

**TEMPERATURE-INDUCED RESPONSES OF XYLEM STRUCTURE
 OF *LARIX SIBIRICA* (PINACEAE) FROM RUSSIAN ALTAY¹**

PATRICK FONTI^{2,5}, MARINA V. BRYUKHANOVA³, VLADIMIR S. MYGLAN⁴,
 ALEXANDER V. KIRDYANOV³, OKSANA V. NAUMOVA⁴, AND EUGENE A. VAGANOV⁴

²WSL Swiss Federal Research Institute, Landscape Dynamics, Zürcherstr 111, CH-8903 Birmensdorf, Switzerland;

³V.N. Sukachev Institute of Forest SB RAS, Akademgorodok 50, bld. 28 660036, Krasnoyarsk, Russia; and ⁴Siberian Federal University, Svobodny av. 79 660041, Krasnoyarsk, Russia

- *Premise of the study:* Xylem structure determines the hydraulic and mechanical properties of a stem, and its plasticity is fundamental for maintaining tree performance under changing conditions. Unveiling the mechanism and the range of xylem adjustment is thus necessary to anticipate climate change impacts on vegetation.
- *Methods:* To understand the mechanistic process and the functional impact of xylem responses to warming in a cold-limited environment, we investigated the relationship between temperature and tracheid anatomy along a 312-yr tree-ring chronology of *Larix sibirica* trees from the Altay Mountains in Russia.
- *Key results:* Climate-growth analyses indicated that warming favors wider earlywood cell lumen, thicker latewood walls, denser maximum latewood, and wider rings. The temperature signal of the latewood was stronger ($r > 0.7$) and covered a longer and more stable period (from June to August) than that of earlywood and tree-ring width. Long-term analyses indicated a diverging trend between lumen and cell wall of early- and latewood.
- *Conclusions:* Xylem anatomy appears to respond to warming temperatures. A warmer early-growing season raises water conduction capacity by increasing the number and size of earlywood tracheids. The higher-performing earlywood tracheids promote more carbon fixation of the latewood cells by incrementing the rate of assimilation when summer conditions are favorable for growth. The diverging long-term variation of lumen and cell wall in earlywood vs. latewood suggests that xylem adjustments in latewood increase mechanical integrity and support increasing tree size under the ameliorated growing conditions.

Key words: climatic conditions; *Larix sibirica*; Pinaceae; tracheid lumen; tracheid wall; tree-ring anatomy; wood density; xylem anatomical traits.

Xylem is a fundamental element of tree hydraulic and mechanical architecture (Tyree and Zimmermann, 2002) and is also an important pathway through which the hydrological and carbon cycles are connected (Fonti and Jansen, 2012). While xylem structure defines water transport and mechanical support to the leaves, which are essential for photosynthetic production, building and maintaining a continuously functional xylem requires a major proportion of the carbon assimilated. Because xylem production occurs under limited resources, the resulting structure is shaped by the competing demands of water transport, mechanical support, and storage of water and nonstructural carbohydrates for defense and resistance to stress (Carlquist, 1975; von Arx et al., 2012).

Xylem functional demands are variable over a tree's lifetime. As a tree grows in size, the hydraulic resistance as well as the

mechanical pressure increases. During the lifespan of a tree, environmental conditions for fixing carbon (such as solar radiation, nutrients, moisture, and warmth) are continuously changing and modifying the carbon pool available for xylem production. In addition, predicted climate changes will strongly modify the growing conditions over the majority of the globe (IPCC, 2007). Thus, to maintain tree performance under changing conditions, appropriate adjustments in the xylem are required.

Through xylem phenotypic plasticity, trees are able to a certain extent to continuously adjust to changing growing conditions (e.g., Bryukhanova and Fonti, 2013). Xylem formation can in fact be plastic enough to produce new tissue fitting changing ontogenetic and environmental demands (Meinzer et al., 2011). These adjustments occur when, year after year, new layers of xylem cells are formed to replace the old and nonfunctional ones. An extensive body of literature has already related the cellular structure of the xylem to its functioning (e.g., Carlquist and Hoekman, 1985; Tyree and Ewers, 1991; Carlquist, 2001; Choat et al., 2007; Wheeler et al., 2007; Sperry et al., 2008; Poorter et al., 2010). For example, variation in anatomical characteristics such as size, density, and grouping of conduits, cell wall thickness, pit structure, and percentage of ray parenchyma have been demonstrated to play a central role in regulating tree hydraulic and mechanical functioning (Baas and Schweingruber, 1987; Larson, 1994; Gartner, 1995; Mattheck and Kubler, 1995; Pittermann et al., 2010; von Arx et al., 2012).

In temperate and boreal conifers, tree rings are mainly composed of two types of tracheids, the earlywood cells, which are produced early in the growing season and are primarily responsible

¹Manuscript received 16 September 2012; revision accepted 22 January 2013.

The authors thank N. S. van Doorn for editing the English. This work has been supported by the Russian Foundation for Basic Research (Project Number 11-04-91153_a) and the Swiss National Science Foundation projects "Identifying seasonal climatic signals from water conducting cells in tree rings" (Nr. IZK0Z3_131408), "Tree growth and forest ecosystem functioning in Eurasia under changing climate" (Nr. IZ73Z0_128035), and "INtra-seasonal Tree growth along Elevational GRAdients in the European Alps" (INTEGRAL, Nr 200021_121859), and the Ministry of Education and Science of the Russian Federation (Scientific School 5327.2012.4).

⁵Author for correspondence (e-mail: patrick.fonti@wsl.ch)

for water transport, and the latewood tracheids, which are produced after the earlywood cells and serve as mechanical support. These cells, resulting from a sequential process of cell division, expansion, and secondary wall thickening, are produced at different times during one growing season and are chronologically disposed along radial files (Wodzicki, 1971; Rossi et al., 2006a). Since the expansion phase determines the size of the tracheid, and the phase of maturation regulates the thickness of the cell wall, depending on the anatomical trait and the tracheid considered, we might expect differing structural adjustments in responses to climatic variations (Vaganov et al., 1999, 2006). The ability of a genotype to adjust the phenotype over the life of a tree is a result of short- to long-term responses to environmental variability and can be used to link the environment with xylem structure.

Tree-ring anatomy, i.e., the study of anatomical structures along dated tree-rings, is a tool available to plant ecologists to better understand xylem phenotypic plasticity (Vaganov et al., 2006; Eilmann et al., 2010; Fonti et al., 2010). Because the radial patterns of wood structure reflect the changing demands placed upon woody plants as they grow and experience differing environmental conditions, the study of their variation over time and in response to differences in climate offers the opportunity to evaluate how well trees will respond to predicted global changes. A limited capacity for phenotypic adjustment might have detrimental consequences for tree productivity and mortality. Widespread forest mortality episodes related to drought or temperature stress have been documented in multiple biomes and on all vegetated continents (Breshears et al., 2009; Allen et al., 2010), demonstrating that many forest ecosystems are already impacted by climate change (Bigler et al., 2006; McDowell et al., 2008). Understanding the mechanism of responses and their functional impact, as well as identifying differences between species and/or genotypes, will contribute to better predictions of vegetation change associated with projected climate change.

In this study, we demonstrate how studies of tree-ring anatomy may help reveal plastic responses in xylem to changing growing conditions. Here we investigated short-term to long-term xylem anatomical changes to varying warming conditions by analyzing a 312-yr tree-ring chronology of high altitude and mature *Larix sibirica* Ldb. (Pinaceae) growing on a temperature limited site from the Altay Mountains in Russia. In particular, we compared the strengths and shifts in climatic signal of differing anatomical traits of cells built at differing times (earlywood or latewood cells) and fulfilling differing functions (hydraulic conductivity or mechanical support).

MATERIALS AND METHODS

Wood samples—The tree-ring material analyzed in this study was collected in 2008 with the primary aim of reconstructing past temperatures by means of several tree-ring proxies (Mygland et al., 2012; Sidorova et al., 2012). The material was collected from *Larix sibirica* Ldb. (Pinaceae) trees growing in the Altay Mountains in southern Siberia (Tuva Republic, Russia; 50°23'N, 89°04'E). The trees, located at the upper tree line between 2280 and 2340 m a.s.l. were alive and up to 600 yr old. Tree architecture and stand structure were typical of a growth-limited site characterized by small, stunted trees that were sparsely and irregularly distributed either singly or in clusters. The forested sites of the area located at this elevation are underlain by permafrost with a seasonally thawing layer of 80–100 cm and are usually snow-free from the end of May to the beginning of September. The region is characterized by a cold and dry continental climate. Records from the nearby climatic station of Mugur-Aksy (1850 m a.s.l., 1970–1994) located 20 km away indicated an annual mean temperature of

~3° and a total precipitation of 152 mm. The original sampling included 25 trees of different ages and consisted of two cores per trees taken at stem breast height using a 5-mm-diameter increment borer.

Tree-ring dating and wood density survey—Tree-ring width (TRW) was measured for one core per tree with a 0.01 mm precision using the LINTAB V 3.0 measuring system connected to TSAP software (Rinn Tech GmbH, Germany). Tree-ring widths of the 25 individual cores were visually cross-dated, and dating was verified using the quality-control program COFECHA (Holmes, 1983). Wood density was measured for the second set of 25 cores from the same trees. Density profiles of tree rings were obtained with a 0.01-mm resolution using the microdensitometer DENDRO-2003 (Walesch Electronics, Switzerland) according to the method described by Schweingruber (1996). The maximum density (MXD) values of each annual ring profile as well as automatically measured tree-rings widths were used to characterize each tree ring and to build chronologies. To check the quality of cross-dating, we compared the TRW chronologies from density measurements and annual ring width measurements.

Cell anatomical survey—Because of the amount of work required to measure detailed features of tracheids over long time series, cell anatomical measurements were performed on a subsample of five trees. Trees in the subsample were chosen based on the length of the tree-ring series (covering at least the last 300 yr) and on their fit with the tree-ring width master chronology ($r > 0.7$). Tracheid anatomy was measured for the cores of the five selected trees along the common period, i.e., for the dated rings from 1695–2007. Cell dimensions were measured ring by ring from digital images of the core transversal microsection using image analysis software AxioVision Rel. 4.8.2 (Carl Zeiss, Oberkochen, Germany). Microsections (20 μm thick) were prepared using a sliding microtome (Reichert, Germany) and stained with methylene blue. Images of each ring cross section were captured using a digital camera connected to a microscope (Axio Imager A1m, Carl Zeiss, Oberkochen, Germany) with 400 \times magnification. For each ring, tracheids were measured cell by cell along five radial files (Fig. 1). The radial files were selected among those with larger tangential cell diameter to avoid bias due to smaller size at the tips of the tracheid. For each selected radial file, we counted the number of tracheids (NT), and for each tracheid we measured the radial lumen size (LD), the cell wall thickness (CWT), and the radial cell diameter (D). From these cell measurements, the cell wall area ($\text{CWA} = 2\text{CWT}(T + D - 2\text{CWT})$) and the cell lumen area ($\text{LUM} = \text{DT} - \text{CWA}$) were calculated assuming a constant tangential cell diameter T of 30 μm , which is characteristic for these radial files. Further, tracheid anatomical traits were calculated for different tree-ring sectors, i.e., by averaging the values of the trait for all the tracheids measured within the same tree-ring sector, defined as the whole ring (WHOLE), composed of the earlywood (EW) and the latewood (LW). In addition, since the cells in the transition zone between earlywood and latewood might encode a mixed signal, analyses were also performed for the sectors considering only the first (First) and last (Last) two cells of the radial files in each ring, respectively. Tracheid assignment to earlywood and latewood was applied according to the ratio between LD and the value of CWT doubled, i.e., a tracheid was assigned to belong to LW if $\text{LD}/\text{CWT} < 3$. The threshold ratio of 3 was determined as the minimum frequency between the bimodal density distribution obtained from the LD to CWT ratio of all measured cells (according to Bryukhanova and Fonti [2013]). Finally, the number of cells (NT) and the ring widths (TRW, EWW, LWLW) were calculated for the respective ring sectors WHOLE, EW, and LW.

Detrending and climate-growth relationships—Before averaging into site chronologies, the density and anatomical individual time-series were standardized to remove age-related trends (Cook and Kairiukstis, 1990). The density series was standardized using Regional curve standardization (RCS)-detrending, Esper et al., 2003). The anatomical time series and TRW were detrended using a negative exponential function.

The effect of temperature and precipitation on the interannual variability of tree-ring growth was quantified by the strength of the common signal (i.e., a measure of the common variability among the tree-ring series) and of the climatic signal (i.e., a measure of the relationship between the common variability among the tree-ring series and the annual climatic variability). The common signal was quantified using the mean correlation between all the trees (Rbt). The climatic signal was quantified through correlations between the detrended chronologies and the monthly climatic data from the nearby meteorological stations. These calculations were performed by calculating Pearson's correlation with each month from May of the previous year to September of the current year, as well as using a moving window of 30 d running over the whole growing

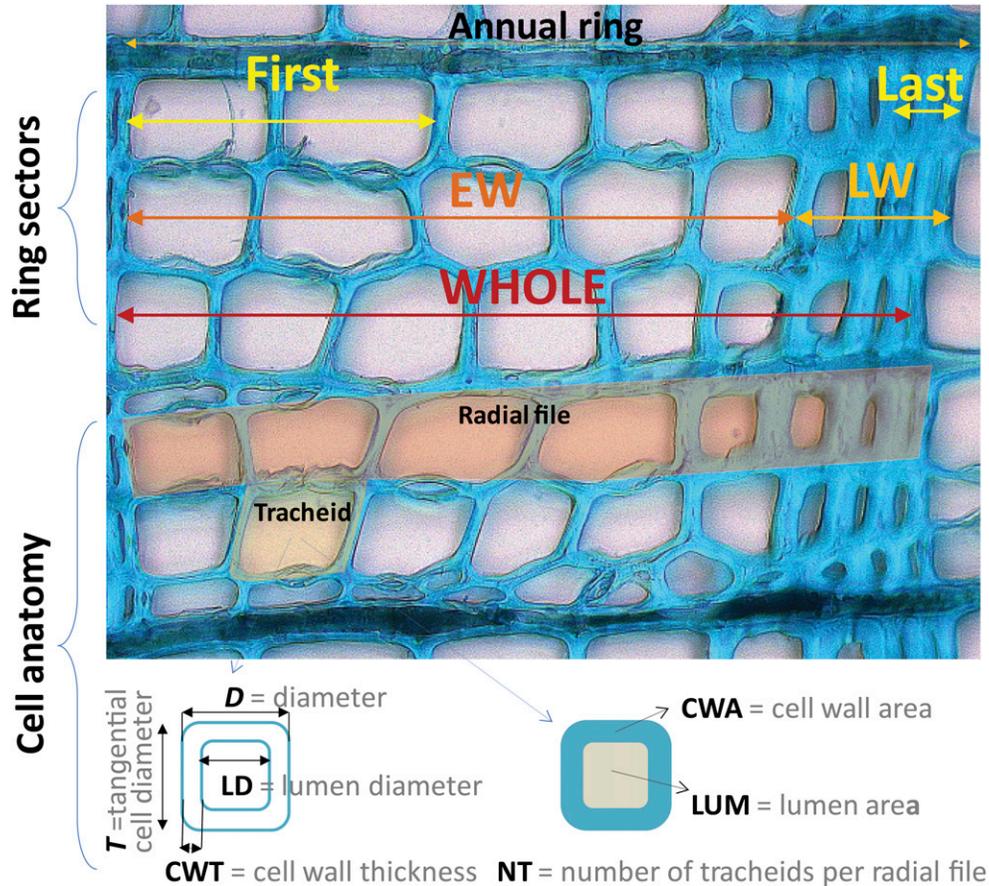


Fig. 1. Example of a tree-ring image and relative descriptions of the xylem anatomical traits and ring sectors considered.

season from February to October. The latter correlations were performed to take into account the effect of short-term weather influences and shifts in xylogenesis. A 30-d window was taken since the phase of cell differentiation usually takes more than 30 d (Anfodillo et al., 2012). Monthly instrumental climate records were available for the stations of Kosch-Agach (50°02'N, 88°68'E, 1758 m a.s.l., 1934–1993 for precipitation and 1958–1993 for temperature); Ak-Kem (49°55'N 86°32'E, 2056 m a.s.l., 1969–1994); and Mugur-Aksy (50°23'N, 90°26'E, 1850 m a.s.l., 1970–1994). Gridded CRU TS3 data (between 45°N–55°N and 70°E–100°E; <http://climexp.knmi.nl>, Mitchell and Jones, 2005) were used to expand the instrumental period from 1901 to 2007. Daily temperature was also obtained from <http://climexp.knmi.nl> for Mugur Aksy and for the low elevation stations of Uch-Aral (46°17'N, 80°93'E, 388 m a.s.l., 1936–2005); Zajsan (47°47'N, 84°92'E, 603 m a.s.l., 1936–2005); Karaganda (49°80'N, 73°15'E, 553 m a.s.l., 1936–2005); and Balhash (46°80'N, 75°08'E, 350 m a.s.l., 1936–2005). In general, temperature trends were similar among the stations with correlation coefficients between 0.6–0.8 for the summer season. In contrast, precipitation records showed different patterns due to strong regional differences (correlations between 0.35–0.45). Thus, for climate-growth relationships we used the high-altitude data of Kosh-Agach because it has the longest coverage and is the closest to the site (within 104 km). The CRU TS3 data were also used to increase the time span of the weather data. Moving window correlations of average daily temperatures were calculated using the Mugur-Aksy data.

RESULTS

The trees selected for the xylem anatomical measurements were growing quite slowly and produced relatively few tracheids per year (Table 1). On average, over the period in common to all five trees (1695–2007), the annual ring was 0.328 ± 0.286 mm

wide and was built from 10 ± 7.5 cells (5.56 ± 2.9 earlywood and 4.45 ± 2.4 latewood cells). The average cell diameter was 2.7 times larger for earlywood than for latewood cells and was $44.25 \pm 6.69 \mu\text{m}$ and $16.50 \pm 2.99 \mu\text{m}$, respectively. The time series of the ring widths clearly demonstrates that at least two of the five trees selected for anatomical measurements showed the typical age-related decreasing trend in ring width during the juvenile period (calendar year < 1680), but over the common period used in this study there is no sign of the presence of juvenile wood (Fig. 2). In contrast, after a period in growth reduction around 1780, the time series shows a slight increase in growth. A similar increasing pattern is also observed for LD of the earlywood and CWT of the latewood (Fig. 3).

The cell anatomical time series displayed a common variability comparable to tree-ring width with $R_{bt} > 0.48$, except for the traits of the ring sectors First and Last, for CWT in EW, and for D, LD, and LUM in LW (Table 2). The common signal proved to be relatively stable over time, since R_{bt} calculated for the period 1901–2007 varied very little in comparison to the entire common period (1695–2007).

The climate-growth relationships shown for all anatomical traits and across all ring sectors are summarized in Fig. 4 with the aim of supplying a global overview of climatic responses over a century-long temperature data set. Only the correlations with the CRU TS3 temperature data from 1901–2007 are shown, since the results from Kosh-Agach (1934–1993) were similar, and since the correlations with precipitation were mostly not significant ($P > 0.05$, $df = 106$) except for a positive signal

TABLE 1. Average and standard deviation (in brackets) of xylem anatomical traits for the common period 1695–2007 of all five cores measured ($N = 1529$).

Ring sector	Width (mm)	NT	D (μm)	LD (μm)	CWT (μm)	LUM (μm^2)	CWA (μm^2)
WHOLE	0.328 (0.286)	10.0 (7.5)	31.84 (5.56)	25.01 (5.04)	3.42 (0.60)	600 (117)	356 (74)
EW	0.254 (0.151)	5.56 (2.9)	44.25 (6.69)	38.77 (6.34)	2.74 (0.39)	951 (148)	377 (74)
LW	0.076 (0.053)	4.45 (2.4)	16.50 (2.99)	7.57 (1.74)	4.24 (0.89)	155 (33)	326 (77)
First	—	2	44.53 (6.71)	40.02 (6.70)	2.25 (0.32)	1019 (173)	317 (53)
Last	—	2	11.37 (3.92)	4.31 (4.06)	3.53 (0.56)	98 (98)	243 (42)

Notes: Width = tree ring width; NT = number of tracheids; D = radial diameter; LD = radial lumen diameter; CWT = cell wall thickness; LUM = lumen area; CWA = cell wall area; WHOLE = all tracheids, EW = earlywood tracheids, LW = latewood tracheids; First = first two tracheids formed in the radial file; Last = last two tracheids formed in the radial file (see Fig. 1).

with April. The correlations with temperature clearly indicated that the major response occurs in current year May to August, with small variation among anatomical traits. The previous year's May, June, and October temperatures also correlated positively with cell size, but the correlations were lower ($r < 0.4$) than those of the current growing season. These last correlations reached maximum values of 0.36 for TRW in June, 0.37 for earlywood LD in June, 0.53 for latewood CWT in July, and 0.48 for MXD in July ($P < 0.01$, $df = 106$). The same climatic signals were reiterated and in some cases even appeared strengthened when June to August (JJA) monthly temperatures were averaged ($r = 0.36$ for TRW, 0.41 for earlywood LD, 0.64 for latewood CWT, 0.60 for MXD, $P < 0.01$, $df = 106$). However, despite the overall common response to the May to August temperatures, xylem anatomical traits varied in the magnitude of their climatic signal. For example, we observed that cell wall thickness related traits responded more strongly

than ring width and tracheid size. Cell anatomical signals appeared stronger in LW than in EW.

To compare the timing of responses between the anatomical traits, we explored the climate–growth relationships for windows between 10 to 60 d moving over the growing season using the nearby, high elevation station of Mugur-Akxy (1970–1994). The results obtained with a 30 d running window are shown in Fig. 5. This detailed analysis fully confirms previous results (Fig. 4); there is a strong summer temperature signal in many of the anatomical traits. Over the period from May to September, responses for CWT and CWA, LD, and LUM and for the number of tracheids (NT) and the respective ring widths (TRW, EWW, and LWW) were similar. We observed that almost all anatomical traits across all ring sectors were increasingly correlated (positively or negatively) during the second half of May. Differences among anatomical traits appeared relative to the sign (negative for D , LD, and LUM in

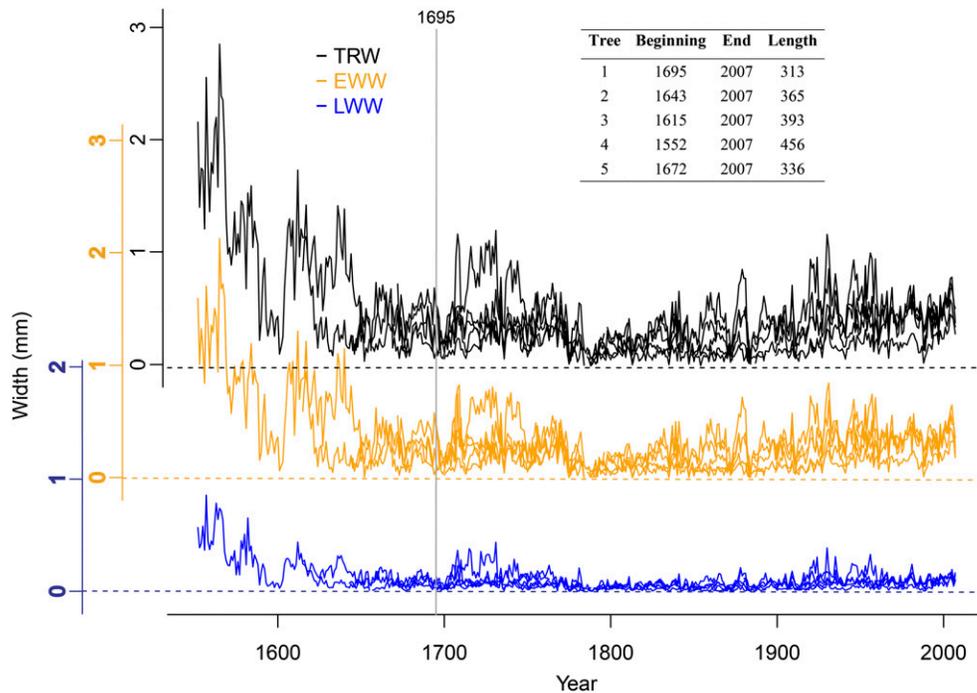


Fig. 2. Undetrended time series of tree-ring width (TRW, black lines), earlywood width (EWW, orange lines), and latewood width (LWW, blue lines) of the five trees considered for xylem anatomical measurements. The horizontal dotted lines indicate the 0 level for LWW (blue), EWW (orange), and TRW (black), respectively. The vertical grey line indicates the left margin of the common period (1695–2007) of the five cores.

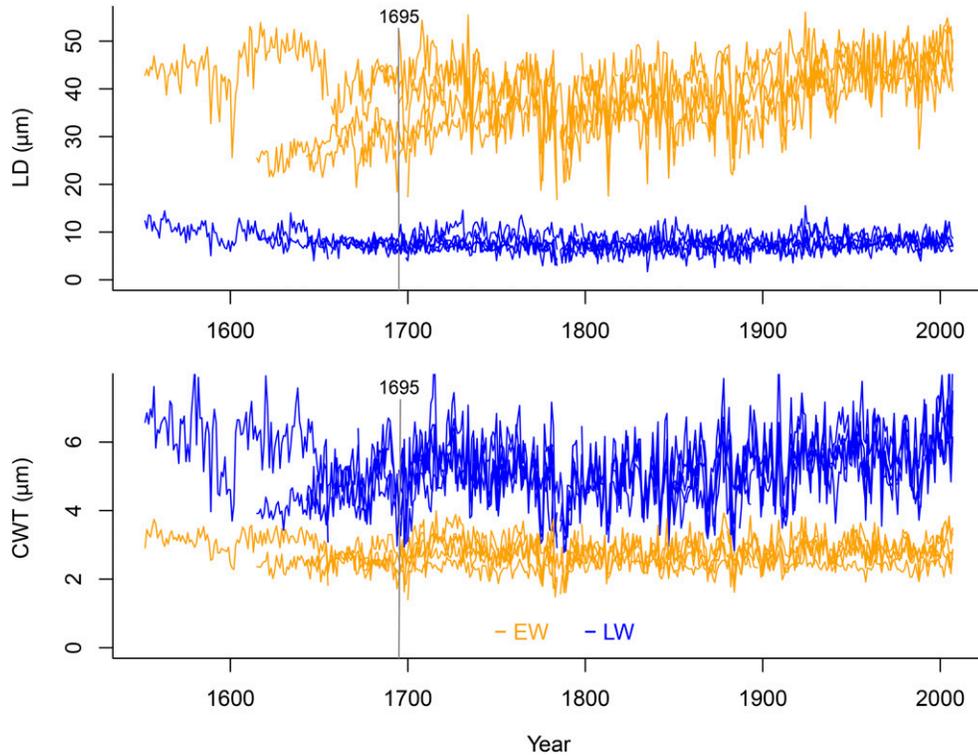


Fig. 3. Undetrended time-series of lumen radial diameter (LD, above graph) and radial cell wall thickness (CWT, below graph) of earlywood (orange lines) and latewood (blue lines) cells of the five trees considered for xylem anatomical measurements. The vertical gray line indicates the left margin of the common period (1695–2007) of the five cores.

WHOLE, LW, and Last), the strength (e.g., from not significant for LD and LUM, to significant for CWT and CWA in WHOLE, significance level of $P = 0.01$) and the duration of the correlation (e.g., decreasing in the first half of July for many traits in EW but keeping strong until mid August for some traits in LW; Fig. 5). A comparison between the correlations obtained in First and Last ring sectors indicated that all anatomical characteristics of the first cell built in the season positively responded to the temperature of late May. Both lumen and wall thickness increased when the second half of May was warm. The correlations in Last, in contrast, indicated that the tracheid size (D , LD, and LUM) of the last cells built in the season was similarly influenced by early growing season temperatures, but with an inverse relationship. With regard to the thickness of the cell wall (CWT and CWA), the response to temperature was positive, and the signal was maintained over

the whole growing season, from mid May to mid August. For EW and LW, comparable results were found, although time discrimination was less apparent for some cells. In EW, the positive temperature signal of cell size remained apparent over a longer period (until early July), since EW usually included more cells than in First. Analogously, the LD and LUM responses in LW instead tended to include more EW-like signal than Last, especially for the second part of the summer (Fig. 5). The signal of the number of cells and their respective widths (EWW and LWW) were similar to the signal of LD. The results obtained for density indicated that MXD displayed similar results to CWT in LW (with a correlation reaching a maximum of 0.73 for CWT in late May). Finally, when the response found for WHOLE was taken into consideration, the correlation of CWT and CWA with temperature reinforced the common response observed for both EW and LW, which was

TABLE 2. Common signal (Rbt) of xylem anatomical traits from the period 1695-2007 common to all the cores and to the period 1901 to 2007 (in brackets).

Ring sector	Width (mm)	NT	D (µm)	LD (µm)	CWT (µm)	LUM (µm ²)	CWA (µm ²)
WHOLE	0.58 (0.53)	0.54 (0.49)	0.54 (0.55)	0.54 (0.52)	0.56 (0.60)	0.50 (0.50)	0.57 (0.57)
EW	0.57 (0.55)	0.54 (0.51)	0.55 (0.49)	0.58 (0.47)	0.38 (0.36)	0.56 (0.41)	0.48 (0.44)
LW	0.50 (0.45)	0.48 (0.45)	0.42 (0.46)	0.16 (0.25)	0.59 (0.62)	0.13 (0.27)	0.57 (0.60)
First	—	—	0.39 (0.22)	0.40 (0.23)	0.16 (0.17)	0.41 (0.22)	0.21 (0.18)
Last	—	—	0.32 (0.12)	0.43 (0.31)	0.36 (0.28)	0.44 (0.34)	0.27 (0.18)

Notes: Width = tree ring width; NT = number of tracheids; D = radial diameter; LD = radial lumen diameter; CWT = cell wall thickness; LUM = lumen area; CWA = cell wall area; WHOLE = all tracheids, EW = earlywood tracheids, LW = latewood tracheids; First = first two tracheids formed in the radial file; Last = last two tracheids formed in the radial file (see Fig. 1).

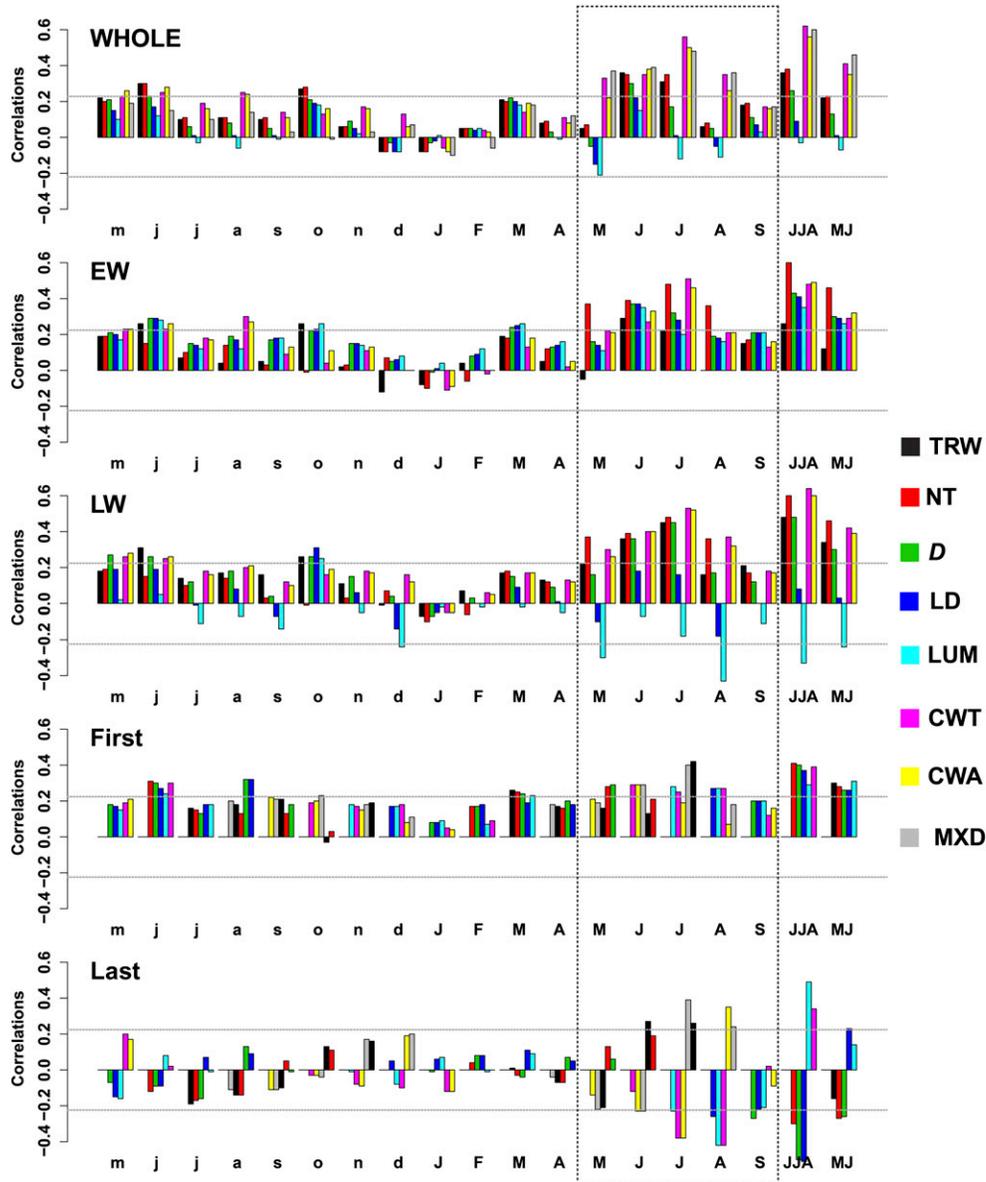


Fig. 4. Climate–growth relationships between the detrended chronologies of the xylem anatomical traits and the monthly average temperatures (Gridded CRU TS3 45°N–55°N and 70°E–100°E for the period 1901–2007) from previous year May (m) to current year September (S), and for the average of June to August (JJA) and May to June (MJ). Lowercase letters refer to months of the previous year, uppercase letters to the months of the current year. Results are presented for the different ring sectors separately. Horizontal gray dotted lines indicate significance level $P < 0.01$. The dashed rectangle indicates a period characterized by strong correlations. WHOLE = whole tree-ring; EW = earlywood, LW = latewood; First = only first two cell in the ring; Last = only last two cell in the ring.

particularly strong from mid May to early July (with maxima of 0.72 and 0.65, respectively). In contrast, the positive response of D, LD, and LUM for EW tended to disappear, due to the confounding opposite responses encoded in the EW and LW cells. Comparing the response of TRW and NT with CWT and CWA, we observed that the climatic signal of TRW and NT tended to disappear in early July, while it remained present about one month longer for CWT and CWA (Fig. 5).

When analyzing how the climatic signal changed along a temperature gradient (Fig. 6), as defined by May–June (MJ) temperatures of the climatic years recorded at the high elevation site of Mugur-Aksy, we observed that warming was anticipating

and extending the temperature signal of those traits encoding an early season response such as TRW and LD. An increase of MJ temperature from 7.2° to 9.9°C induced the temperature signal of TRW to expand from June–July to May–July, while slightly decreasing in strength (blue vertical sector enclosed by dashed lines in June, top left plot). Similarly, the LD signal appeared earlier in the season, but with a slightly increasing strength of the correlations. The magnitude of the shift corresponds to an anticipation of ~1 wk/°C of warming. In contrast, the pattern of correlation observed for MXD and CWT, which are very similar to each other, were more stable over time. The correlations (red band from May to August in the right plots) remained >0.4

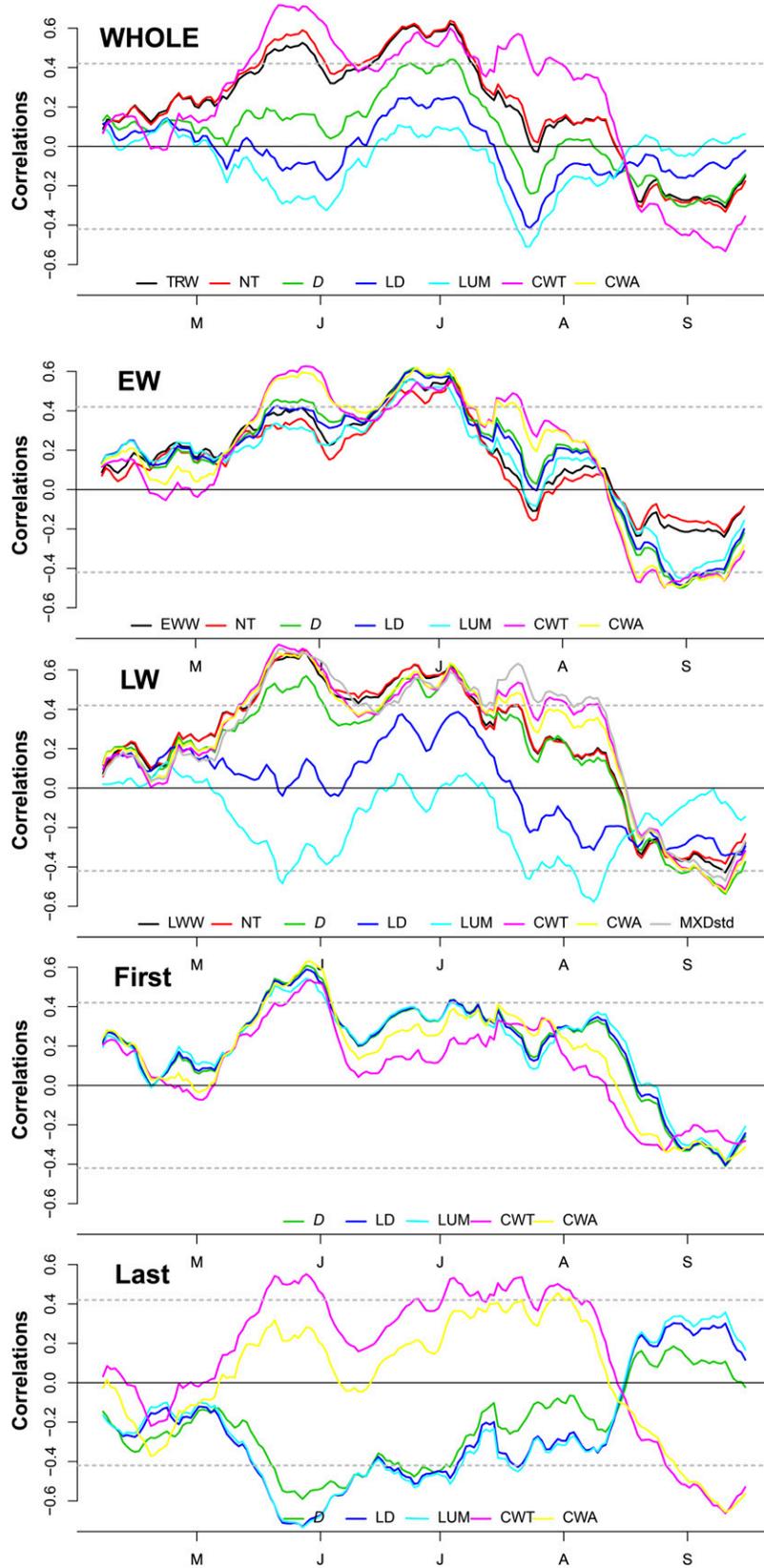


Fig. 5. Moving correlations between the detrended chronologies of the xylem anatomical traits and the daily average temperature from the climatic station of Muger-Aksy (50°23'N, 90°26'E, 1850 m a.s.l., 1969–1994). The window is 30 d wide and is moved in steps of 1 d from mid April to mid September of the current year. Horizontal dotted lines indicate significance level $P < 0.01$.

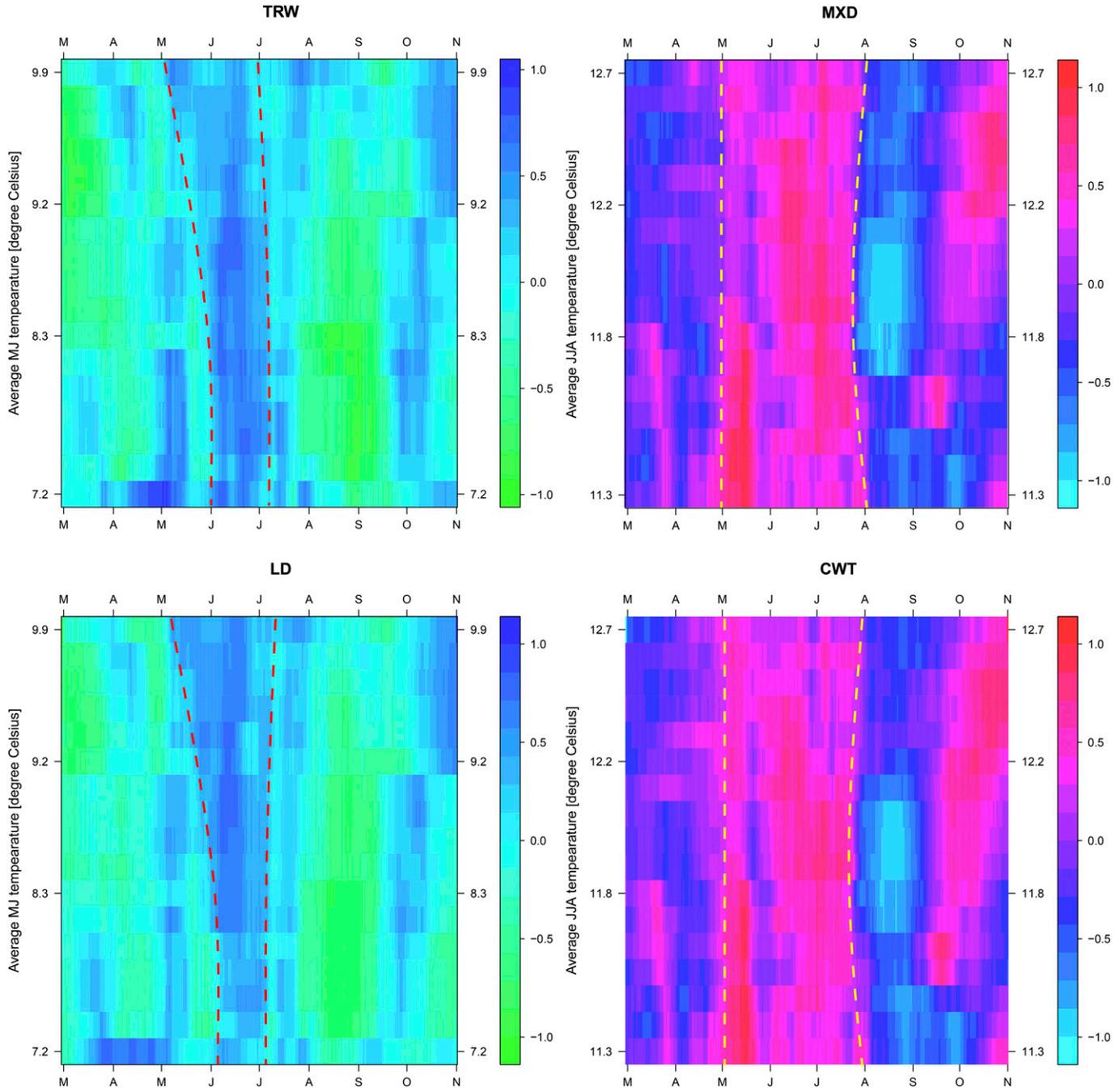


Fig. 6. Correlations of detrended tree-ring width (TRW), maximum latewood density (MXD), earlywood lumen diameter (LD), and latewood cell wall thickness (CWT) with 30-d moving window of Mugur-Aksy daily temperature (1850 m a.s.l., 1969–1994) from March to November (along the x-axis) calculated for a 10-yr window moving along the 26 yr ranked according to their MJ (left plots) and JJA temperature (right plots), from the coldest to the warmest (along the y-axis). The y-axis label indicates the MJ (left graphs) and JJA temperature (right graphs) averaged over the selected 10 yr. Dotted lines delineate the main response to temperature as discussed in the text.

and were stable over the growing season independently from the temperature gradient.

A long-term perspective of the variability in TRW, earlywood LD, latewood CWT, and MXD in relation to the climatic data, is shown in Fig. 7. When detrending was applied, the TRW, MXD, earlywood LD, and latewood CWT displayed a long-term increase starting around 1780 with highest values in the last decades. A similar increase is, however, not present for

the earlywood CWT and latewood LD, indicating diverging long-term trends between lumen and cell wall.

DISCUSSION

Responses to temperature—The results obtained in this study showed that the five analyzed trees, which were growing

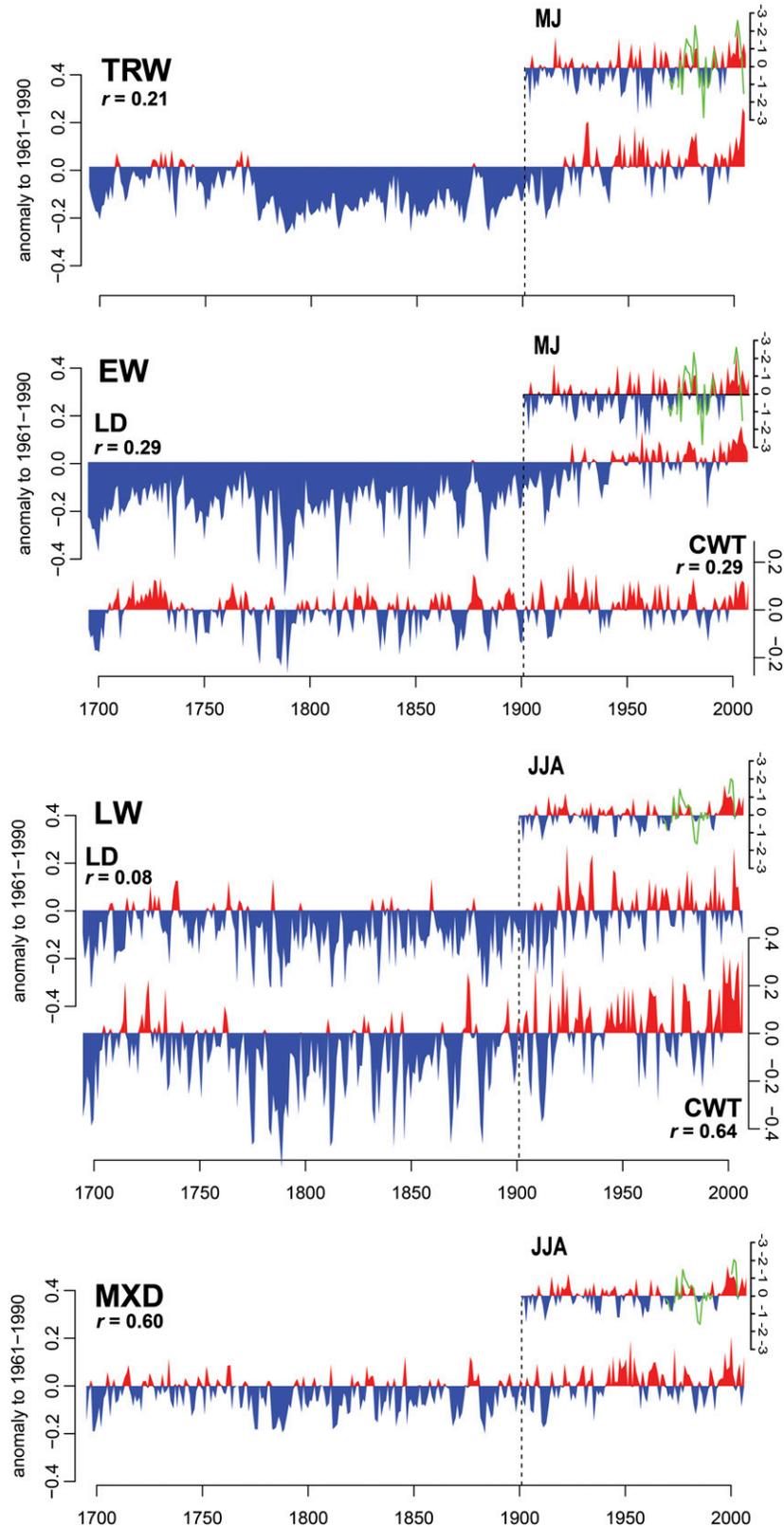


Fig. 7. Anomalies from the 1961–1990 average for the detrended tree-ring width (TRW), lumen diameter (LD), and cell wall thickness (CWT) for earlywood (EW) and latewood (LW), and maximum latewood density (MXD) chronologies over the common period (1695–2007). Detrending was applied using a negative exponential function. Temperature anomalies for May–June (MJ for TRW and LD) and June to August (JJA for CWT and MXD) of CRU TS3 (colored polygons, 1901–2007) and for Mugur-Aksy (green line, 1850 m a.s.l., 1969–1994) are shown for comparison. The r value indicates the correlation between the anatomical traits and the climatic data (CRU TS3).

in a temperature-limited environment, were able to continuously adjust their xylem structure. In the long-term, we observed a diverging pattern of variation between the increasing earlywood lumen and latewood cell wall thickness and the rather stable earlywood cell wall thickness and latewood lumen. The climate-growth analyses of the short-term plastic responses indicated that warmer growing seasons promoted the production of wider earlywood lumen, thicker latewood walls, denser latewood, and wider ring widths. These results are in line with numerous previous observations demonstrating that the long-term variation of xylem cell anatomy in tree rings responds to various changing environmental conditions (Fonti et al., 2010) including temperature (Yasue et al., 2000), date of snow melt (e.g., Kirilyanov et al., 2003; Panyushkina et al., 2003), and moisture availability (e.g., Eilmann et al., 2009; Campelo et al., 2010; Bryukhanova and Fonti, 2013). The strength of the common signal obtained for the cell anatomical traits, often with R_{bt} values > 0.48 , was relatively high in comparisons with previous studies, confirming that the growth processes of all the trees were responding to a common factor. The generalized and also relatively strong response of all traits to temperature, but not to precipitation, with correlation to growing season temperature in some cases reaching values > 0.7 , clearly indicated that growth processes were mainly determined by temperature. The temperature signal was strongly expressed in the current year, but some influence of the previous season could be detected (Fig. 4), likely due to a carry-over effect of the previous growing conditions on current wood production via the mobilization of previously stored assimilates (Kagawa et al., 2006; Vaganov et al., 2009).

Mechanism of response—Despite the generalized common response to temperature observed for all anatomical traits and the various ring sectors, differences observed in the timing and strength of the responses to temperature increase our understanding of the mechanism behind xylem structural adjustments.

The climate-growth relationships revealed differing climatic responses between earlywood LD and latewood CWT. Our results indicated that earlywood cells positively respond to early season temperature (Figs. 4, 5) and that their response moves forward in the season by 1 wk/°C of warming in May to June (MJ, Fig. 6). This observed shift is comparable to the earlier growing season onset of ~ 7 d/°C quantified by monitoring cambial activity for mature *Larix decidua* trees growing along an altitudinal gradient in the Alps (Moser et al., 2010) and confirms observations of an offset in the timing of the initiation of cambial activity and of rapid cambial cell division at the beginning of the vegetation period along the northern timberline in Eurasia (Kirilyanov et al., 2003). This earlier onset has consequences also for TRW, since the extension of duration of the growing season translates into more numerous earlywood cells, as confirmed by our results. The temperature signal in TRW was from May to June and was shorter than that observed for CWA and MXD (Figs. 4, 5), indicating that latewood production relies on the carbon assimilation of the whole growing season, likely spanning from May to August. This timing corresponds to the time for cellular differentiation usually observed for northern hemisphere trees growing in cold-limited conditions (e.g., Deslauriers et al., 2008; Rossi et al., 2006b). Identification of a varying time-window for the temperature responses suggests the existence of causal relationships between the anatomical adjustments. The differing climatic fingerprint between earlywood LD and latewood CWT is related to the timing of the physiological processes: during the first part of the growing

season, variations in temperature affect radial expansion, whereas latewood cell wall thickness depends on the total amount of assimilates accumulated during the season, which depends on the duration and rate of carbon assimilation (Rossi et al., 2012). Determination of the onset, duration, and termination of cell differentiation, radial expansion, wall thickening, and lignification for high-elevation conifers has indicated that the formation of thicker cell walls in latewood tracheids is also related to the duration of the process (Rossi et al., 2006b).

Our results strongly support the hypothesis that early-season temperature in cold-limited environments primarily determines the water conduction capacities of the earlywood cells by influencing both the number and the lumen diameter of tracheids. The wall thickness of the latewood cells, which are produced later in the season, benefit in two ways from the warming conditions: first, the longer and warmer growing season favors more carbon assimilation, and second, more efficient water transport of the earlywood cells allow maximization of the assimilation rate during favorable conditions.

These response mechanisms could also partially explain why the latewood density signal (measured either as MXD or as CWA) from sites above 1000 m a.s.l. is in general stronger than that of other cell traits (Figs. 4, 5) and for TRW (Buntgen et al., 2010). The latewood density signal encompasses the additive effect of temperature on the duration and rate of carbon assimilation, as well as being more stable over time than TRW (Fig. 6) such that the correlation is less influenced by the year-to-year variability of the growth onset.

Functional impact—In conifers, earlywood cells are primarily responsible for water transport, while latewood cells serve as mechanical support. Relatively small changes in the wood structure can have a large functional impact (Tyree and Zimmermann, 2002; Fonti and Jansen, 2012). The widening of earlywood lumina with increasing temperatures would result in improved conduction of water in earlywood tracheids (Figs. 4, 5). According to the Hagen-Poiseuille equation, the volumetric flow rate passing through a cylinder is proportional to the fourth power of its diameter. Thickening of latewood cells leads to increased mechanical strength, since many mechanical wood properties are directly related to wood density (Zobel and Buijtenen, 1989).

We therefore interpret the observed long-term changes in xylem including widening of the earlywood lumen and thickening the latewood cell walls as long-term adjustments of the xylem structure to global warming. This adjustment is consistent with increased demands of mechanical integrity as the tree increases in size (including the leaf volume) due to ameliorating growing conditions. We admit the role of ameliorated growing conditions remains speculative, as it is difficult to attribute the response only to climate-related changes, especially when the climate data coverage as limited as in our case study. Wood properties at a specific location also reflect the plant's hydraulic and mechanical requirements at the time when the wood was formed (Lachenbruch et al., 2011). The typical age-size related radial pattern of variation of xylem anatomical traits usually involves rapid interannual changes in the first few years of tree growth followed by a reduction in the changes as trees age (Zobel and Buijtenen, 1989). Ontogenetic trends thus should be clearly detectable in the juvenile wood, but they should disappear as the tree height and canopy mass stabilize with aging. Ontogenetic changes might be due not only to an increasing demand for water and support as the trees grows, but also for

other reasons. For example, according to the West, Brown and Enquist (WBE) model (West et al., 1999) increased size of water conducting cells compensates for increased flow resistance caused by friction over longer paths and increased effect of gravity (Anfodillo et al., 2006). Ability to identify deviations from the radial ontogenetic pattern of variation would allow us to identify how trees use structural plasticity to adjust function.

In this study, we removed age-size related trends by applying a negative exponential function (Fig. 7). Although important to interpreting the remaining long-term variation, it is still notable that cell wall thickness of earlywood cells (CWT), despite showing a common year-to-year variation to LD, does not follow the same long-term increase as LD (Fig. 3). Similarly, but with an inverse relationship, the diverging long-term trend occurred for the latewood cells. Assuming that cell wall thickness of water-conducting earlywood tracheids has the primary function of supplying resistance to (drought-induced) embolism, we suggest that in the Altay Mountains the trees we measured did not experience enough drought stress to require hydraulic reinforcement, and thus adjustments were solely oriented at improving the water transport capacity.

Conclusion and take-home message—This study demonstrates the potentials and limitations of our approach. On one hand, the results provide a detailed understanding of how tree-ring anatomy can provide a mechanistic and functional understanding of xylem plastic responses for a conifer species growing in a cold-limited environment. In particular, the analysis of climate–growth relationships performed separately for the earlywood and latewood tracheids (i.e., for cells built at differing times and fulfilling differing functions) help to identify the causal relationships for plastic responses in xylem and their possible role. On the other hand, due to limitations in our knowledge of the ontogeny of radial patterns of anatomical traits and how they are influenced by the environment, we need more information to interpret the functional significance of short-term and long-term variability in xylem anatomy.

For a broader application of tree-ring anatomy in global change research, a concerted effort involving diverse disciplines (functional ecology, wood anatomy, plant physiology and dendrochronology) is required to better link cause–effect relationships between genesis and structure and function of xylem. We also need to explore plasticity of other functionally relevant anatomical features (e.g., amount of radial rays, cell grouping, pit characteristics) in many climates and species to assemble a complete picture of the range of possible xylem adjustments and their functional impacts. By elucidating a mechanistic understanding of the connections between the hydrological and carbon cycles in different species in the context of climate, we will be able to better predict the response of vegetation to anticipated changes in global climate.

LITERATURE CITED

- ALLEN, C. D., A. K. MACALADY, H. CHENCHOUNI, D. BACHELET, N. McDOWELL, M. VENNETIER, T. KITZBERGER, ET AL. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259: 660–684.
- ANFODILLO, T., V. CARRARO, M. CARRER, C. FIOR, AND S. ROSSI. 2006. Convergent tapering of xylem conduits in different woody species. *The New Phytologist* 169: 279–290.
- ANFODILLO, T., A. DESLAURIERS, R. MENARDI, L. TEDOLDI, G. PETTIT, AND S. ROSSI. 2012. Widening of xylem conduits in a conifer tree depends on the longer time of cell expansion downwards along the stem. *Journal of Experimental Botany* 63: 837–845.
- BAAS, P., AND F. H. SCHWEINGRUBER. 1987. Ecological trends in the wood anatomy of trees, shrubs and climbers from Europe. *Iawa Bulletin* 8: 245–274.
- BIGLER, C., O. U. BRAKER, H. BUGMANN, M. DOBBERTIN, AND A. RIGLING. 2006. Drought as an inciting mortality factor in Scots pine stands of the Valais, Switzerland. *Ecosystems* 9: 330–343.
- BRESHEARS, D. D., O. B. MYERS, C. W. MEYER, F. J. BARNES, C. B. ZOU, C. D. ALLEN, N. G. McDOWELL, ET AL. 2009. Tree die-off in response to global change-type drought: Mortality insights from a decade of plant water potential measurements. *Frontiers in Ecology and the Environment* 7: 185–189.
- BRYUKHANOVA, M., AND P. FONTI. 2013. Xylem plasticity allows rapid hydraulic adjustment to annual climatic variability. *Trees—Structure and Function*. doi:10.1007/s00468-012-0802-8.
- BUNGTEN, U., D. FRANK, V. TROUET, AND J. ESPER. 2010. Diverse climate sensitivity of Mediterranean tree-ring width and density. *Trees—Structure and Function* 24: 261–273.
- CAMPELO, F., C. NABAIS, E. GUTIERREZ, H. FREITAS, AND I. GARCIA-GONZALEZ. 2010. Vessel features of *Quercus ilex* L. growing under Mediterranean climate have a better climatic signal than tree-ring width. *Trees—Structure and Function* 24: 463–470.
- CARLQUIST, S. 2001. Wood anatomy of the endemic woody Asteraceae of St Helena I: Phyletic and ecological aspects. *Botanical Journal of the Linnean Society* 137: 197–210.
- CARLQUIST, S., AND D. A. HOEKMAN. 1985. Ecological wood anatomy of the woody southern Californian flora. *International Association of Wood Anatomists Bulletin* 6: 319–347.
- CARLQUIST, S. J. 1975. Ecological strategies of xylem evolution. University of California Press, Berkeley, California, USA.
- CHOAT, B., L. SACK, AND N. M. HOLBROOK. 2007. Diversity of hydraulic traits in nine *Cordia* species growing in tropical forests with contrasting precipitation. *New Phytologist* 175: 686–698.
- COOK, E. R., AND L. A. KAIRIUKSTIS. 1990. Methods of dendrochronology applications in the environmental sciences. Kluwer, Dordrecht, Netherlands.
- DESLAURIERS, A., S. ROSSI, T. ANFODILLO, AND A. SARACINO. 2008. Cambium phenology, wood formation and temperature thresholds in two contrasting years at high altitude in southern Italy. *Tree Physiology* 28: 863–871.
- EILMANN, B., N. BUCHMANN, R. SIEGWOLF, M. SAURER, P. CHERUBINI, AND A. RIGLING. 2010. Fast response of Scots pine to improved water availability reflected in tree-ring width and $\delta^{13}\text{C}$. *Plant, Cell & Environment* 33: 1351–1360.
- EILMANN, B., R. ZWEIFEL, N. BUCHMANN, P. FONTI, AND A. RIGLING. 2009. Drought-induced adaptation of the xylem in Scots pine and pubescent oak. *Tree Physiology* 29: 1011–1020.
- ESPER, J., E. R. COOK, P. J. KRUSIC, K. PETERS, AND F. H. SCHWEINGRUBER. 2003. Tests of the RCS method for preserving low-frequency variability in long tree-ring chronologies. *Tree-Ring Research* 59: 81–98.
- FONTI, P., AND S. JANSEN. 2012. Xylem plasticity in response to climate. *New Phytologist* 195: 734–736.
- FONTI, P., G. VON ARX, I. GARCIA-GONZALEZ, B. EILMANN, U. SASS-KLAASSEN, H. GARTNER, AND D. ECKSTEIN. 2010. Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. *New Phytologist* 185: 42–53.
- GARTNER, B. L. 1995. Plant stems physiology and functional morphology. Academic Press, San Diego, California, USA.
- HOLMES, R. 1983. Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin* 43: 69–78.
- IPCC [INTERNATIONAL PANEL ON CLIMATE CHANGE]. 2007. Climate Change 2007: The physical science basis. Cambridge University Press, Cambridge, UK.
- KAGAWA, A., A. SUGIMOTO, AND C. MAXIMOV. 2006. ^{13}C pulse-labelling of photoassimilates reveals carbon allocation within and between tree rings. *Plant, Cell & Environment* 29: 1571–1584.
- KIRDYANOV, A., M. HUGHES, E. VAGANOV, F. SCHWEINGRUBER, AND P. SILKIN. 2003. The importance of early summer temperature and date of snow

- melt for tree growth in the Siberian Subarctic. *Trees—Structure and Function* 17: 61–69.
- LACHENBRUCH, B., J. MOORE, AND R. EVANS. 2011. Radial variation in wood structure and function in woody plants, and hypotheses for its occurrence. In F. Meinzer, B. Lachenbruch, and T. Dawson [eds.], *Size and age-related changes in trees structure and function*, 121–164. Springer Sciences+Business Media, Dordrecht, Netherlands.
- LARSON, P. R. 1994. *The vascular cambium development and structure*. Springer, Berlin, Germany.
- MATTHECK, C., AND H. KUBLER. 1995. *Wood—The internal optimization of trees*. Springer, Berlin, Germany.
- MCDOWELL, N., W. T. POCKMAN, C. D. ALLEN, D. D. BRESHEARS, N. COBB, T. KOLB, J. PLAUT, ET AL. 2008. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytologist* 178: 719–739.
- MEINZER, F. C., LACHENBRUCH, B., AND T. DAWSON. 2011. *Size and age-related changes in trees structure and function*. Springer Sciences+Business Media, Dordrecht, Netherlands.
- MITCHELL, T. D., AND P. D. JONES. 2005. An improved method of constructing a database of monthly climate observations and associated high-resolution grids. *International Journal of Climatology* 25: 693–712.
- MOSER, L., P. FONTI, U. BÜNTGEN, J. ESPER, J. LUTERBACHER, J. FRANZEN, AND D. FRANK. 2010. Timing and duration of European larch growing season along altitudinal gradients in the Swiss Alps. *Tree Physiology* 30: 225–233.
- MYGLAN, V. S., O. CH. OIUDPAA, AND E. A. VAGANOV. 2012. A 2367-year tree-ring chronology for the Altai–Sayan Region (Mongun-Taiga mountain massif). *Archaeology, Ethnology & Anthropology of Eurasia* 40: 76–83.
- PANYUSHKINA, I. P., M. K. HUGHES, E. A. VAGANOV, AND M. A. R. MUNRO. 2003. Summer temperature in northeastern Siberia since 1642 reconstructed from tracheid dimensions and cell numbers of *Larix cajanderi*. *Canadian Journal of Forest Research* 33: 1905–1914.
- PITTERMANN, J., B. CHOAT, S. JANSEN, S. A. STUART, L. LYNN, AND T. E. DAWSON. 2010. The relationships between xylem safety and hydraulic efficiency in the Cupressaceae: The evolution of pit membrane form and function. *Plant Physiology* 153: 1919–1931.
- POORTER, L., I. McDONALD, A. ALARCÓN, E. FICHTLER, J.-C. LICONA, M. PEÑA-CLAROS, F. STERCK, Z. VILLEGAS, AND U. SASS-KLAASSEN. 2010. The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytologist* 185: 481–492.
- ROSSI, S., A. DESLAURIERS, AND T. ANFODILLO. 2006a. Assessment of cambial activity and xylogenesis by microsampling tree species: An example at the alpine timberline. *International Association of Wood Anatomists Journal* 27: 383–394.
- ROSSI, S., A. DESLAURIERS, T. ANFODILLO, H. MORIN, A. SARACINO, R. MOTTA, AND M. BORGHETTI. 2006b. Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. *New Phytologist* 170: 301–310.
- ROSSI, S., H. MORIN, AND A. DESLAURIERS. 2012. Causes and correlations in cambium phenology: Towards an integrated framework of xylogenesis. *Journal of Experimental Botany* 63: 2117–2126.
- SCHWEINGRUBER, F. 1996. *Tree rings and environment dendroecology*. Haupt, Bern, Switzerland.
- SIDOROVA, O. V., M. SAURER, V. S. MYGLAN, A. EICHLER, M. SCHWIKOWSKI, A. V. KIRDYANOV, M. V. BRYUKHANOVA, ET AL. 2012. A multi-proxy approach for revealing recent climatic changes in the Russian Altai. *Climate Dynamics* 38: 175–188.
- SPERRY, J. S., F. C. MEINZER, AND K. A. MCCULLOH. 2008. Safety and efficiency conflicts in hydraulic structure: Scaling from tissues to trees. *Plant Cell and Environment* 31: 632–645.
- TYREE, M. T., AND F. W. EWERS. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* 119: 345–360.
- TYREE, M. T., AND M. H. ZIMMERMANN. 2002. *Xylem structure and the ascent of sap*, 2nd ed. Springer, Berlin, Germany.
- VAGANOV, E. A., M. K. HUGHES, A. V. KIRDYANOV, F. H. SCHWEINGRUBER, AND P. P. SILKIN. 1999. Influence of snowfall and melt timing on tree growth in subarctic Eurasia. *Nature* 400: 149–151.
- VAGANOV, E. A., M. K. HUGHES, AND A. V. SHASHKIN. 2006. Growth dynamics of conifer tree rings images of past and future environments. In M. M. Caldwell, G. Heldmaier, R. B. Jackson, O. L. Lange, H. A. Mooney, E.-D. Schulze, and U. Sommer [eds.], *Ecological studies series*, vol. 183. Springer, Berlin, Germany.
- VAGANOV, E. A., E. D. SCHULZE, M. V. SKOMARKOVA, A. KNOHL, W. A. BRAND, AND C. ROSCHER. 2009. Intra-annual variability of anatomical structure and $\delta^{13}\text{C}$ values within tree rings of spruce and pine in alpine, temperate and boreal Europe. *Oecologia* 161: 729–745.
- VON ARX, G., S. R. ARCHER, AND M. K. HUGHES. 2012. Long-term functional plasticity in plant hydraulic architecture in response to supplemental moisture. *Annals of Botany* 109: 1091–1100.
- WEST, G. B., J. H. BROWN, AND B. J. ENQUIST. 1999. A general model for the structure and allometry of plant vascular systems. *Nature* 400: 664–667.
- WHEELER, E. A., P. BAAS, AND S. RODGERS. 2007. Variations in dicot wood anatomy: A global analysis based on the insidewood database. *International Association of Wood Anatomists Journal* 28: 229–258.
- WODZICKI, T. J. 1971. Mechanism of xylem differentiation in *Pinus silvestris* L. *Journal of Experimental Botany* 22: 670–687.
- YASUE, K., R. FUNADA, O. KOBAYASHI, AND J. OHTANI. 2000. The effects of tracheid dimensions on variations in maximum density of *Picea glehnii* and relationships to climatic factors. *Trees—Structure and Function* 14: 223–229.
- ZOBEL, B. J., AND J. P. V. BUIJTENEN. 1989. *Wood variation its causes and control*. Springer, Berlin, Germany.