

High responsiveness of wood anatomy to water availability and drought near the equatorial rear edge of Douglas-fir

Marcos González-Cásares, J. Julio Camarero, Michele Colangelo, Angelo Rita, and Marín Pompa-García

Abstract: In trees, wood anatomy is a more precise and informative measure of hydraulic responses to drought than radial growth. Tree populations located near the equatorial-range edge of their distribution (rear edge) are used to monitor responses to changes in hydroclimate; however, few studies have assessed the wood anatomy of these populations. We measured tracheid lumen diameter (LD) and cell wall thickness (CWT) in a rear-edge Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) population. We also evaluated the formation of intra-annual density fluctuations (IADFs), characterized by earlywood (EW) tracheids with narrow lumens. We expected that EW LD would be particularly sensitive to hydroclimate variability. We found that EW LD was directly related to precipitation during the previous year's late winter and the current year's spring. Wet conditions were associated with wider EW tracheids and improved growth. These correlations peaked when considering cumulative precipitation from January to May. EW IADFs were formed in response to dry conditions during March and April, suggesting that dry spring conditions limit tracheid expansion. The described associations in a rear-edge Douglas-fir population confirm that wood anatomy is highly responsive to drought. We conclude that EW LD is a valuable proxy for hydroclimate reconstruction in Douglas-fir.

Key words: drought, intra-annual density fluctuations, lumen diameter, Mexico, *Pseudotsuga menziesii*, wood anatomy.

Résumé : Chez les arbres, l'anatomie du bois est une mesure des réactions hydrauliques à la sécheresse plus précise et instructive que la croissance radiale. Les populations situées près de la limite équatoriale de l'aire de répartition (bord arrière) constituent des indicateurs des réactions à l'hydroclimat. Cependant, leur anatomie du bois est peu étudiée. Nous avons mesuré le diamètre du lumen et l'épaisseur de la paroi cellulaire des trachéides dans une population de bord arrière de douglas de Menzies (*Pseudotsuga menziesii* (Mirb.) Franco). Nous avons aussi évalué le développement des fluctuations intra-annuelles de la densité, caractérisées par des trachéides du bois initial avec un lumen étroit. Nous anticipons que le diamètre du lumen dans le bois initial serait particulièrement sensible à la variabilité de l'hydroclimat. Nous avons trouvé que le diamètre du lumen dans le bois initial était directement relié aux précipitations durant la fin de l'hiver précédent et le printemps de la saison de croissance en cours. Les conditions humides étaient associées à des trachéides du bois initial plus larges et à une meilleure croissance. Ces corrélations étaient les plus fortes lorsqu'on tenait compte de la somme des précipitations de janvier à mai. Les fluctuations intra-annuelles de la densité dans le bois initial survenaient en réaction à des conditions sèches en mai et avril, ce qui suggère qu'un printemps sec limite l'expansion des trachéides. Les associations décrites dans une population de bord arrière de douglas de Menzies confirment que l'anatomie du bois réagit fortement à la sécheresse. Nous concluons que le diamètre du lumen dans le bois initial est un substitut utile pour la reconstitution de l'hydroclimat chez le douglas de Menzies. [Traduit par la Rédaction]

Mots-clés : sécheresse, fluctuations intra-annuelles de la densité, diamètre du lumen, Mexico, *Pseudotsuga menziesii*, anatomie du bois.

Introduction

Tree populations acclimate, migrate, or become extinct in response to climate changes (Aitken et al. 2008). In drought-prone regions of the Northern Hemisphere, tree populations forming the southernmost limits of species distribution (rear-edge populations) are expected to show more reduced growth rates than core populations in response to warmer and drier conditions (Loehle 1998). In fact, some rear-edge populations have already displayed long-term radial growth reductions and high mortality rates in response to drought (Camarero et al. 2015a, 2015b), sup-

porting the hypothesized sensitivity of rear-edge forests to water scarcity.

Patterns in radial growth can be used as a surrogate measure of tree resilience in response to drought (Gazol et al. 2018). Dendrochronological studies on the equatorial-range edge of tree populations (rear edge) have demonstrated drought stress as one of the major growth constraints in these marginal forests (Camarero et al. 2013); however, a more mechanistic view of how drought limits forest productivity should be based on wood anatomy, which reflects the effects of water shortage on tree functioning because the size of xylem conduits determines hydraulic conduc-

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tivity and growth (Fonti et al. 2010). Nevertheless, few studies have assessed how wood anatomy (the product of xylem dynamics; Vaganov et al. 2006) responds to hydroclimate in American rear-edge populations. Most studies on quantitative wood anatomy and xylogenesis take place in Eurasian forests (Cuny et al. 2015), whereas few studies have been done in American regions, despite these regions being major centers of diversity for important tree species such as pines (*Pinus* L. spp.; Millar 1998). Rear-edge populations are suitable for exploring the climate sensitivity of trees and analyzing wood-anatomy proxies for hydroclimate reconstruction (Pellizzari et al. 2016).

Wood-anatomy analyses have detected rapid responses of conduit diameter to seasonal changes in water availability (von Wilpert 1991; Pacheco et al. 2015). In conifers, dry conditions usually lead to the formation of tracheids with smaller lumens and thinner cell walls (Dünisch and Bauch 1994; Abe et al. 2003; Eilmann et al. 2011); however, the responsiveness of transversal lumen diameter (LD) and cell wall thickness (CWT) to hydroclimate and drought has not been quantified in rear-edge Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) populations. The ample distribution range of Douglas-fir along western North America suggests that this species may display large growth plasticity in response to hydroclimate variability. In addition, this research is of regional and global interest, as this conifer has been widely planted and is a major commercial timber species in America and Europe (Hermann and Lavender 1999; Sedjo 2017).

Several studies have assessed the radial-growth responses of Douglas-fir to hydroclimate variability and showed that wood formation is restricted by water shortage during spring and summer in both natural (Zhang and Hebda 2004; Littell et al. 2008; Griesbauer and Green 2010a, 2010b; Restaino et al. 2016) and planted stands (Bower et al. 2005; Sergent et al. 2014). Quantitative analyses of wood anatomy for rear-edge species could allow for forecasting of the effects of projected climate warming on Douglas-fir radial growth in its core distribution area. These analyses would benefit from using techniques that rapidly assess anatomical features such as intra-annual density fluctuations (IADFs), which reflect seasonal responses of the xylem to hydroclimate conditions (Vieira et al. 2010; De Micco et al. 2016). In Douglas-fir, IADFs are potential hydroclimate proxies (Acosta-Hernández et al. 2019).

In this study, we explore the sensitivity of wood anatomical proxies to climate variability and drought to assess their suitability for hydroclimate reconstruction in a rear-edge Douglas-fir population. First, we provide an assessment of the responses of LD and CWT to interannual hydroclimate variability. Second, we evaluate how these variables respond to intra-annual changes in precipitation, temperature, and drought. Third, we introduce a novel method to detect earlywood (EW) IADFs using LD data. We focused on EW because (i) it accounts for most of the hydraulic conductivity within the ring and (ii) it contains IADFs formed by bands of latewood (LW)-like tracheids (i.e., cells with small lumens and thick walls; De Micco et al. 2016). We hypothesized that seasonally derived wood-anatomy variables, particularly EW tracheid LD, are mainly controlled by water availability.

Materials and methods

Study area

Most rear-edge Douglas-fir populations are fragmented and restricted to wet, high-elevation sites that are subjected to seasonal drought (González-Elizondo et al. 2012). This is the case for the sampled forest ("Las Cebollas"), which is situated in the Sierra Madre Occidental mountain range in Durango, northern Mexico. The study site is located at 23°35'N and 105°23'W, at an elevation of 2840 m above sea level (a.s.l.). The nearby climate station of El

Salto, Mexico (23°48'N, 105°21'W, 2543 m a.s.l., period 1946–2016), was used to describe the climate conditions of the site. The study area presented a cool temperate climate with a mean annual temperature of 11.5 °C and a mean annual precipitation of 1100 mm (Supplementary Fig. S1a¹). The wet and dry seasons last from June to September and from October to May, respectively. Early spring (April), which corresponds to the beginning of the growing season, is the driest period of the year. Trend analyses indicate that the climate of the study area is warming, whereas its precipitation shows no long-term trend but does show high interannual variability (Supplementary Fig. S1b¹).

Tree species

Douglas-fir is a very productive species that is subjected to ample climatic (mean annual temperature ranges from -5 °C to 22 °C, and mean annual precipitation ranges from 350 to ca. 3000 mm) and ecological gradients along its natural range of distribution in western North America, from northern British Columbia (Canada) to the mountain ranges of Mexico (Thompson et al. 1999).

Field sampling

We used a subset of five trees from those previously studied by Acosta-Hernández et al. (2019). These five trees did not produce reaction wood and showed the highest correlation values with the site mean chronology for the 1990–2016 period. The Pearson correlation of the selected trees ranged from 0.77 to 0.88, whereas the mean correlation of all measured trees was 0.71.

Three cores were taken from each sampled tree at breast height (1.3 m) using a 5 mm Pressler increment borer. Two of the cores were mounted, sanded to fine grit, measured, and averaged to build a site mean tree ring width (TRW) series chronology, whereas the third core was used for wood-anatomy analyses and was not sanded. The mean diameter at breast height and height of the whole set of trees were 44.7 cm and 22.1 m, respectively, and the mean age was 68 years (Acosta-Hernández et al. 2019). The diameter, height, and age ranges for the five studied trees were 43.0–47.1 cm, 21.0–24.0 m, and 65–70 years, respectively.

We used standard dendrochronological methods (Fritts 1976) to obtain TRW measurements. Cores were visually cross-dated following the Yamaguchi (1991) method, and TRWs were measured at 0.01 mm resolution using the LINTAB-TSAP system (Rinntech, Heidelberg, Germany). The visual cross-dating was checked using the COFECHA program (Holmes 1983).

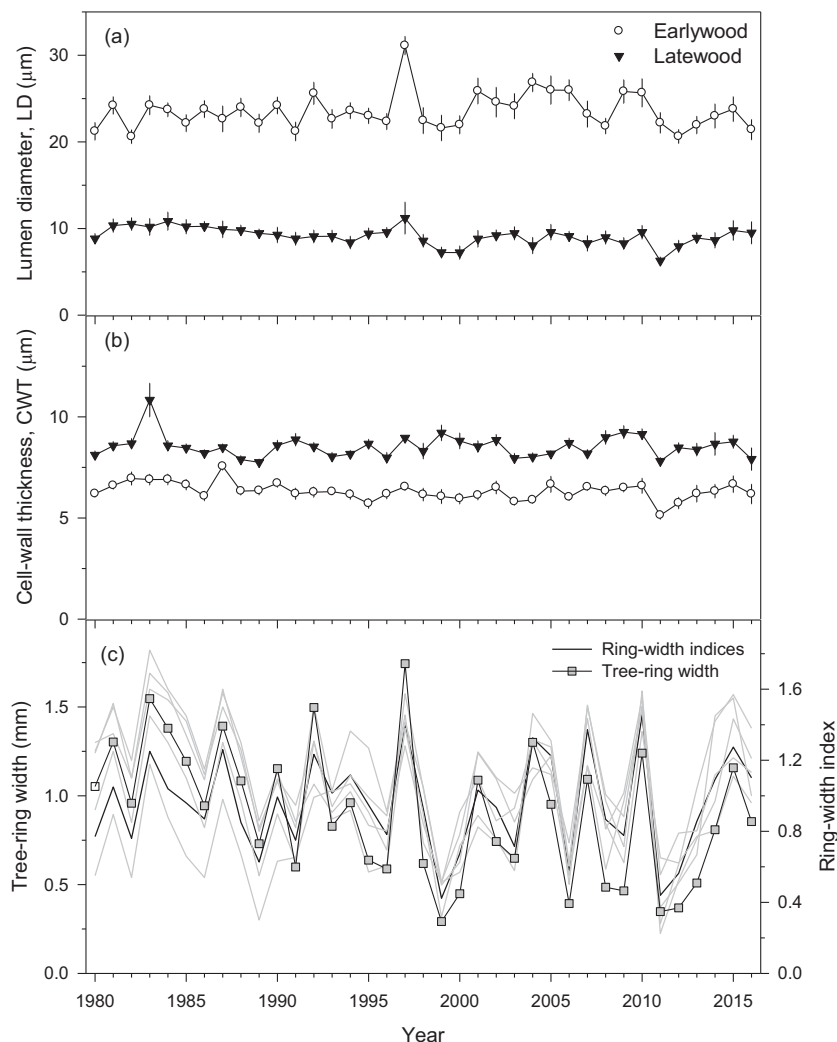
Wood anatomical measurements

The cores selected for wood-anatomy analyses were sectioned using a sledge microtome (Anglia Scientific AS 2000, UK). Thin sections (10–20 μm thick, 4–5 cm long) containing several rings were mounted on glass slides, stained with safranin (0.5% in distilled water), and fixed with Eukitt. Images of sections (see an example in Supplementary Fig. S2¹) were first taken at 40×–100× magnification with a digital camera mounted on a light microscope (Olympus BH2) and then stitched with the PTGui software (New House Internet Services, Rotterdam, Netherlands). It must be noted that differences in section thickness and magnification could impact wood-anatomy measurements (Prendin et al. 2017).

We studied the rings formed between 1980 and 2016, which corresponded to mature wood of the trunk sapwood. We measured LD and tangential double CWT along the radial direction in five radial lines of tracheids that were randomly selected from each tree ring using the ImageJ software (Schneider et al. 2012). These two anatomical variables reflect xylem dynamics in conifers (Cuny et al. 2014). We also calculated the ratio of LD to CWT (LD/CWT) to define LW when LD/CWT < 2 according to the Mork index (Denne 1989). To compare patterns of tracheid dimensions

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjfr-2019-0120>.

Fig. 1. Mean annual values of (a) lumen diameter (LD) and (b) cell wall thickness (CWT) presented separately for earlywood and latewood tracheids. (c) Mean series of tree ring width (black line with symbols) and ring-width indices (black line without symbols). The error bars represent standard error (SE). The grey lines in the lowermost plot show the individual series of ring-width indices for the trees selected for wood-anatomy analyses.



(LD and CWT) among rings and years, the number of cells was standardized by using the tracheidogram method (Vaganov et al. 2006) with the tgram package in R (DeSoto et al. 2011; de la Cruz and DeSoto 2017). Following Pacheco et al. (2015), each ring was divided into 10 radial sectors or deciles of equal width along the tangential direction, with the first and last deciles corresponding to the early EW and the late LW, respectively (see Supplementary Fig. S3¹). We also calculated mean and standardized values of LD, CWT, and LD/CWT for each ring ($n = 190$ rings) and year ($n = 37$ years) following Deslauriers et al. (2003). The mean values of LD and CWT were also computed separately for EW and LW.

Detection of IADFs

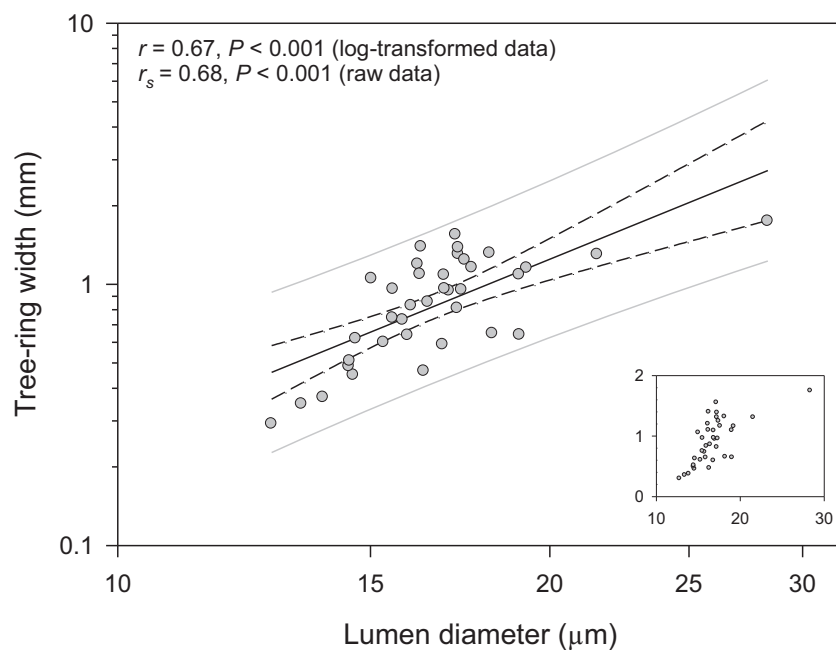
To detect IADFs, we calculated standardized mean tracheidograms for each year using LD data. As previously outlined, we used 10 sectors to quantify the LD variability within each ring. Then, we applied a method to remove the (linear) trend and low-frequency variation from the LD data. The degree of smoothing for lower frequency variation (seasonal component) was selected using an additive decomposition technique. We applied a seasonal-trend decomposition procedure based on loess (STL) smoothing to analyze the trend and changes in LD over the 1980–2016 period. The loess fit at a particular x value of the LD series was obtained using

weighted least squares to fit a polynomial of degree λ to the n_α observations nearest x , where α is the smoothing parameter (Cleveland et al. 1988). STL is a filtering procedure for decomposing a time series into different components (trend, seasonal, and remainder components) by applying loess smoothing (Cleveland et al. 1988, 1990). The time series of LD was formed by several components: $Y_t = f(S_t, T_t, R_t)$, where Y_t is the raw LD data at period t , S_t is the seasonal component at period t , T_t is the trend component at period t , and R_t is the remainder (error) component at period t . This decomposition technique was used to distinguish the aforementioned components in the LD data. Then, we calculated a random component as $R_t = Y_t - T_t - S_t$, which corresponds to standardized LD residuals. These residuals followed normality. We calculated the lowermost (in the lowest 5% of the distribution) values of the LD residuals. These residuals are LD departures from the seasonal mean and corresponded to EW IADFs formed by bands of tracheids with very narrow lumens.

Statistical procedures

An analysis of variance (ANOVA) with multiple pairwise Tukey's tests was used to compare LD values between years. We removed the long-term trends of wood-anatomy variables by detrending the raw series based on fitting negative linear or exponential

Fig. 2. Relationship observed between the mean annual values of tracheid lumen diameter and tree ring width. Correlations and significance levels (P values) are presented for either log-transformed (Pearson r) or raw (Spearman r_s) data. The linear regression is presented with 95% confidence intervals (dashed lines) and prediction intervals (grey lines). The inset shows the raw data relationship.



functions and then dividing the observed values by fitted values to obtain standardized values. To remove first-order temporal autocorrelation, we fitted autoregressive models and then averaged the prewhitened indices using biweight robust means. Thus, we obtained mean residual series (or chronologies) of LD and CWT for the entire ring and also separately for the EW and LW. A similar procedure was carried out with TRW data to obtain ring-width indices. These analyses were done using the program ARSTAN (Cook and Krusic 2005).

Because precipitation data did not follow a normal distribution (Supplementary Fig. S1a¹), we used Spearman correlation coefficients to relate monthly and seasonal climate data (mean temperature and total precipitation) from the El Salto station with indexed LD and CWT values for both EW and LW. Based on a previous dendrochronological study in this tree population (Acosta-Hernández et al. 2019), we considered the temporal window for the correlation analyses to be from September of the previous year to September of the current year.

We also correlated climate data with intra-annual LD and CWT values to detect climate–anatomy signals on short temporal scales. Then, we calculated correlations between cumulative monthly precipitation data of the growth year (January–September) and the mean LD of each decile. Finally, to assess drought signals in the wood’s anatomical traits, we correlated the series of EW LD, LW CWT, and the annual ratio of LD to CWT (LD/CWT) with the standardized precipitation evapotranspiration index (SPEI), a multiscalar drought index with positive and negative values indicating wet and dry conditions, respectively (Vicente-Serrano et al. 2010). We again considered the months of the growth year (January–September) and used 1- to 21-month-long SPEI scales (i.e., corresponding to droughts with cumulative water deficits for the past 1–21 months). The SPEI was calculated using the SPEI version 1.7 library (Beguería et al. 2014) based on climate data from the El Salto station. All statistical analyses were done in the R statistical environment (R Core Team 2017).

Table 1. Spearman correlation (r_s) values calculated for the mean series of lumen diameter (LD) and cell wall thickness (CWT) measured in earlywood (EW) and latewood (LW) for the period 1980–2016.

Variable	Wood type	LD		CWT
		EW	LW	EW
LD	LW	0.303 (0.068)	—	—
CWT	EW	0.201 (0.231)	0.616 (0.001)	—
	LW	0.151 (0.368)	0.061 (0.717)	0.302 (0.068)

Note: P values are shown in parentheses ($P < 0.05$ was considered significant).

Results

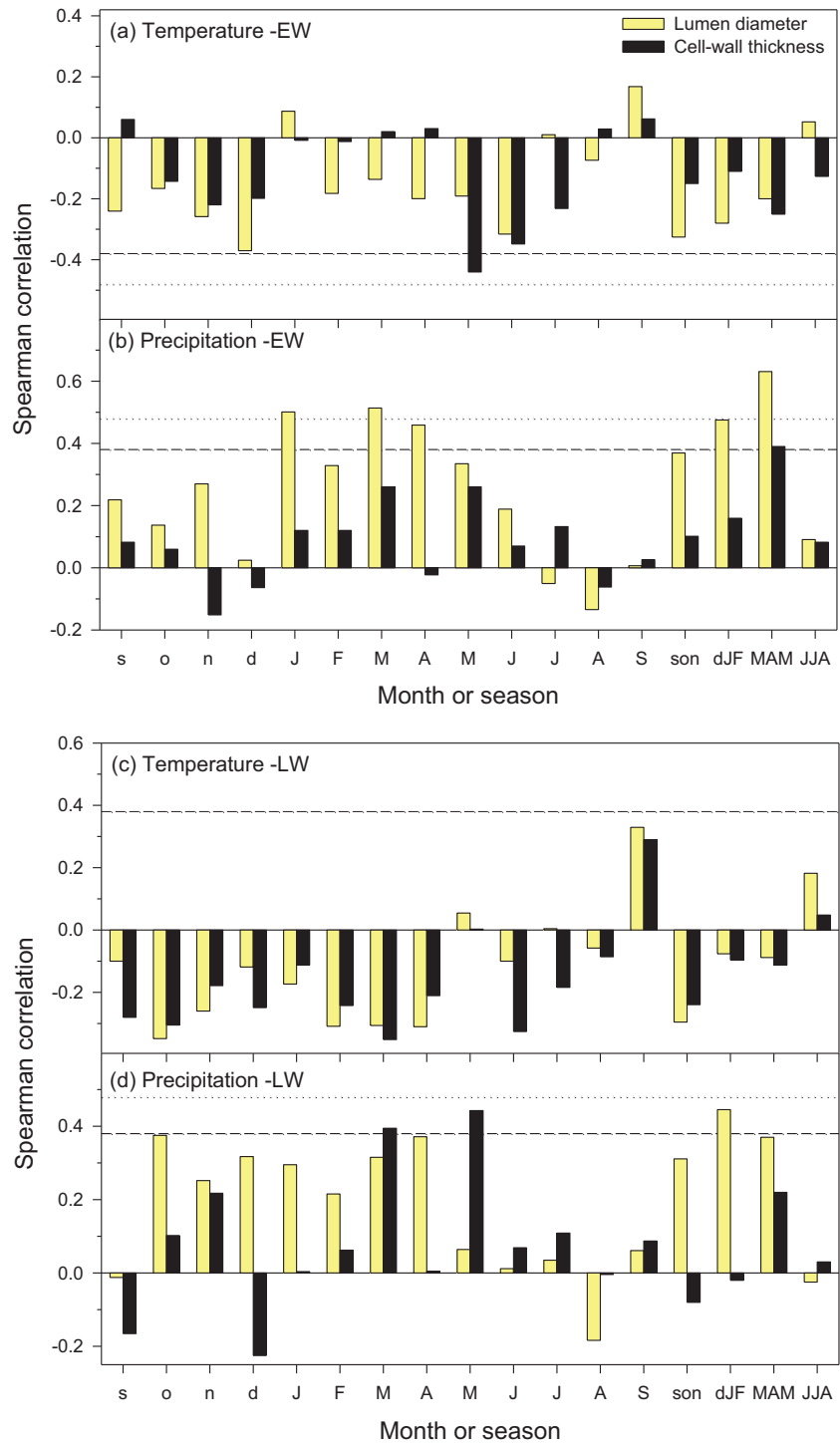
Relationship between wood anatomy and radial growth

The mean (\pm standard error (SE)) LD was $16.34 \pm 0.12 \mu\text{m}$, and the mean CWT was $7.42 \pm 0.05 \mu\text{m}$ (Figs. 1a and 1b). The mean LD and CWT for EW tracheids were $23.42 \pm 1.18 \mu\text{m}$ and $6.32 \pm 0.62 \mu\text{m}$, respectively, whereas the mean LD and CWT for LW tracheids were $9.26 \pm 0.80 \mu\text{m}$ and $8.52 \pm 0.28 \mu\text{m}$, respectively (Figs. 1a and 1b). The mean TRW of the five selected trees was $1.35 \pm 0.37 \text{ mm}$ (the mean for all trees was $1.27 \pm 0.36 \text{ mm}$; Fig. 1c).

High LD values and growth rates were observed in 1992, 1997, and 2010 (see Supplementary Fig. S4¹), whereas low LD values and growth rates were observed in 1999, 2006, and 2011 (Figs. 1a and 1c). Indeed, LD and TRW were significantly positively related (data were previously log-transformed to achieve normality; for both cases, $r = 0.67$, $r_s = 0.68$, and $P < 0.001$; Fig. 2). A similar association was observed with residual TRW indices ($r = 0.65$), confirming that the association was not due to first-order autocorrelation in TRW data, as raw and indexed TRW data were tightly associated ($r = 0.82$, $P < 0.001$; Fig. 1c).

LD reached maximum values in 1997 (31.11 and $11.20 \mu\text{m}$ for EW and LW, respectively), a year that was characterized by a very high growth rate (1.7 mm). The high LD values in EW and LW tracheids in 1997 agreed with the correlation between EW LD and LW LD, and this relationship was similar to that observed between EW CWT and LW CWT (Table 1).

Fig. 3. Relationships among climate and wood-anatomy traits (lumen diameter and cell wall thickness). Spearman correlation coefficients and significance levels are shown for earlywood (EW; *a* and *b*) and latewood (LW; *c* and *d*). Dashed and dotted horizontal lines indicate the 0.05 and 0.01 significance levels, respectively. Monthly and seasonal climate data are mean temperatures (*a* and *c*) and total precipitation (*b* and *d*) for the period from the previous year (months abbreviated with lowercase letters) to September of the current year (months abbreviated with uppercase letters). son: September, October, and November of the previous year; dJF: December of the previous year and January and February of the current year; MAM: March, April, and May of the current year; JJA: June, July, and August of the current year. [Color online.]



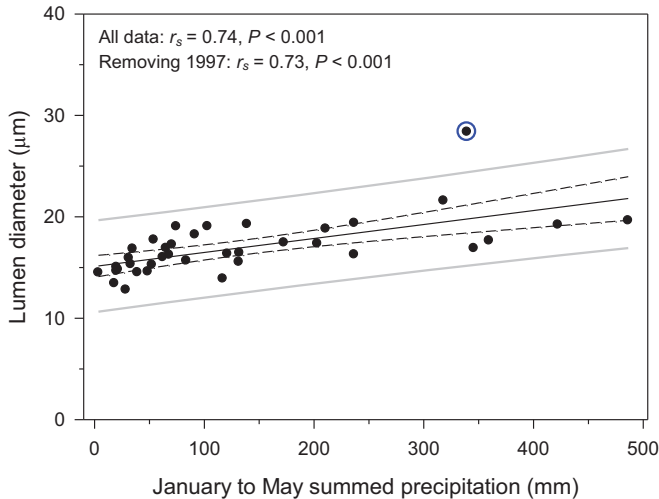
Relationships among climate, drought, and wood anatomy

For EW, CWT decreased with warm May conditions (Fig. 3a) and LD increased in response to high previous-winter (January) and current-spring (March and April) precipitation (Fig. 3b). For LW, warm current-September conditions and wet conditions in the

previous autumn and winter were associated with high LD values and cool previous-October and wet March and May conditions were related to high CWT (Fig. 3c). Wet and warm conditions during the previous winter also favored the formation of LW tracheids with wide lumens (large LD) and thick walls (high CWT)

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Fig. 4. Positive association found between the summed precipitation from January to May and tracheid lumen diameter. The Spearman correlation value (r_s) is presented with its significance levels (P values) for all data and for data with the 1997 outlier (data point circled in blue) removed. The linear regression is presented with 95% confidence intervals (dashed lines) and prediction intervals (grey lines). [Color online.]



(Fig. 3d). These findings were also observed when considering the whole tree ring. Again, significant positive associations between LD and precipitation from the previous autumn and winter and current spring were observed for the whole ring (Supplementary Fig. S5¹). Overall, we found strong links between LD and precipitation. In fact, the cumulative precipitation from January to May was significantly associated with LD (Fig. 4).

In addition, we found a maximum correlation value ($r_s = 0.59$ – 0.61 , $P < 0.0001$) between the cumulated precipitation from January to May and the LD value of the third and fourth deciles, which correspond to tracheids formed in EW (Fig. 5).

Lastly, the EW LD responded positively to high SPEI values from January to June, mainly at 4- to 11-month-long scales (the correlation peaked in March–April considering 8- to 9-month SPEI scales; Fig. 6a), but no significant correlations were found between LW CWT and SPEI (Fig. 6b).

EW IADFs

We could detect EW IADFs using our automatic method based on the analysis of LD data. The IADFs were characterized by several rows (usually 3–5) of tracheids with narrow lumens (see Supplementary Fig. S2¹). IADFs were very noticeable in 1998, 2003, and 2007, when the residuals of LD reached minimum values (Fig. 7), but IADFs were also detected in 1995, 1996, 1999, 2000, 2002, 2006, and 2008, years that were characterized by dry spring conditions. For instance, precipitation during March and April of years with evident IADFs (mean \pm SE = 4 ± 2 mm) was 10% of the mean for years without IADF (39 ± 11 mm).

Discussion

We found support for our hypothesis that wet conditions favor the formation of xylem conduits with wider lumens and therefore increase the theoretical hydraulic conductivity. High precipitation during the late previous winter and early current spring promoted the formation of EW tracheids with wide transversal diameter in the rear-edge Douglas-fir population. Specifically, the correlations peaked when considering cumulative precipitation from January to May and the LD of tracheids located in the second decile (i.e., corresponding to the early EW). When considering the SPEI for drought, the correlation with LD peaked from January to

June and corresponded to water balance accumulated since the previous October. Lastly, the method used (STL) allowed for detecting EW IADFs, characterized by several rows of tracheids with narrow lumens, which were formed in response to dry conditions in March and April. This approach offers an efficient and reliable method to detect anomalies in LD, including IADFs. It could be used to detect changes in LD or CWT in response to other stress factors such as drought, frost, or insect defoliation.

Overall, our findings support the hypothesis that precipitation from winter to spring affects the LD and therefore influences the potential hydraulic conductivity (Fonti et al. 2010). We found a close relationship between LD (a proxy of hydraulic conductivity) and radial growth, in line with findings from a previous study on a high-elevation pine species (Camarero et al. 1998). Consequently, drought during the growing season decreases tracheid expansion, hydraulic conductivity, and radial growth (Eilmann et al. 2009; Bryukhanova and Fonti 2013). Such a decrease in LD would result in denser wood with greater strength (Zobel and van Buijtenen 1989). The associations found between climate and LD are consistent with the finding that EW IADFs were formed in response to dry spring conditions (Acosta-Hernández et al. 2019). We show that automatic methods based on LD data are promising tools for developing and quantifying new, seasonal hydroclimate proxies such as IADFs in Douglas-fir and other conifers subjected to seasonal drought (De Micco et al. 2016).

Interestingly, cool, wet conditions during the previous autumn and winter also enhanced the LW LD, which may be an indirect consequence of the positive effects on EW LD. Both EW LD and LW LD were positively correlated, suggesting that the formation of wider tracheids in the early growing season, when conductivity probably peaks, determines to some degree the formation of wider tracheids in the late growing season when conditions become wetter because of the summer monsoon (Adams and Comrie 1997). In fact, LW production in forests of southwestern North America is a proxy of summer monsoon precipitation (Griffin et al. 2013), but future studies should investigate how this climate signal depends on wood-anatomy features such as EW LD. Xylogenesis studies in similar rear-edge tree populations would allow for determining the timing of EW and LW formations and the responses of wood-anatomy features (LD, CWT, and LD/CWT) to short-term climate variability.

We found that CWT increased in response to wet current-spring and previous-winter conditions in the EW and LW, respectively. In contrast with LD, CWT did not show significant correlations with the SPEI for drought. Unexpectedly, we did not detect significant associations between LW CWT and summer climate conditions. This could indicate that LW CWT depends mainly on LW LD and indirectly on EW LD. In general, our results are in agreement with previous studies on conifer species in which CWT was found to decrease in both EW and LW under warmer and drier conditions (Eilmann et al. 2009; Fonti et al. 2013; Martín-Benito et al. 2013; Heres et al. 2014).

The observed relationships among climate, LD, and CWT suggest that Douglas-fir wood has evolved to provide hydraulic safety. This interpretation is supported by the positive association between hydraulic capacitance and LW proportion observed in this species (Domec and Gartner 2002). Therefore, LW may act as water-storage tissue whenever low pressures and related xylem embolism do not occur. The strong responsiveness of EW LD to water availability agrees with Douglas-fir ecophysiological strategies: Douglas-fir is considered to be an isohydric species because it avoids drought stress by rapidly reducing stomatal conductance to limit water loss through transpiration but maintains a constant leaf water potential (Bauerle et al. 1999). In addition, water deficit during the growing season reduces Douglas-fir photosynthesis rates (Lassoie and Salo 1981), which may contribute to reduced EW formation.

Fig. 5. Spearman correlations calculated between cumulative monthly precipitation and tracheid lumen diameter (LD). LD was calculated as mean values for the radial deciles comprising the tree ring (x axis). The grey scale indicates cumulative precipitation for each month (e.g., the July bar is the precipitation accumulated from January to July). The dashed and dotted lines indicate the 0.05 and 0.01 significance levels, respectively. [Color online.]

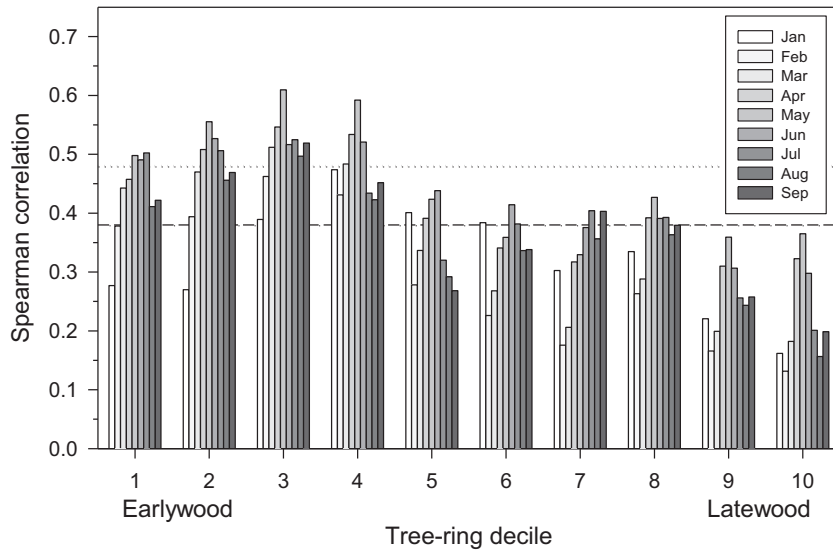
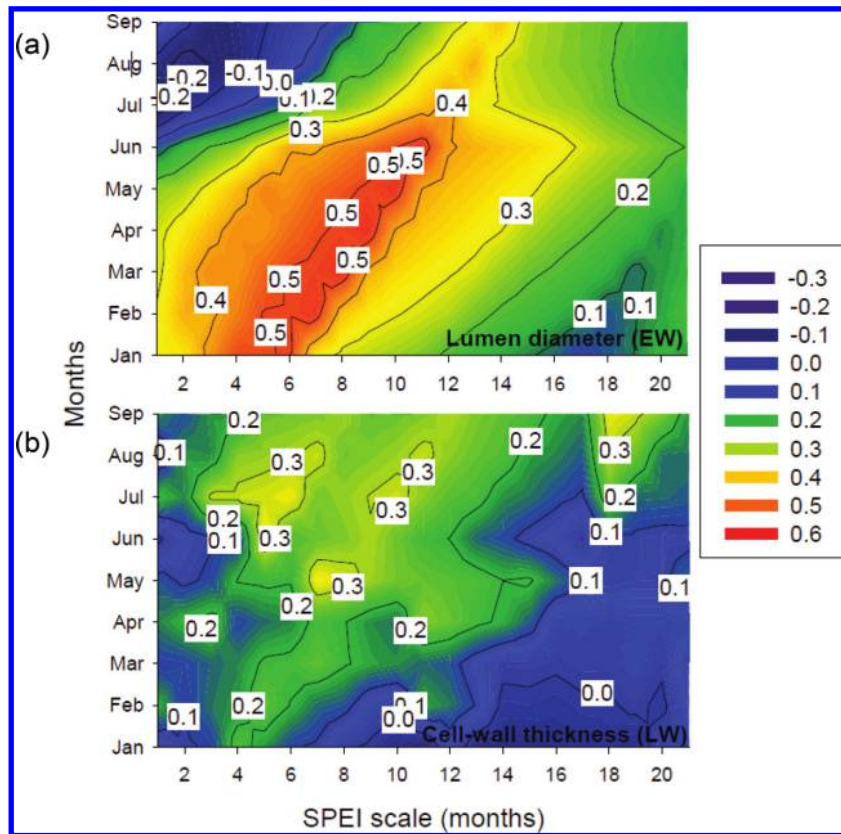


Fig. 6. Associations between drought and wood anatomy assessed in Douglas-fir for the lumen diameter of earlywood (EW) tracheids (a) and the cell wall thickness of latewood (LW) tracheids (b). Drought was quantified using the standardized precipitation evapotranspiration index (SPEI) for drought, which was calculated at 1- to 20-month-long scales (x axes) from January to September of the growth year (y axes). Correlations (scale color and legend) greater than 0.38 and 0.46 were significant at the 0.05 and 0.01 levels, respectively. [Color online.]

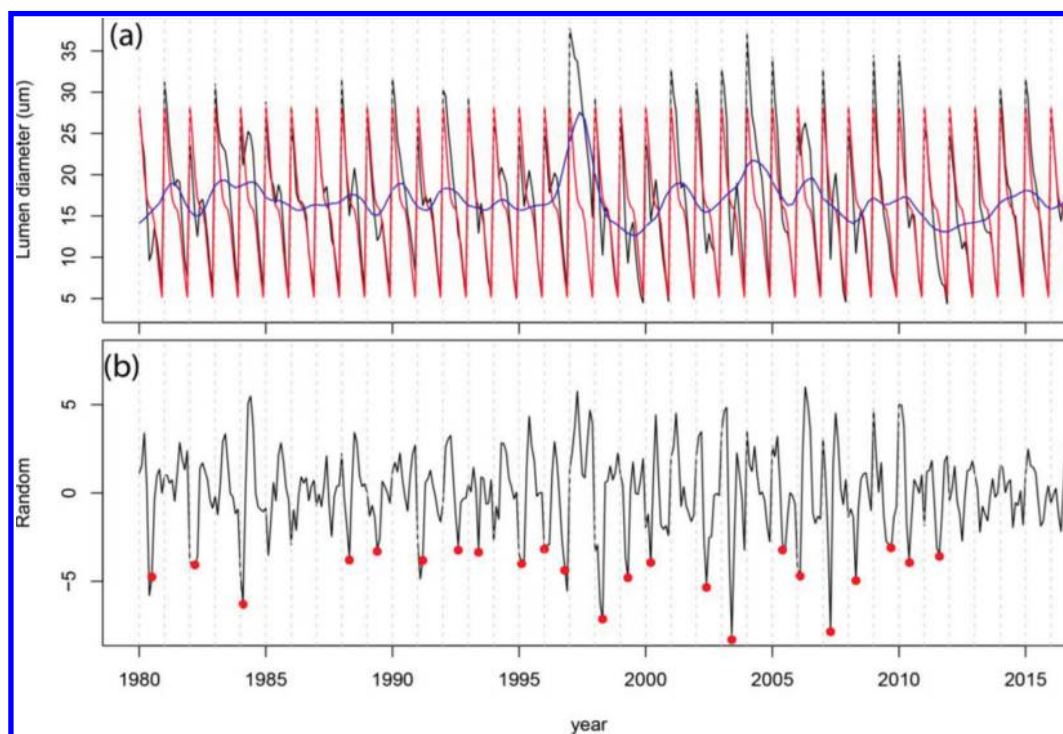


The responses of the two Douglas-fir wood-anatomy features to hydroclimate variability were significant. These responses could be mediated by low soil-moisture values at the study site, which would reduce water availability to trees given the high evapotranspiration rates of Douglas-fir (Andrews et al. 2012). Neverthe-

less, the soil water-holding capacity can only partially explain the responsiveness of Douglas-fir xylem to drought, even if some trees are able to access deep water sources (Andrews et al. 2012).

Our results agree with previous dendrochronological analyses of Douglas-fir populations in Mexico that aimed to reconstruct

Fig. 7. Results of the analysis of intra-annual density fluctuations (IADFs) based on lumen diameter (LD) data following the tracheidogram approach. (a) Normalized values of LD (black line) with trend (blue line) and seasonal (red line) components. (b) Random component of LD extracted after removing the trend component, seasonal component, and lowermost values (residuals lower than the 5% distribution) corresponding to earlywood IADFs (red dots). [Color online.]



hydroclimate variability and found a close relationship between EW formation and the rainfall accumulated from the late previous winter to the early current spring (Villanueva-Díaz et al. 2009). However, Villanueva-Díaz et al. (2009) could not achieve robust reconstructions of summer precipitation, which could be more linked to LW production. Our findings indicate that their interpretation may be correct, as we did not find significant associations between LW anatomical variables and summer precipitation. However, our study also explains how the enhancement of EW production under wet early growing-season conditions may be due to the production of wider and more numerous EW tracheids. This finding could be extended to other conifers that inhabit drought-prone areas, as wet and cool winter to spring conditions have been found to promote the growth of pine species near the study area (González-Cásares et al. 2017).

Douglas-fir shows natural genetic adaptation to local climate and drought (Bansal et al. 2015). Accordingly, the Douglas-fir populations best adapted to cope with forecasted drought conditions would be those from regions with relatively cool winters and arid summers. However, the frequency of extreme climate events such as severe droughts can increase in the forthcoming decades. Such drying trends could negatively impact Douglas-fir growth and productivity. A similar loss in productivity was observed in Europe during the 2003 drought and heat, when dieback and mortality episodes affected Douglas-fir plantations (Sergent et al. 2014). Reductions in LD and radial growth could be used as early warning indicators of forest productivity loss in response to dry spells. We also show that the xylem responsiveness to hydroclimate is high in rear-edge tree populations when it is properly analyzed and quantified. Although we found a pronounced xylem sensitivity to early growing-season precipitation in a rear-edge Douglas-fir population, we note that other forests located near the “core” of the distribution range but subjected to seasonal droughts or located in xeric sites (e.g., in steep slopes with shallow soils) could show similar responses (Griesbauer and Green 2010a). Our results could

also be used to suggest strategies for forest management as climate changes become more apparent. Understanding the influences of water availability on conduit size (lumen and cell wall dimensions) can provide useful proxies to forecast physiological adjustments to future environmental conditions.

To conclude, the cumulative precipitation from the previous winter to the current spring influences both the EW LD (a proxy of the theoretical hydraulic conductivity; Hacke et al. 2015) and radial growth. EW IADFs reflect dry spring conditions. Wet winter to spring conditions enhance the formation of wider tracheids, which provide more hydraulic conductivity and allow for increased growth. Because most Douglas-fir forests are water limited (Restaino et al. 2016), our wood-anatomy investigations on a rear-edge Douglas-fir population provide relevant information to project forest productivity in response to a warmer and drier climate at the core of the species distribution area.

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