Research paper

Linking wood anatomy and xylogenesis allows pinpointing of climate and drought influences on growth of coexisting conifers in continental Mediterranean climate

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Forecasted warmer and drier conditions will probably lead to reduced growth rates and decreased carbon fixation in long-term woody pools in drought-prone areas. We therefore need a better understanding of how climate stressors such as drought constrain wood formation and drive changes in wood anatomy. Drying trends could lead to reduced growth if they are more intense in spring, when radial growth rates of conifers in continental Mediterranean climates peak. Since tree species from the aforementioned areas have to endure dry summers and also cold winters, we chose two coexisting species: Aleppo pine (*Pinus halepensis* Mill., Pinaceae) and Spanish juniper (*Juniperus thurifera* L., Cupressaceae) (10 randomly selected trees per species), to analyze how growth (tree-ring width) and wood-anatomical traits (lumen transversal area, cell-wall thickness, presence of intra-annual density fluctuations—IADFs—in the latewood) responded to climatic variables (minimum and maximum temperatures, precipitation, soil moisture deficit) calculated for different time intervals. Tree-ring width and mean lumen area showed similar year-to-year variability, which indicates that they encoded similar climatic signals. Wet and cool late-winter to early-spring conditions increased lumen area expansion, particularly in pine. In juniper, cell-wall thickness increased when early summer conditions became drier and the frequency of latewood IADFs increased in parallel with late-summer to early-autumn wet conditions. Thus, latewood IADFs of the juniper capture increased water availability during the late growing season, which is reflected in larger tracheid lumens. Soil water availability was one of the main drivers of wood formation and radial growth for the two species. These analyses allow long-term (several decades) growth and wood-anatomical responses to climate to be inferred at intra-annual scales, which agree with the growing patterns already described by xylogenesis approaches for the same species. A plastic bimodal growth behavior, driven by dry summer conditions, is coherent with the presented wood-anatomical data. The different wood-anatomical responses to drought stress are observed as IADFs with contrasting characteristics and responses to climate. These different responses suggest distinct capacities to access soil water between the two conifer species.

Keywords: intra-annual density fluctuation, *Juniperus thurifera*, *Pinus halepensis*, quantitative wood anatomy, radial growth, xylem.

Introduction

Anthropogenic climate warming represents a global challenge for forest productivity in drought-prone areas (Reichstein et al. 2013). Models predict a worldwide increase of mean air temperature of ~2–4 °C with significant drying over regions such as

the Mediterranean basin (IPCC 2013). Climate warming is expected to magnify drought stress in that area by raising air temperatures and evapotranspiration rates, thus increasing the frequency and severity of droughts and heat waves (Meehl and Tebaldi 2004). Arid and hot conditions could be more intense

during spring, when radial growth rates of most Mediterranean conifers peak (Camarero et al. 2010, Vicente-Serrano et al. 2010), with negative consequences on forest growth (Sarris et al. 2007) and related ecosystem services such as carbon fixation in long-lasting wood pools. In addition to summer drought, low winter temperatures also constrain forest growth in continental Mediterranean areas (Gimeno et al. 2012, Granda et al. 2014). Several conifer species respond to these dual stressors with a bimodal radial growth pattern, i.e., with higher wood-formation rates in periods with optimal climatic conditions, namely spring and autumn (Camarero et al. 2010). This double seasonal climatic stress also implies that spring and autumn climatic conditions can have contrasting effects (formation of earlywood-tracheids at the final part of the ring) on growth and wood-anatomical traits (e.g., tracheid lumen area (LA) and cell-wall thickness (CWT)) (De Luis et al. 2007, Olano et al. 2012, Pasho et al. 2012). In fact, the formation of intra-annual density fluctuations (IADFs) in Mediterranean conifers could be related to this bimodal pattern of growth. For instance, latewood IADFs are produced when climate induces the formation of earlywood-like tracheids with wide LAs and thin cell walls in summer or autumn when typically thick-wall tracheids with narrow lumen are formed (Campelo et al. 2007). Despite IADFs having been climatically characterized in many Mediterranean conifer species (Vieira et al. 2010, Novak et al. 2013), we still lack a more mechanistic approach to determining how their formation and anatomy respond to climate. A deeper understanding of the processes forming the IADFs justified them as a valuable wood-anatomical feature to be studied to link climate, xylogenesis and wood anatomy as advocated in previous research (Vaganov et al. 2006). Since the bimodal growth pattern has been described following cambium phenology over the course of a few years (cf. Camarero et al. 2010), longer records of IADF production and wood anatomy could identify whether that pattern is reflected in the responses of xylem traits to climate variability. Specifically, it could be investigated whether LA or CWT respond differently to seasonally varying climatic stress.

The long-term climate effects on radial growth of conifers are usually analyzed by measuring annually or seasonally resolved variables such as tree-ring, earlywood or latewood widths using dendrochronology (Fritts 2001). However, quantitative wood anatomy allows information to be obtained on tree functioning (e.g., carbon uptake, water use) and growth to be encoded at much finer temporal scales (Fonti et al. 2010, Von Arx and Carrer 2014). Here, we aim to characterize how climate influences growth and wood anatomy in two coexisting conifer species (Spanish juniper and Aleppo pine) subjected to continental Mediterranean conditions but presenting different phenological (e.g., xylogenesis), morphological (e.g., root depth) and physiological (e.g., drought tolerance) features (Gauquelin et al. 1999, Ne'eman and Trabaud 2000, Baquedano and Castillo 2007, Camarero et al. 2010, DeSoto et al. 2011). To achieve this, we quantify wood-anatomical traits (LA, CWT) and relate

them to climate variables (temperature, precipitation, water balance, vapor pressure deficit (VPD), soil moisture) by contrasting correlation analyses results and xylogenesis data available for both species. We also analyze how climate drives seasonal changes in wood anatomy by evaluating the formation of IADFs in response to specific climatic conditions. We hypothesize that the bimodal pattern of growth is idiosyncratically expressed by the two coexisting conifer species, which should show different growth and wood-anatomy responses to climate. We discuss how these different wood responses to climate relate to contrasting species-specific traits, mainly those related to the root depth and the ability of trees to access soil water.

Materials and methods

Study site and species

We selected a mixed Mediterranean forest (Vedado de Peñaflor) located close to Zaragoza city (41°47′N, 0°43′W, 560 m above sea level (a.s.l.)), in the Middle Ebro Basin, northeastern Spain. Based on climate data for the period 1970–2013 (see below), the climate at the site is continental Mediterranean with an average yearly rainfall of 313 mm (semi-arid conditions), of which 66 mm were recorded between June and August. Annual mean temperature was 13.8 °C with frosts occurring frequently during winter (see Online Resource 1, Appendix S1 available as Supplementary Data at *Tree Physiology* Online). The estimated annual water balance was −476 mm with water deficit occurring from March to October. No significant trend was observed in precipitation data of the study area since 1970, but mean maximum temperatures have been steadily rising leading to drier conditions (see Appendix S1 available as Supplementary Data at *Tree Physiology* Online). Gypsum and marls comprise the parent rock material, while the topography of the terrain consists of small hills and valleys with west and east-facing slopes. Two co-occurring evergreen conifer species belonging to different families were selected: Spanish juniper (*Juniperus thurifera* L., Cupressaceae) and Aleppo pine (*Pinus halepensis* Mill., Pinaceae). Spanish juniper is endemic to the Western Mediterranean basin, being most abundant in Spain and Morocco, and it usually grows under continental Mediterranean climate conditions on poor or rocky soils (Gauquelin et al. 1999). Spanish juniper growth is enhanced by wet winter and warm spring conditions, whereas summer water deficit is associated with low growth rates (DeSoto et al. 2012). Aleppo pine is the most widely distributed Mediterranean pine species and is well adapted to growth under xeric conditions by tolerating water shortage (Ne'eman and Trabaud 2000). In the study area, Aleppo pine growth is mainly enhanced by wet and cool conditions during spring and in the winter prior to tree-ring formation (Pasho et al. 2012). At the studied site, *J. thurifera* forms a relict population mostly located on valley bottoms and west-facing slopes, and is intermingled with *P. halepensis*, which is mostly present on

east-facing slopes where xeric conditions are stronger. More details on the study site can be found in Camarero et al. (2010).

Climate data

Daily climatic records were obtained from the Peñaflor-Aula Dei (41°45′N, 0°49′W, 280 m a.s.l.) weather station located at 5 km from the study site. In order to fill some missing temperature data gaps from Peñaflor we used the Zaragoza-airport (41°40′N, 1°00′W, 263 m a.s.l.) weather station located at 30 km from the study site. We considered the daily-resolved records for mean, maximum and minimum temperatures, relative humidity and total precipitation for the period 1970–2013. Vapor pressure deficit was estimated using temperature and humidity data (Abtew and Melesse 2013). We also calculated the water balance or difference between precipitation and potential evapotranspiration (PET) following Hargreaves and Samani (1982), and the soil moisture deficit (SMD) was obtained as the difference between cumulative evapotranspiration and cumulative rainfall (Cocozza et al. 2012). Daily data were also averaged (temperatures, VPD) or summed (precipitation, water balance) at various time intervals (5, 7, 10 and 15 days) to analyze climate–growth relationships at multiple temporal scales.

Dendrometer and xylogenesis data

To characterize the phenology of radial growth and xylogenesis (phases of xylem formation) we used data of radial stem changes based on manual band dendrometer records (Agricultural Electronics, Tucson, AZ, USA) and observations of xylem formation (xylogenesis) based on repeated wood sampling (micro cores), respectively. These data were recorded biweekly or monthly in 10 trees per species. Part of the dendrometer data (2006–12 period) and all xylogenesis data (years 2006 and 2010) have been already published (Camarero et al. 2010, Pasho et al. 2012). Dendrometer data were used to determine the times of maximum increment and decrement rates that were defined when >50% of trees showed positive or negative increment rates, respectively. Xylogenesis allowed the main phases of xylem formation to be characterized in both species by counting different cell developmental phases (cambium cells, radially enlarging tracheids indicating cambium resumption, thickening tracheids indicating the onset of wall lignification, and mature tracheids (cf. Antonova and Stasova 1993, Cuny et al. 2014). More details on these data can be found in Camarero et al. (2010) and Pasho et al. (2012).

Wood-anatomy data acquisition and analyses

In January 2014, a total of 20 dominant trees, between 40 and 60 years old (10 per species), were randomly selected and sampled with a Pressler increment borer taking three radial cores per tree at 1–1.3 m. During sampling we avoided damaged stem areas with visible scars or showing signs of recent injuries and forming compression wood. In the laboratory, two cores per tree

were prepared following standard dendrochronological methods (fixed in woody mounts, glued and sanded). These samples were visually cross-dated and then measured to a precision of 0.01 mm using a LINTAB measuring system (F. Rinn, Heidelberg, Germany). Cross-dating was statistically validated using the COFECHA program (Holmes 1983). For histological analyses, we selected the five best cross-dated trees cores per species, that is, trees with cores showing the highest correlation with the mean ring-width series of each tree species. We also considered cores without visible anomalies (e.g., reaction wood, resin pockets) and containing the pith in order to obtain complete tree-ring sequences. Samples were processed following Gärtner and Schweingruber (2013). Cores were cut in to small pieces (3–5 cm long), boiled in water to soften the wood and then cut in to thin slices (10–20 μm thick) using a rotary microtome. Slices were then cleaned, stained with a safranin (1%) and astra blue (0.5%) (both in distilled water), rinsed with water and alcohol and finally permanently fixed with a synthetic resin (Eukitt™; Merck, Darmstadt, Germany).

Image acquisition was performed with a digital camera mounted on a light microscope; images were captured at 100× magnification. Overlapping images were taken from each sample and stitched together using the PTGui software (New House Internet Services, Rotterdam, The Netherlands) to obtain high-resolution images (0.833 pixels μm^{-1}) of the entire section. Tree-ring borders were manually drawn on the images that were semi-automatically analyzed using the ROXAS software (Von Arx and Carrer 2014).

We measured the following wood-anatomical traits in the transversal xylem sections: mean LA and radial CWT. The radial dimension of CWT was chosen because it has a higher year-to-year variability than the tangential dimension (Vysotskaya and Vaganov 1989). Then, following Hereş et al. (2014), we calculated several parameters related to the hydraulic performance of the tree: (i) mean hydraulic diameter (d_h); (ii) mean percentage conductive area; and (iii) theoretical hydraulic conductivity (K_h) computed according to the Hagen–Poiseuille law (Tyree and Zimmermann 2002). The d_h was calculated by assuming that LAs are circular and using the formula $d_h = \Sigma d^5 / \Sigma d^4$, where d is the tracheid diameter (Pockman and Sperry 1997).

To better define the time windows over the phases of radial growth we created 10 chronologies out of LA and CWT based on the relative radial position of each tracheid along the tree ring. This was done by dividing each tree ring into 10 sectors (deciles) along the radial direction and then computing the median LA and CWT values for each decile (see Online Resource 2, Figure S2 available as Supplementary Data at *Tree Physiology* Online). We decided to use deciles because they provided a good trade-off between sample size (number of cells per sector) and spatial and temporal resolution.

To assess the presence of latewood IADFs and calculate their relative frequency (%), we graphed the LA and CWT mean values

along the tree-ring for each IADF and compared them with the Mork index, which is commonly used to define the latewood presence (Denne 1988). In this case, the Mork index proved to have a low efficiency in accurately defining earlywood and latewood limits due to the presence of latewood IADFs (see Online Resource 3, Figure S3 available as Supplementary Data at *Tree Physiology* Online). Intra-annual density fluctuations exclusively found within the latewood were therefore identified visually.

Lastly, we built chronologies of tree-ring width and wood-anatomical variables for each tree species using the ARSTAN program, which was specifically developed for the removal of biologically induced age–size related growth trends (Cook 1985), tendencies that are also present in wood-anatomical data (Carrer et al. 2015). Individual series were fitted using a cubic smoothing splines function with 50% frequency–response cutoff of 50 years. Afterwards, observed values were divided by expected values to obtain detrended and dimensionless indices, which were then subjected to autoregressive modelling to remove part of the temporal autocorrelation. These indices were averaged to create the mean chronology of each variable for the two species.

The climate–growth associations were quantified by calculating Pearson correlations between the detrended growth and wood-anatomical chronologies and the different time windows of climatic data for the best-replicated period (1975–2013). In the case of IADF frequencies, we related them to climatic variables using the Spearman non-parametric correlation coefficient

(r_s). The calculations were computed from previous October to current September based on a previous study (Camarero et al. 2010).

Results

Wood-anatomical features

The quantitative description of wood anatomy was based on rings formed between 1970 and 2013 and included the measurement of 3.0×10^5 and 4.6×10^5 tracheids in Aleppo pine and Spanish juniper, respectively. The two species significantly differ in the distributions of LA and CWT (Figure 1), with pine typically forming tracheids with larger lumens (pine 276 μm^2 vs juniper 147 μm^2) and thicker walls (pine 4.1 μm vs juniper 3.4 μm) (Table 1). Consequently, both the d_h and K_h were higher in Aleppo pine than in Spanish juniper, despite the fact that mean conductive area (34%) did not significantly differ between the two species (Table 1). The chronologies based on these anatomical variables showed lower correlation values between conspecific trees as compared with tree-ring width series, except for K_h. The annual mean production of IADFs in Spanish juniper was twice (32%) that in Aleppo pine (15%), but the mean IADF series of both species were highly related ($r = 0.33$, $P = 0.04$) indicating that they encoded similar climatic information.

We found a highly significant ($P < 0.001$) relationship between tree-ring width and the number of tracheids forming a ring, slightly
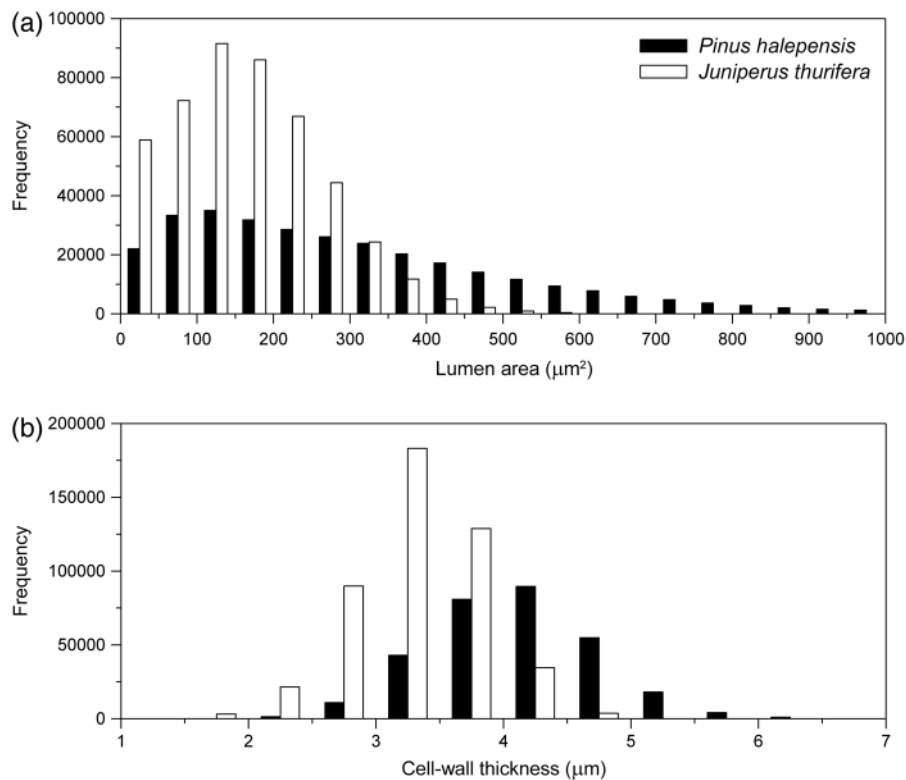

Figure 1. Distribution of (a) tracheid LA and (b) CWT for the two conifers (Aleppo pine, *P. halepensis*; Spanish juniper, *J. thurifera*). Data correspond to the 1975–2013 period.

Table 1. Main descriptive variables obtained for the trees considering size, age, tree-ring width and wood-anatomical variables. Values are means and standard deviation calculated for the period 1970–2013. Different letters indicate significant ($P < 0.05$) differences between species based on Mann–Whitney U-tests. IADF, intra-annual density fluctuation; LA, lumen area; CWT, cell-wall thickness; d_h, hydraulic diameter; K_h, theoretical hydraulic conductivity.

Variables	Spanish juniper (J. thurifera)		Aleppo pine (P. halepensis)	
	Variable	Correlation between trees	Variable	Correlation between trees
Diameter at 1.3 cm (cm)	14.8 ± 1.0	–	16.7 ± 1.0	–
Tree height (m)	5.1 ± 0.2a	–	6.0 ± 0.2b	–
Age at 1.3 m (years)	60 ± 15	–	54 ± 12	–
Tree-ring width (mm)	1.26 ± 0.72	0.42	1.82 ± 1.14	0.53
Frequency of IADFs (% year⁻¹)	32 ± 20b		15 ± 12a	
No. tracheids per ring	4264 ± 1053		2966 ± 835	
LA (µm²)	147.26 ± 36.55a	0.14	275.78 ± 86.46b	0.22
CWT (µm)	3.43 ± 0.31a	0.28	4.14 ± 0.36b	0.10
d_h (µm)	17.00 ± 1.97a	0.30	24.82 ± 4.48b	0.39
K_h (kg m MPa⁻¹ s⁻¹ 10⁻¹³)	6.22 ± 3.93a	0.45	12.50 ± 7.59b	0.60
Conductive area (%)	33.58 ± 6.34	0.24	34.44 ± 5.47	0.25

stronger in juniper ($r = 0.93$, $n = 244$) than in pine ($r = 0.90$, $n = 179$). As expected, for a similar number of tracheids pine formed rings approximately twice the width of those of juniper (see Online Resource 4, Figure S4 available as Supplementary Data at *Tree Physiology* Online). In addition, d_h and CWT were negatively related in juniper ($r = -0.40$, $n = 237$, $P = 0.015$), whereas this association was positive in pine ($r = 0.26$, $n = 176$, $P < 0.001$).

Phenology of radial growth and xylem formation

On average, both species started growing in early April and finished in late October (Table 2). Maximum radial growth increment rates were observed in mid (Spanish juniper) to late May (Aleppo pine), whilst maximum stem decrement rates were detected in early (Spanish juniper) to late August (Aleppo pine) in response to summer drought. Xylogenesis data showed that the first tracheids were formed in late March in both species, i.e., almost 15 days before the first stem radial increment was detected (Table 2). However, the maximum xylem growth rates, corresponding to the formation of radially enlarging tracheids, occurred in mid-to late May, and they almost coincided with those observed using dendrometers. The wall-thickening tracheids started and finished forming from early May to mid-October in Aleppo pine, and from late May to late October in Spanish juniper.

Inter-annual temporal patterns in growth and wood anatomy

Raw chronologies of tree-ring width and LA showed negative and positive significant ($P < 0.001$) trends, respectively, in both species (pine, $r = -0.45$ and $r = 0.77$; juniper, $r = -0.62$ and $r = 0.46$). The year-to-year variability in ring width was similar between the two species ($r = 0.73$, $P < 0.001$) indicating a likely common climatic signal (Figure 2). In fact, growth peaked in years characterized by wet spring conditions (e.g., 1997)

whilst wood production was very low during years with a warm and dry spring (e.g., 2005; see Online Resource 1 available as Supplementary Data at *Tree Physiology* Online). The patterns of mean LA, per ring, were also consistent over time between the two coexisting species ($r = 0.71$, $P < 0.001$), but this was not the case for CWT ($r = 0.23$, $P < 0.001$).

Climate influences on wood anatomy

The results obtained using a 15-day window produced the most robust and significant correlations. In general wet and cool conditions during the previous winter (recharge of soil water) and in spring (Spanish juniper) or summer (Aleppo pine) enhanced LA expansion, while dry spring-to-summer conditions (negative water balance) reduced the conduit size (see Online Resource 5, Figure S5 available as Supplementary Data at *Tree Physiology* Online). Warm summer to autumn conditions preceded by low SMD enhanced CWT in the case of Spanish juniper, whereas wet and warm late-winter and summer conditions were associated with high CWT values in Aleppo pine. The first of these two associations was also observed in juniper. Considering the analyses based on tree-ring deciles, wet conditions from February to July enhanced LA of tracheids located in the first four deciles (early to mid-early-wood) and also in the last decile (late latewood) in both species, with maximum positive associations observed in May and June (Figure 3).

Contrastingly, the positive role of warm February conditions on LA affected most deciles distributed throughout the ring in juniper, but mainly the fifth to sixth deciles in the pine (mid- to late early-wood). In the case of CWT, warm February minimum temperatures enhanced wall thickening in Aleppo pine along the entire ring but the signal was stronger in the first three and last two deciles. Wet February to March conditions also enhanced CWT in both species, but July to September wet conditions only increased CWT in the last two deciles (latewood) of pine tracheids.

Table 2. Dates (day of the year) summarizing the xylem phenology (xylogenesis) of the two studied species (Spanish juniper, *J. thurifera*; Aleppo pine, *P. halepensis*) in the study site based on manual band dendrometers (2006–12 period) or xylogenesis (2006 and 2010) data. Abbreviations referring to dendrometer data: F, first positive growth rate; MG, maximum radial increment rate; MD, maximum radial decrement rate; L, last positive rate. Abbreviations of xylogenesis phases: EE, start of growth (first enlarging tracheids are formed); ME, maximum production of radially enlarging tracheids; EL, beginning of wall-thickening tracheids; LL, last wall-thickening tracheids are formed.

| Year | Dendrometer data | | | | | | | | Xylogenesis data | | | | | | | |
| | *J. thurifera* | | | | *P. halepensis* | | | | *J. thurifera* | | | | *P. halepensis* | | | |
	F	MG	MD	L	F	MG	MD	L	EE	ME	EL	LL	EE	ME	EL	LL
2006	112	163	201	334	110	175	195	304	95	133	142	315	75	155	135	290
2007	115	141	200	292	90	134	257	290								
2008	102	136	247	302	103	152	255	307								
2009	90	135	205	272	89	135	225	330								
2010	97	131	183	321	114	160	257	322	88	137	150	290	88	142	122	285
2011	94	124	257	331	90	134	260	290								
2012	103	132	222	278	94	131	221	296								
Mean	102	137	216	304	99	146	239	306	92	135	146	303	82	149	129	288
SD	30	37	34	17	10	17	25	16	5	3	6	18	9	9	9	4
Julian day	11 April	16 May	3 August	30 October	7 April	24 May	25 August	31 October	31 March	14 May	25 May	28 October	21 March	27 May	7 May	13 October

Climatic drivers of IADF formation

A positive water balance in late July (pine) or early September (juniper) increased the formation of IADFs in the latewood (Figure 4). However, cold conditions in March to June and in autumn (only in juniper) were negatively related to IADF formation. Lastly, a higher SMD in autumn was associated to the production of IADFs, being more important from September to October in the case of juniper. Considering monthly values, in pine IADF frequency was positively related to wet July to August conditions, while in juniper IADFs were formed in response to years with wet (high water balance and low VPD) August conditions (Figure 5).

Discussion

Here we evidence that wood-anatomical features encode long-term and high-resolution climatic information on tree functioning and growth of two conifer species coexisting under continental Mediterranean conditions. The observed relationships between climate and anatomical traits confirm previous information on xylogenesis of the two species. This allowed the major wood responses to water shortage to be pinpointed. We found that wet conditions during the early growing season in spring or before (winter) result in the formation of wide tree rings that consist of numerous tracheids with relatively large lumens in the case of pine, while LAs are smaller in juniper. The small LAs of juniper species make them less vulnerable to cavitation as compared with coexisting pine species forming tracheids with larger lumens (Willson et al. 2008). This seems to explain why drought-induced die-off mainly affects pines in mixed pine–juniper forests on semi-arid sites (McDowell et al. 2008). However, not only tracheid dimensions but also small alterations on pit traits (frequency, geometry and biomechanics) between coexisting species can affect tree performance on water transport, embolism resistance and capacitance (Hacke 2015). Considering only our measurements, wood-anatomical data therefore show that junipers will be less vulnerable to die-off than pines since many Cupressaceae have evolved towards a drought-resistant xylem (Pittermann et al. 2012). On the contrary, pines will show higher growth rates and K_h if conditions are wet enough in the early growing season.

As it ages Aleppo pine forms wider tracheids compared with Spanish juniper. This is also related to the usual bigger size of adult trees (tree height and deeper root system (Willson et al. 2008)). The shallower root system of junipers could make them more dependent on summer and autumn rains than Aleppo pine, and this could explain why junipers tend to form latewood IADFs more frequently (Table 1) whereas pines are able to sustain low growth rates during the summer dry period (see also Camarero et al. 2010). This agrees with the different microsite location of the species on the study site. The
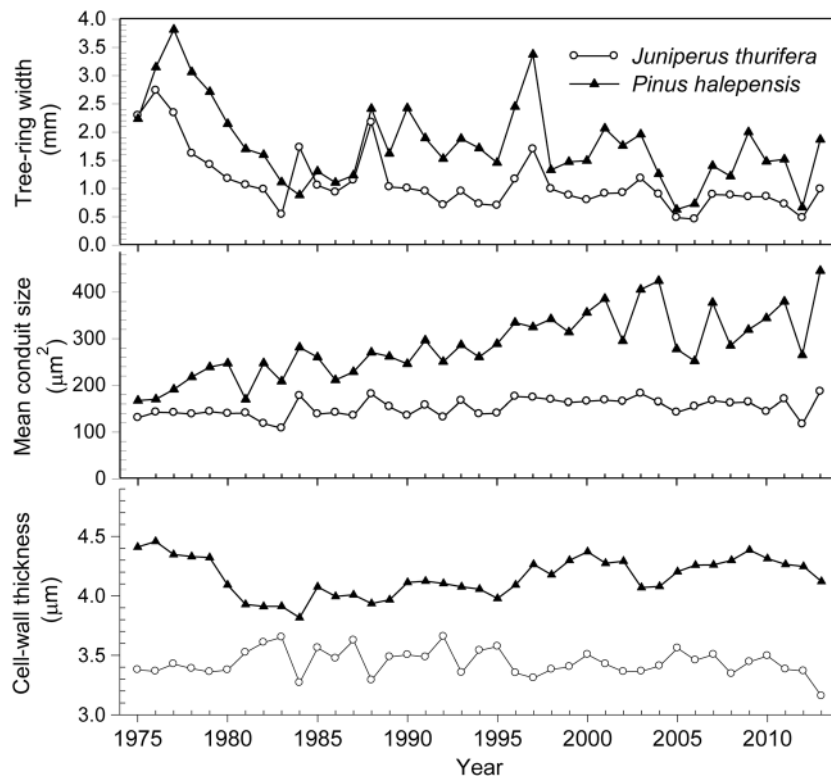
Figure 2. Mean tree-ring width, LA (mean conduit size) and CWT chronologies for the two species (Spanish juniper, *J. thurifera*; Aleppo pine, *P. halepensis*).

soil-water conditions play a central role from late March to June for the expansion of tracheid lumens in both species (Figure 3). However, the responsiveness of LA to SMD lasted until summer in juniper. In the case of CWT, correlations with climate were more variable than in the case of LA and differed between the two species. In both species, wet and warm late-winter conditions were associated with thicker earlywood and latewood tracheids, confirming lagged climate–anatomy relationships since latewood starts forming from the end of May onwards (Table 2). Warm summer to autumn conditions preceded by low soil moisture enhanced CWT in the case of Spanish juniper, whereas wet summer conditions were associated with thick cell walls in Aleppo pine. Such a response has been explained by the low intercellular variability in wall thickness among neighboring tracheids in Spanish juniper (Olano et al. 2012). These authors attributed the formation of latewood tracheids with smaller LAs in this species to limited carbohydrate availability because of competition with other carbon sinks (Oribe et al. 2003). However, our interpretation is that the amount of carbon invested for each tracheid is fairly similar over the entire growing season and the change in LA from earlywood to latewood is mainly responsible for the changes in CWT, which could represent an allometric relationship related to constraints of conduit size and wall reinforcement (Hacke et al. 2001), and not a carbon-related limitation of xylogenesis (Cuny et al. 2014). This argument is also supported for the pine species based on the associations observed between LA

or hydraulic diameter and CWT (see Online Resource 4, Figure S4 available as Supplementary Data at *Tree Physiology* Online).

Soil water availability during the early growing season, which depends on precipitation but also on soil temperatures and water-holding capacity, is one of the main drivers of wood formation and radial growth under continental Mediterranean climates (Martin-Benito et al. 2013). We found that wet and cool May conditions at a 15-day scale enhanced lumen expansion in both species as a result of improved cumulative soil water reserves, but high precipitation in June was associated with narrow lumens, particularly in Aleppo pine (see Online Resource 5, Figure S5 available as Supplementary Data at *Tree Physiology* Online). At a monthly scale, Aleppo pine formed larger lumens in response to a high water balance during the early spring when xylem formation starts (Figure 3, Table 1), which is a similar signal to that observed for the tree-ring width (Pasho et al. 2012). This implies that those climatic conditions favorable to lumen enlargement also enhance radial growth and suggests that wood formation and a higher K_h in the early growing season are interrelated and largely determine the annual growth rate in this species. These findings confirm the importance of winter soil-water recharge for Aleppo pine prior to cambial resumption in spring and also suggest that this species can reach deeper soil-water pools than those accessible to junipers (Sarris et al. 2013). This vertical segregation of soil-water resources
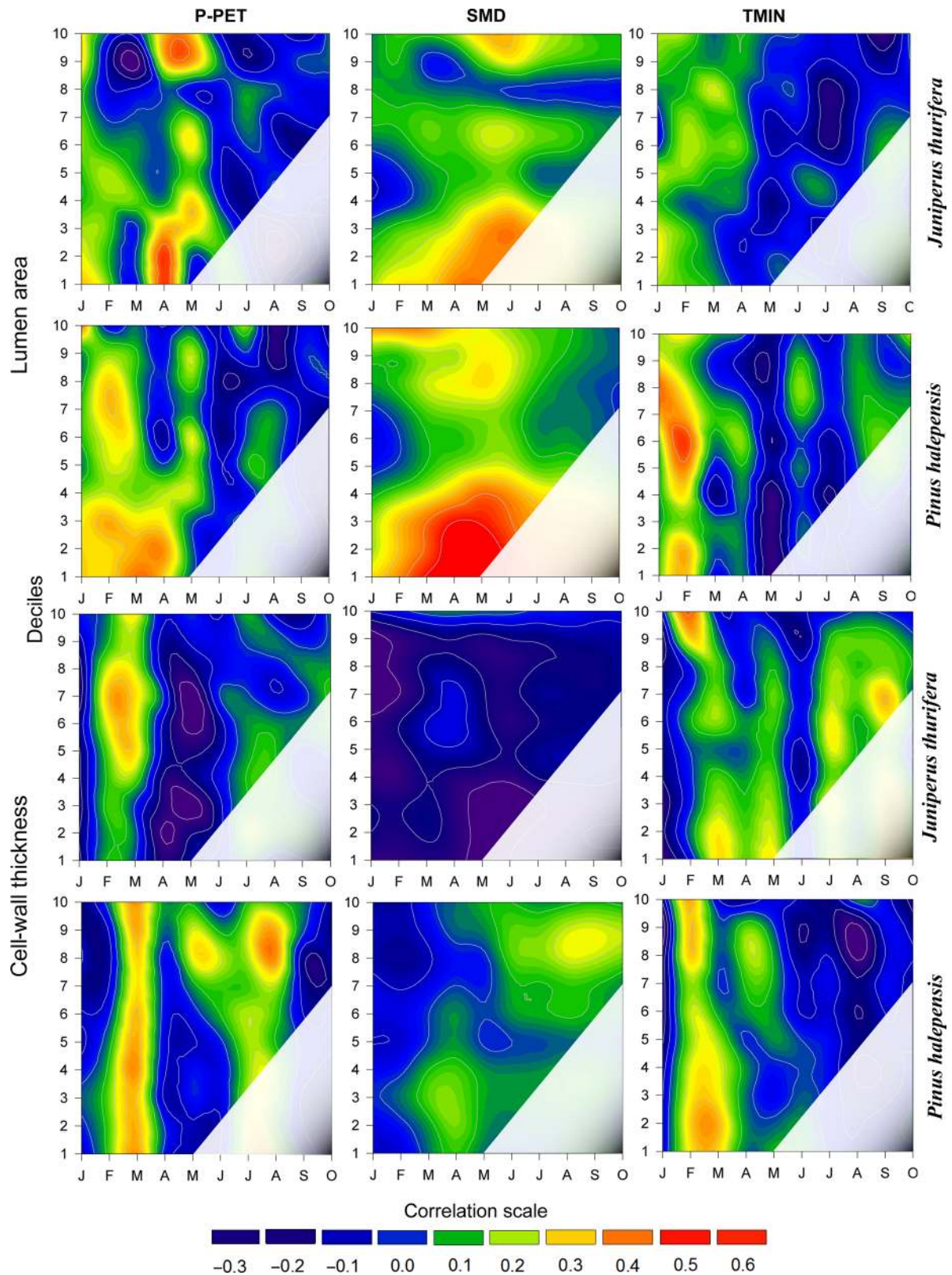
Figure 3. Climate–growth associations computed by comparing LA and CWT with monthly climatic variables (P-PET, water balance; SMD, soil moisture deficit; and T_{min}, minimum temperature) in Spanish juniper (*J. thurifera*) and Aleppo pine (*P. halepensis*). Correlations were calculated from January to October of the year of tree-ring formation (*x* axes) and partitioning the tree-ring in deciles along the radial direction from the beginning (Decile 1) to the end (Decile 10) of the ring (*y* axes). Correlations coefficient above |0.31| are significant at $P < 0.05$. Chronologically inconsistent correlations (bottom-right triangle in each plot) are not shown.
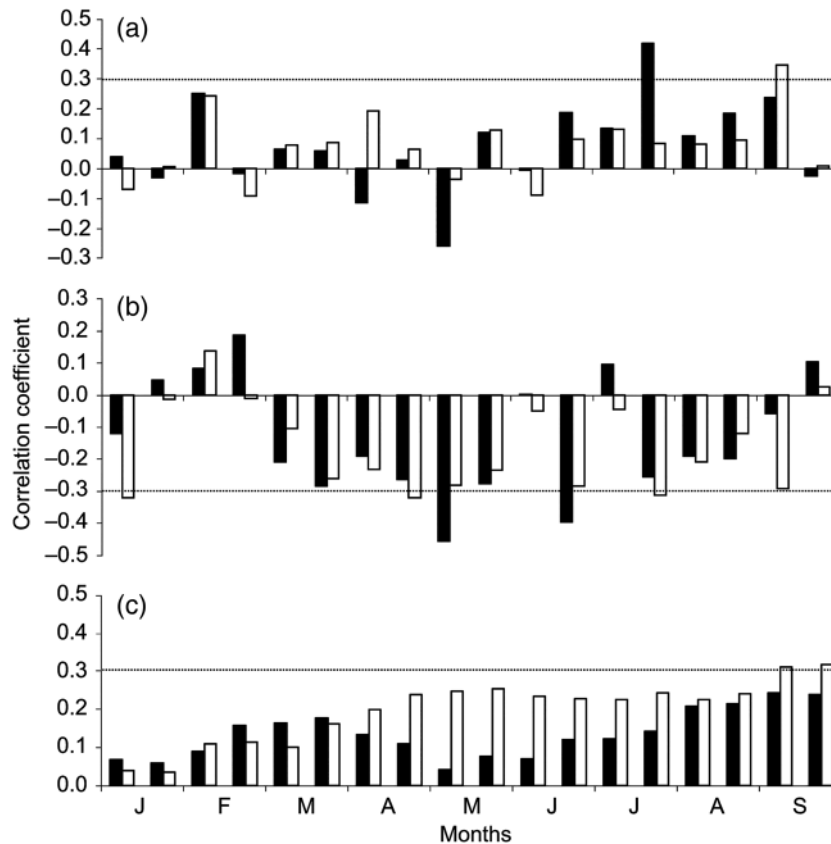
Figure 4. Spearman correlations computed for the two species (filled bars, Aleppo pine, *P. halepensis*; empty bars, Spanish juniper, *J. thurifera*) by relating the frequency of IADF and the 15-day summed water balance (a), averaged mean minimum temperatures (b) and summed SMD (c). Horizontal lines indicate significance levels at *P* < 0.05.

would explain the observed growth behavior differences by allowing a minimal growth of Aleppo pine in summer, while making juniper more dependent on spring rainfall to grow (Figure 3).

The longer season of latewood formation would explain a most active cambial activity of juniper in autumn and the frequent formation of IADFs. The positive effect of spring precipitation and summer SMD on the LA of juniper latewood tracheids indicates that the main driver of IADFs in this species is an increase in the water balance and a decrease on VPD, i.e., a reduction in soil and atmospheric dry conditions, which leads to the formation of tracheids with ample LAs in late summer and early autumn. This clearly represents a carryover effect in juniper response having first the punctual events of spring precipitations inducing a long-lasting effect on soil moisture which finally permits the species to produce the second peak of cambial activity. Contrastingly, latewood IADF formation in Aleppo pine is mainly favored by high precipitation in mid-summer in agreement with its earlier latewood development (Camarero et al. 2010).

The formation of latewood IADFs represents a valuable ecological proxy of late-summer to early-autumn water availability, although this is still little explored and IADF formation is not fully mechanistically understood. The relatively rapid formation of wide-lumen tracheids within the latewood suggests that the cambium, apparently dormant during the summer drought, reacts quickly to a significant amount of precipitation by forming an IADF. However, this response is similar to what is observed in the earlywood despite both occurring in completely different xylogenetic phases, namely radial enlargement of earlywood tracheids in the early growing season vs active lignification and maturation in the late growing season.

To conclude, tree-ring width and LA responded to wet previous winter to current spring conditions, respectively, in two conifers of different genera coexisting under continental Mediterranean conditions. The formation of latewood IADFs was favored by late-summer to early-autumn wet conditions which triggered the formation of tracheids with ample LAs. The relationships found between climate, growth and wood-anatomical data concur with the bimodal pattern of xylogenesis described for conifers in continental Mediterranean climates. The plastic responses of wood-anatomical variables to climate, and particularly to water deficit, could be caused by the different accessibility of soil water reserves for the species since junipers develop more shallow root systems than pines.
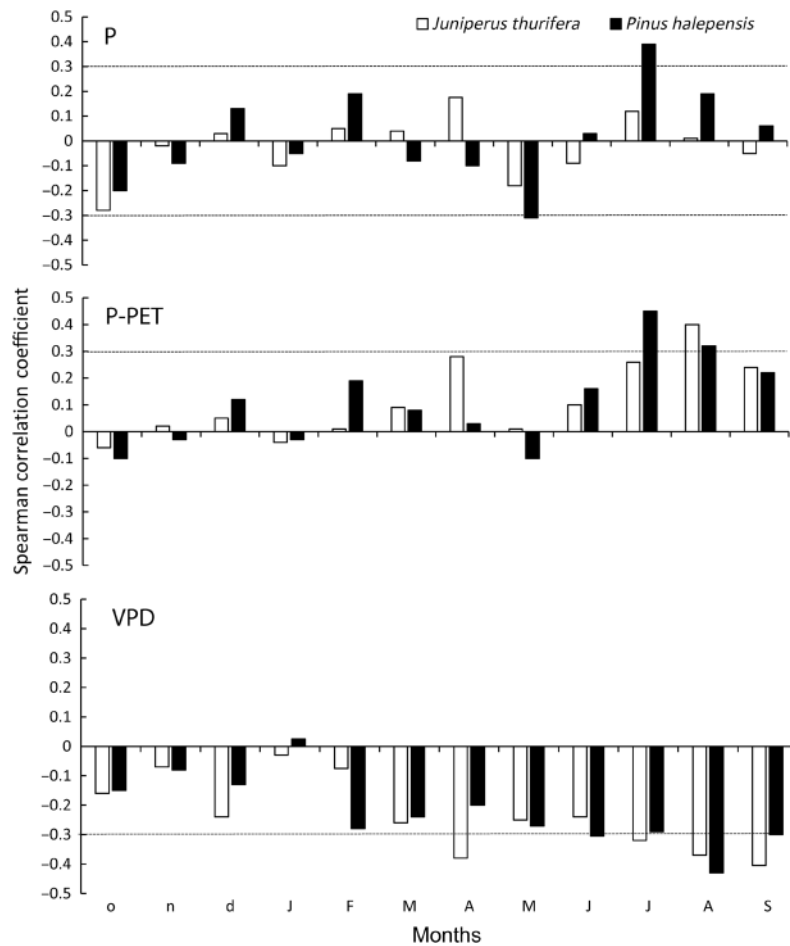
Figure 5. Relationships calculated between the frequency of latewood IADF of Aleppo pine (*P. halepensis*, filled bars) and Spanish juniper (*J. thurifera*, empty bars) vs monthly climatic variables: precipitation (P), water balance (P-PET) and VPD. Climatic variables include the time window from previous October to current September. Months prior to or concurrent with the growing season are abbreviated by lowercase and uppercase letters, respectively. Bars located outside the horizontal lines indicate significant correlations ($P < 0.05$).

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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