths to attain similar reliability to ring width-based chronologies, which implies a huge effort in comparison to the measurement of ring widths that is usually performed in dendroclimatic studies. The suitability of the measured anatomical parameters must be carefully evaluated, since previous studies have shown that even with very low inter-tree correlations and common signals, strong and sound climatic responses can be found in anatomical variables (Yasue et al. 2000). A reason for the weak common signal in these variables may be due to the low inter- and intraindividual variability of these parameters in comparison to ring widths.

Our results highlight the fact that tracheid size at the beginning of xylogenesis depends on conditions during late winter, with larger tracheids occurring after a warmer February. A strong signal of climatic conditions before the onset of the growing period has been shown to negatively influence the size of conductive elements in ring-porous trees (Fonti et al. 2007). This correlation has been interpreted as an increasing sensitivity of overwintering cambial derivatives by fostering the reactivation of auxin carriers (Fonti et al. 2007). Since tracheid size is highly correlated within earlywood growth along a radial file, the climatic signal influencing tra-

cheid size at the beginning of xylogenesis is maintained along the entire earlywood, probably leading to spurious correlations of cell size with February temperature and March rainfall. Tracheid size is also controlled by conditions during the cell expansion phase, which occurs during a brief period immediately following cell differentiation (Gričar 2009). Tracheids expand sequentially, which leads to a temporal shift in climatic conditions recorded by cell size during the expansion phase. The temporal shift is displayed by the climatic responses for CEL1, CEL2, and CEL3, with rainfall effects on cell size shifting from June in the second segment of the earlywood to July in the third segment. This timing is in accordance with previous knowledge on the phenology of earlywood tracheid enlargement for *J. thurifera*, which occurs from May to July (Camarero et al. 2010). The absence of any significant effect of rainfall on cell formation in the first segment of earlywood growth might be related to relatively low water stress, which is characteristic of May, when such cells are formed. Water deficit conditions in May occur in just 5% of the years, compared with 50% in June and 70% in July.

Two complementary mechanisms may explain, at least in part, the critical role of water availability for cell size. First, since cell turgor is associated with water supply during the cell expansion phase, cell enlargement may primarily result from a direct effect of short-term water availability (Kramer 1964; Von Wilpert 1991). Second, since plants respond to water stress by enhancing abscisic acid production, an indirect effect of water availability on cell size mediated by fitohormones is also possible (Xiong et al. 2002). Abscisic acid is involved in controlling the size of xylem conductive cells by blocking the action of indole-3-acetic acid, which in turn is responsible for tracheid expansion (Little and Savidge 1987). Although the details of the mechanisms involved in the process of cell expansion are still under discussion (Vaganov et al. 2011), this process would lead to an optimum adjustment of the hydraulic system to water availability.

Similarly, wall thickness was also controlled by June–July rainfall, but there was a minor delay in the timing, since the first and second parts of earlywood growth were correlated to June rainfall, whereas the third part of earlywood growth was affected by July rainfall. Such a time lag could be related to the fact that the cell maturation phase in our study area, including wall thickening, was initiated immediately after full cell expansion, which typically occurs during June and July (Camarero et al. 2010). Although final lumen size is fixed at the same time as wall thickness, earlywood lumen diameter chronologies and climatic response were almost identical to those corresponding to tracheid diameter (fig. 5). This may be related to the fact that the lumen diameter of earlywood cells is closely linked to tracheid diameter ($r = 0.98$, $P < 0.001$, $n = 44,131$) but shows little correlation to wall thickness ($r = 0.19$, $P < 0.001$, $n = 44,131$), which suggests that cell size—but not wall thickness—is determined during the cell expansion phase.

Like other evergreen Mediterranean conifers, *J. thurifera* is able to maintain photosynthetic activity throughout the entire year (Larcher 2000). Trees accumulate carbohydrates in winter, which are stored and subsequently allocated to early-

wood formation in spring, after cambial reactivation (Kagawa et al. 2006). The seasonal variation of carbohydrates in *J. thurifera* stems is coherent with this hypothesis, with resources being accumulated during winter and subsequently depleted at the start of xylogenesis (Camarero et al. 2010; DeSoto 2010). The observed climate-growth relationships suggest that cambial activity is strongly affected by winter conditions, particularly rainfall from December to March. The number of cells is higher after dry winters, a fact that has been previously interpreted as the effect of cloudy and snowy conditions during wet winters, in addition to the subsequent reduction of both maximum daily temperatures and solar radiation received by the trees, which results in reduced photosynthetic rates and cambial activity (Rozas et al. 2009; DeSoto 2010). Total cell number and ring width may also be determined by a prolonged growing season caused by an earlier onset of xylogenesis following warm April conditions and improved division rates due to rainy summer conditions (Camarero et al. 2010).

A certain degree of dissimilarity in the climatic signal shown by earlywood and latewood chronologies was expected, given the distinct phenology of their xylogenesis (see Camarero et al. 2010). Nevertheless, the strong differential signal that was observed is related to a dramatic change in the factors that control tracheid development. These factors showed a shift from a positive control of water availability during June–July in the earlywood phase to a positive control of temperature in August–September in the latewood phase. This relationship might seem similar to that observed in alpine environments, where latewood formation is limited by summer temperature, with cool summers leading to “light rings,” almost completely constituted by earlywood (Gindl 1999; Yasue et al. 2000; Wang et al. 2002). Nevertheless, our results on *J. thurifera* are not comparable, since temperature exerts a positive effect on both wall thickness and lumen size, which does not necessarily lead to “dense” rings.

The results on latewood parameters should be interpreted with caution because of high interindividual variability, which suggests the need for a much larger sample depth than used in this exploratory study. A potential interpretation is that our results reflect the different nature of latewood in junipers compared with other gymnosperms. *Juniperus thurifera* showed a very low percentage of latewood in annual rings, while wall thickness barely varied across the complete tree ring. Both results suggest that latewood may simply result from a reduction of tracheid enlargement at the end of the growing season, rather than a true differentiation of a particular cell type characteristic of the latewood, as is usual in other conifers, that is, showing a considerably higher wall thickness of latewood than earlywood cells (Yasue et al. 2000). Usually, earlywood has ~11 times the specific conductivity of latewood, and up to 90% of total water flow occurs through the earlywood. Latewood has a lower vulnerability to embolism, playing a strong role on hydraulic security (Domec and Gartner 2002). In addition, latewood shows higher density and a key structural function, whereby its formation constitutes a sink for carbohydrates, since it requires a continuous supply of energy, either from storage tissues or from photosynthesis (Oribe et al. 2003). However, the need for these latewood functions may be reduced because of the

combination of the small size of *J. thurifera* trees (with a maximum height of ~11 m at the study site; Rozas et al. 2009), the high specific conductivity and low vulnerability to embolism of its earlywood (Willson et al. 2008), and exiguous carbohydrate availability in a very restrictive environment, thus explaining the reduced development of latewood in this species.

The Potential of Anatomical Features as Climate Proxies

In accordance with our findings, a close link between tree ring width and cell number has been previously described for gymnosperm tree species (Gregory 1971; Camarero et al. 1998). Ring width may be interpreted as resulting from the combination of cell number and cell size, and therefore it should encode similar information to that of both parameters. The relative strength of tracheid number and size in determining tree ring width may be species or even context dependent (Wang et al. 2002; Panyushkina et al. 2003). In our case, the low variability found in tracheid diameter compared with cell number resulted in the observed close relationship between cell number and ring width chronologies and between their respective climatic signals. Therefore, a separate analysis of climatic signals related to cell number and tracheid diameter provided a more powerful tool toward understanding tree climatic sensitivity of wood structure than tree ring width chronologies alone. Moreover, this analysis allowed us to obtain a higher number of climatic proxies, in addition to several proxies for the same climatic parameter, which may be used together (Meko et al. 1980). The diversification of biological proxies with different characteristics allowed the power of the calculated models to be improved for performing climatic reconstructions. This improvement was shown in this work for June rainfall, with an additional 13.5% of variance being explained when anatomical variables were accounted for.

Lumen diameters and wall-to-cell ratios generally show a direct functional meaning related to xylem hydraulic properties, such as conductivity or resistance to implosion (Tyree and Zimmerman 2002). Consequently, they constitute standard measurements in ecophysiological studies (Hacke and Sperry 2001), which have been recently incorporated into dendroclimatic studies on conifers (Fonti et al. 2010). Nevertheless, since these parameters result from the combined effects of two separate xylogenetic processes—namely, tracheid expansion and maturation—the interpretation of their link to particular climatic parameters is complex. Hence, the use of tracheid diameter and wall thickness seems more straightforward, since both parameters respond to prevailing climatic conditions during cell expansion and maturation phases, respectively. Consequently, we suggest that the use of these or other simple anatomical parameters may be appropriate for evaluating the intraseasonal climatic influence on anatomical features during xylogenesis. The inclusion of derived variables, such as lumen diameter or wall-to-cell ratios, should be accomplished in the context of adequate statistical techniques, such as structural equation modeling, where the additive effect of different climatic parameters on these features throughout cell expansion and wall thickening can be satisfactorily incorporated.

Standardization through the tracheidogram method allows tree rings with different cell numbers to be comparable (Vaganov 1990). However, cambial activity initiation, intensity, and duration can show strong yearly, intra-annual, and even interindividual variability (Lupi et al. 2010). Thus, standardization to a common number of cells does not ensure that cells in the same position have been formed simultaneously. Despite the accuracy of existing knowledge about xylogenesis in our study species, this uncertainty increases with the number of standardized cells. The number of standardized cells is a compromise between the required intra-annual temporal resolution and the temporal homogeneity in the within-ring cell position. Consequently, the number of standardized tracheids that may be generated from raw data is a key aspect in climatic studies of anatomical features. The optimum number of tracheids may depend on the specific goals of the study and should be kept low enough to guarantee a high within-standardized tracheid temporal homogeneity, if the goal is to assess climate-growth relationships or to obtain a reliable proxy for climatic reconstruction.

Conclusions

The combination of anatomical features, cell number, and ring width data depicted the multiplicity of factors that control secondary growth under a continental Mediterranean climate. Winter conditions determine the pattern of carbohydrate accumulation before cambial activity onset, consequently affecting final ring width. Late winter temperature serves as a cue for initial tracheid size, while warm spring temperature determines the onset of cambial activity. Cambial activity and intensity, as well as earlywood tracheid and wall size, are positively related to early summer rainfall, whereas latewood tracheid and wall features are positively controlled by late summer temperature. Our exploratory study shows the strong potential of anatomical-based chronologies to improve our understanding of climatic effects on growth and tree ring formation under really complex situations, such as continental Mediterranean climates, where multiple control factors shift across the year. Moreover, our ability to reconstruct past climatic time series may be greatly enhanced by using novel climatic proxies and, particularly, by the existence of multiple uncorrelated biological proxies of the same climatic parameters. Further research should focus on confirming and validating the general applicability of tracheid-based chronologies in Mediterranean environments. Finally, our study reinforces the potential use of SEM models in the context of dendrochronology.

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Literature Cited

- Arbuckle JL 2003 Amos 5.0 update to the Amos user's guide. Smallwaters, Chicago.
- Bertraudière V, N Montès, T Gauquelin, J-L Édouard 1999 Dendroécologie du genévrier thurifère (*Juniperus thurifera* L.) exemple de la thuriferaie de la montagne de Rié (Pyrénées, France). *Ann For Sci* 56:685–697.
- Bouriaud O, J-M Leban, D Bert, C Deleuze 2005 Intra-annual variations in climate influence growth and wood density of Norway spruce. *Tree Physiol* 25:651–660.
- Camarero JJ, J Guerrero-Campo, E Gutierrez 1998 Tree-ring growth and structure of *Pinus uncinata* and *Pinus sylvestris* in the Central Spanish Pyrenees. *Arct Alp Res* 30:1–10.
- Camarero JJ, JM Olano, A Parras 2010 Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. *New Phytol* 185:471–480.
- Chave J, D Coomes, S Jansen, SL Lewis, NG Swenson, AE Zanne 2009 Towards a worldwide wood economics spectrum. *Ecol Lett* 12:351–366.
- Cook ER, RL Holmes 1996 Guide for computer program ARSTAN. Pages 75–87 in HD Grissino-Mayer, RL Holmes, HC Fritts, eds. The international tree-ring data bank program library version 2.0 user's manual. Laboratory of Tree-Ring Research, University of Arizona, Tucson.
- Cook ER, K Peters 1981 The smoothing spline: a new approach to standardizing forest interior tree-ring width series for dendroclimatic studies. *Tree-Ring Bull* 41:45–53.
- De Luis M, J Gričar, K Čufar, J Raventós 2007 Seasonal dynamics of wood formation in *Pinus halepensis* from dry and semi-arid ecosystems in Spain. *IAWA J* 28:389–404.
- Denne MP 1988 Definition of latewood according to Mork (1928). *IAWA Bull* 10:59–62.
- DeSoto L 2010 Global change effect on the dioecious tree *Juniperus thurifera* in the Iberian Peninsula. PhD diss. University of Valladolid, Soria.
- DeSoto L, JJ Camarero, JM Olano, R Rozas 2012 Geographically structured and temporally unstable growth responses of *Juniperus thurifera* to recent climate variability in the Iberian Peninsula. *Eur J For Res*, doi:10.1007/s10342-011-0564-7.
- DeSoto L, M de la Cruz, P Fonti 2011 Intra-annual pattern of tracheid size in the Mediterranean *Juniperus thurifera* as indicator for seasonal water stress. *Can J For Res* 41:1280–1294.
- Domec JC, BL Gartner 2002 How do water transport and water storage differ in coniferous earlywood and latewood? *J Exp Bot* 53: 2369–2379.
- Eilmann B, R Zweifel, N Buchmann, E Graf Pannatier, A Rigling 2011 Drought alters timing, quantity, and quality of wood formation in Scots pine. *J Exp Bot* 62:2763–2771.
- Fonti P, N Solomonoff, I García-González 2007 Earlywood vessels of *Castanea sativa* record temperature before their formation. *New Phytol* 173:562–570.
- Fonti P, G von Arx, I García-González, B Eilmann, U Sass-Klaassen, H Gärtner, D Eckstein 2010 Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. *New Phytol* 185:42–53.
- Fritts HC 1976 Tree rings and climate. Kluwer Academic, New York.
- García-González I, P Fonti 2006 Selecting earlywood vessels to maximize their environmental signal. *Tree Physiol* 26:1289–1296.
- García-Plazaola JI, T Faria, J Abadia, A Abadia, MM Chaves, JS Pereira 1997 Seasonal changes in xantophyll composition and photosynthesis of cork oak (*Quercus suber* L.) leaves under Mediterranean climate. *J Exp Bot* 48:1667–1674.
- Gindl W 1999 Climatic significance of light rings in timberline spruce, *Picea abies*. *Arct Alp Res* 31:242–246.
- Grace JB, TM Anderson, H Olf, SM Scheiner 2010 On the specification of structural equation models for ecological systems. *Ecol Monogr* 80:67–87.
- Gregory RA 1971 Cambial activity in Alaskan white spruce. *Am J Bot* 58:160–171.
- Gričar J 2009 Significance of intra-annual studies of radial growth in trees. In R Kaczka, I Malik, P Owczarek, H Gärtner, G Helle, J Heinrich, eds. TRACE: tree rings in archaeology, climatology and ecology. Vol 7. Proceedings of the Dendrosymposium, April 27–30, 2008. GeoForschungsZentrum, Zakopane.
- Grissino-Mayer HD 2001 Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-Ring Res* 57:205–221.
- Hacke UG, JS Sperry 2001 Functional and ecological xylem anatomy. *Perspect Plant Ecol* 4:97–115.
- Hughes MK, TW Swetnam, HF Diaz 2011 Dendroclimatology: progress and prospects. Springer, New York.
- Iriondo JM, MJ Albert, A Escudero 2003 Structural equation modelling: an alternative for assessing causal relationships in threatened plant populations. *Biol Conserv* 113:367–377.
- Kagawa A, A Sugimoto, TC Maximov 2006 ¹³CO₂ pulse-labelling of photoassimilates reveals carbon allocation within and between tree rings. *Plant Cell Environ* 29:1571–1584.
- Kirilyanov A, M Hughes, EA Vaganov, F Schweingruber, P Silkin 2003 The importance of early summer temperature and date of snow melt for tree growth in the Siberian Subarctic. *Trees* 17:61–69.
- Kramer PJ 1964 The role of water in wood formation. Pages 519–532 in MH Zimmermann, ed. The formation of wood in forest trees. Academic Press, New York.
- Larcher W 2000 Temperature stress and survival ability of Mediterranean sclerophyllous plants. *Plant Biosyst* 134:279–295.
- Legendre P, L Legendre 1998 Numerical ecology. Elsevier, Amsterdam.
- Little CHA, RA Savidge 1987 The role of plant growth regulators in forest tree cambial growth. *Plant Growth Regul* 6:137–169.
- Lupi C, H Morin, A Deslauriers, S Rossi 2010 Xylem phenology and wood production: resolving the chicken-or-egg dilemma. *Plant Cell Environ* 33:1721–1730.
- McCarroll D, NJ Loader 2004 Stable isotopes in tree rings. *Quat Sci Rev* 23:771–801.
- Meko DM, CW Stockton, WR Boggess 1980 A tree-ring reconstruction of drought in southern California. *Water Res Bull* 16:594–600.
- Mitrakos KA 1980 A theory for Mediterranean plant life. *Acta Oecol* 1:245–252.
- Novak M, J Mikova, M Krachler, J Kosler, L Erbanova, E Prechova, I Jackova, D Fottova 2010 Radial distribution of lead and lead isotopes in stem wood of Norway spruce: a reliable archive of pollution trends in Central Europe. *Geochim Cosmochim Acta* 74: 4207–4218.
- Oksanen J, FG Blanchet, R Kindt, P Legendre, RB O'Hara, GL Simpson, P Solymos, MH Stevens, H Wagner 2010 vegan: community ecology Package. R package, version 1.17-4. <http://CRAN.R-project.org/package=vegan>.
- Olano JM, V Rozas, D Bartolomé, D Sanz 2008 Effects of changes in traditional management on height and radial growth patterns in a *Juniperus thurifera* L. woodland. *For Ecol Manag* 255:506–512.
- Oribe Y, R Funada, T Kubo 2003 Relationships between cambial activity, cell differentiation and the localisation of starch in storage tissues around the cambium in locally heated stems of *Abies sachalinensis* (Schmidt) Masters. *Trees* 17:185–192.
- Panyushkina IP, MK Hughes, EA Vaganov, MAR Munro 2003 Summer temperature in northeastern Siberia since 1642 reconstructed

- from tracheid dimensions and cell numbers of *Larix cajanderi*. Can J For Res 33:1905–1914.
- Pittermann J, JS Sperry, J Wheeler, U Hacke, E Sikkema 2006 Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. Plant Cell Environ 29:1618–1628.
- R Development Core Team 2011 R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Rigling A, OU Braker, G Schneider, FH Schweingruber 2002 Intra-annual tree-ring parameters indicating differences in drought stress of *Pinus sylvestris* forests within the *Erico-Pinion* in the Valais (Switzerland). Plant Ecol 163:105–121.
- Rivas-Martínez S, J Loidi 1999 Bioclimatology of the Iberian Peninsula. Itin Geobot 13:41–47.
- Rossi S, A Deslauriers, T Anfodillo 2006 Assessment of cambial activity and xylogenesis by microsampling tree species: an example at the Alpine timberline. IAWA J 27:383–394.
- Rossi S, A Deslauriers, J Gričar, J-W Seo, CB Rathgeber, T Anfodillo, H Morin, T Levanic, P Oven, R Jalkanen 2008 Critical temperatures for xylogenesis in conifers of cold climates. Glob Ecol Biogeogr 17:696–707.
- Rozas V, L DeSoto, JM Olano 2009 Sex-specific, age-dependent sensitivity of tree-ring growth to climate in the dioecious tree *Juniperus thurifera*. New Phytol 182:687–697.
- Samuels AL, M Kaneda, H Rensing 2006 The cell biology of wood formation: from cambial divisions to mature secondary xylem. Can J Bot 84:631–639.
- Schweingruber FH 1996 Tree rings and environment: dendroecology. Haupt, Berne.
- Schweingruber FH, P Poschold 2005 Growth rings in herbs and shrubs: life span, age determination and stem anatomy. For Snow Landsc Res 79:195–415.
- Sperry JS 2003 Evolution of water transport and xylem structure. Int J Plant Sci 164:115–127.
- Tanaka JS 1987 “How big is enough?”: sample size and goodness of fit in structural equation models with latent variables. Child Dev 58:134–146.
- Tyree MT, MH Zimmerman 2002 Xylem structure and the ascent of sap. 2nd ed. Springer, New York.
- Vaganov EA 1990 The tracheidogram method in tree-ring analysis and its application. Pages 63–76 in ER Cook, LA Kairiukstis, eds. Methods of dendrochronology: applications in the environmental sciences. Kluwer Academic, Dordrecht.
- Vaganov EA, KJ Anchukaitis, MN Evans 2011 How well understood are the processes that create dendroclimatic records? a mechanistic model of the climatic control on conifer tree-ring growth dynamics. Pages 37–75 in MK Hughes, TW Swetnam, HF Diaz, eds. Dendroclimatology: progress and prospects. Vol 2. Springer, New York.
- Vieira J, F Campelo, C Nabais 2009 Age-dependent responses of tree-ring growth and intra-annual density fluctuations of *Pinus pinaster* to Mediterranean climate. Trees 23:257–265.
- Von Wilpert K 1991 Intra annual variation of radial tracheid diameters as a monitor of site specific water stress. Dendrochronologia 9:95–114.
- Wang L, S Payette, Y Bégin 2002 Relationships between anatomical and densitometric characteristics of black spruce and summer temperature at tree line in northern Quebec. Can J For Res 32:477–486.
- Willson CJ, PS Manos, RB Jackson 2008 Hydraulic traits are influenced by phylogenetic history in the drought-resistant, invasive genus *Juniperus* (Cupressaceae). Am J Bot 95:299–314.
- Xiong L, KS Schumaker, J-K Zhu 2002 Cell signaling during cold, drought, and salt stress. Plant Cell 14:S165–S183.
- Yamaguchi DK 1991 A simple method for crossdating increment cores from living trees. Can J For Res 21:414–416.
- Yasue K, R Funada, O Kobayashi, J Ohtani 2000 The effects of tracheid dimensions on variations in maximum density of *Picea glehnii* and relationships to climatic factors. Trees 14:223–229.
- Zweifel R, L Zimmermann, F Zeugin, DM Newbery 2006 Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. J Exp Bot 57:1445–1459.