ORIGINAL PAPER

Climatic impacts and drought control of radial growth and seasonal wood formation in *Pinus halepensis*

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Received: 3 November 2011/Revised: 26 June 2012/Accepted: 28 June 2012 © Springer-Verlag 2012

Abstract Short- and long-term growth responses to drought and climatic influences still remain poorly understood. In this study, we investigated the impact of climatic drivers (temperature, precipitation) and drought, using the Standardized Precipitation Index (SPI) calculated at different time scales (1-48 months), on earlywood (EW) and latewood (LW) widths in Pinus halepensis. Nine forests subjected to dry summer conditions were sampled in Mediterranean semi-arid areas from north-eastern Spain. In addition, we explored the seasonal dynamics of cambial activity and wood formation in relation to short-term climate variability. We found two peaks of tracheid cell production corresponding to EW (May-June) and LW (mid-July-August) growth phases, associated with a sharp decrease in enlarging cells in early July in response to low water availability. In the period of analysis (1970-2005), EW growth was positively correlated with precipitation in previous December and current January, April, May and June, while it was negatively correlated with temperature

Communicated by S. Mayr.

Electronic supplementary material The online version of this article (doi:10.1007/s00468-012-0756-x) contains supplementary material, which is available to authorized users.

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J. Julio Camarero ARAID-Instituto Pirenaico de Ecología (CSIC), Avda. Montañana 1005, Zaragoza 50080, Spain in June and July. LW was correlated positively with minimum temperatures in January. Probably this was an indirect relationship as a consequence of increased EW width at higher January temperatures. Drought affected more negatively EW than LW formation as evidenced the higher SPI-EW correlation (r = 0.72) than the SPI-LW one (r = 0.54). The strongest EW response to drought was observed in July, whereas the highest LW response to drought occurred in September; and this seasonal pattern matched the phases of lowest EW and LW tracheid production. Under a future reduction of winter and spring precipitation, the studied forests may show a decrease in tracheid cell production, causing a decline of radial growth, a reduction in hydraulic conductivity and, indirectly, a hampered carbon uptake in such semi-arid woodlands.

Keywords Climate · Drought · Earlywood · Latewood · *Pinus halepensis* · Standardized Precipitation Index · Xylogenesis

Introduction

In Circum-Mediterranean forests, drought is considered as the main driver of the tree growth variability (Sarris et al. 2007). Climate change models project a decrease in annual mean precipitation and rising air temperatures over the Mediterranean Basin for the late twenty-first century leading to an increase in evapotranspiration (IPCC 2007; Giorgi and Lionello 2008; García-Ruiz et al. 2011). These trends towards increasing arid conditions in the region are expected to cause more frequent episodes of forest growth decline and mortality events as those already observed by some authors (Macias et al. 2006; Linares et al. 2009; Galiano et al. 2010; Sánchez-Salguero et al. 2010). Various studies indicate contrasting tree growth responses to drought (Martín-Benito et al. 2008; Koepke et al. 2010; Linares et al. 2010; Sánchez-Salguero et al. 2010). For instance, some authors (Abrams et al. 1998; Sarris et al. 2007) noted that trees growing in xeric sites showed declining radial growth trends and increased crown dieback and mortality in response to long-lasting droughts. However, other researchers argued that species growing in xeric locations develop adaptive features to withstand the negative effects of drought on growth by reducing water loss or increasing hydraulic efficiency, and by showing lagged growth responses or recovering rapidly after the drought (Bréda et al. 2006; Eilmann et al. 2009; McDowell et al. 2008).

Dendrochronology is a useful tool for providing information on climate–growth relationships based on year-toyear correlative approaches. However, these relationships must be compared with xylogenesis studies for capturing short term climatic influences on radial tree growth (Camarero et al. 1998; Rossi et al. 2008). Therefore, information regarding the phenology of xylem growth is required to better understand the processes underlying the effects of climate and drought stress on seasonal wood formation.

Pinus halepensis forests provide a valuable system to explore how drought measured at different time scales constrains radial growth. This conifer is one of the dominant tree species in the Western Mediterranean Basin and the most ecologically important species in semi-arid woodlands (Néeman and Trabaud 2000). P. halepensis is considered as a species well adapted to withstand drought by reducing growth as water availability decreases (Serre-Bachet 1992; Borghetti et al. 1998; Nicault et al. 2001; Rathgeber et al. 2005; De Luis et al. 2007; Camarero et al. 2010). The increasing aridity in the Mediterranean region could have important implications for the intra-annual growth dynamics of P. halepensis which could be reflected also in long-term (inter-annual) growth trends in this species. In addition, there are no studies analyzing jointly the intra- and inter-annual long-term responses of earlywood (hereafter abbreviated as EW) and latewood (hereafter abbreviated as LW) formation to climate and drought in P. halepensis forests from semi-arid areas.

In Circum-Mediterranean drought-prone forests, a deeper knowledge on the EW and LW responses to recurrent water shortages at several time scales is important for understanding how amplified precipitation variability will drive radial growth trends (Andreu et al. 2007). In a previous study on growth–drought relationships assessed at multiple time scales, we found that *P. halepensis* growth is highly sensitive to mid-term cumulative drought stress, particularly during summer (Pasho et al. 2011b). In this study, we aim at understanding how drought and climate variability affects the patterns of seasonal wood formation (EW and LW widths) of this species. We aim at providing a mechanistic basis concerning the long-term impacts of drought and climate on EW and LW production by studying xylogenesis processes (periods and production rates of different tracheid types) in a semi-arid *P. halepensis* forest. In addition, the current and post-drought (up to 2 years later) effects on growth of both wood types are investigated to quantify possible lagged effects of water deficit on seasonal wood production.

Our objectives were (1) to quantify the long-term (interannual) effects of climate and drought on EW and LW growth in P. halepensis forests from north-eastern Spain, and (2) to describe the intra-annual xylogenesis processes, including the seasonal wood production, for explaining the differential long-term responses of EW and LW production to climate and drought. The hypothesis of this study was that severe and long-term drought conditions will impact more EW than LW formation since EW tracheids mature when water deficit usually starts and their number and transversal lumen size reflect well the theoretical hydraulic conductivity of the tree, and, consequently its potential capacity to uptake carbon and grow (von Wilpert 1991). We also expect that the drought-induced reduction in EW production and hydraulic conductivity will lead to an indirect decrease in LW formation. The evaluation of these ideas is relevant for predicting the responses of Circum-Mediterranean P. halepensis forests from semi-arid areas to the forecasted aridification trends (IPCC 2007).

Materials and methods

Study sites

The study sites are located in central (Middle Ebro Basin) and southern (Teruel) Aragón, north-eastern Spain (see Table S1 in Supporting Information). The area is characterized by semi-arid Mediterranean continental conditions with a seasonal variability in precipitation and temperature, mostly evidenced in the Middle Ebro Basin (mean annual temperature is 13.1 °C, total annual precipitation is 558 mm) and less pronounced in southern Teruel (mean annual temperature is 10.6 °C, total annual precipitation is 1,021 mm) although summer is the predominant dry season in all studied sites. In the Middle Ebro Basin the water balance is strongly negative, as a consequence of the high potential evapotranspiration which reaches annual values of 1,300 mm in some of the driest sectors of this valley (Vicente-Serrano and Beguería 2003) and up to 800 mm in Teruel sites (Peña Monné et al. 2002). Moreover, the high year-to-year temporal variability in precipitation introduces additional constraints on tree growth, since severe droughts

are frequent and periods of more than 80 days without precipitation are common (Vicente-Serrano and Beguería 2003). Furthermore, some of the forests in the Middle Ebro Basin grow on gypsum substrates which improve water penetration into soil, contributing to local aridity (Navas and Machín 1998).

Pinus halepensis Mill. is the dominant forest tree species in the study sites. This species commonly occurs in semi-arid sites, on the top and slopes of structural platforms developed on Miocene carbonate and marl sediments. The understory species are mainly Mediterranean shrubs and sub-shrubs such as *Quercus coccifera* L., *Rosmarinus officinalis* L., *Linum suffruticosum* L., *Genista scorpius* L. and *Thymus* species. In the less xeric sites (TA, PU, MP, PL and MA) (see Table S1), *P. halepensis* co-exists with other tree species such as *Quercus ilex* L. subsp. *ballota* (Desf.) Samp., *Quercus faginea* Lam., *Juniperus thurifera* L., *Pinus pinaster* Ait. and *Pinus nigra* Arn. subsp. *salzmannii* (Dunal) Franco.

Climatic data

To quantify climate-growth relationships at a local scale, we explored a large database of local meteorological stations and selected 14 of them located near the sampled sites (mean site-station distance is 14.5 km) with the longest climate series. From these stations, monthly and yearly variables of mean temperature (minimum, average and maximum) and total precipitation were collected for the period 1950-2005. We carefully controlled the data quality, including the reconstruction and homogenization processes to guarantee the data reliability. Details on the dataset can be found in Vicente-Serrano et al. (2010b) and El Kenawy et al. (2011). Trends in temperature and precipitation series were analysed by means of non-parametric Spearman's rank correlation test (p < 0.05) because it is less affected by the presence of outliers and non-normality of the series than parametric coefficients (Lanzante 1996).

Drought index calculation

To quantify the impact of drought on EW, LW growth we employed the Standardized Precipitation Index (SPI) (McKee et al. 1993), which has been accepted by the World Meteorological Organization as the universal meteorological drought index for effective drought monitoring and climate risk management (Hayes et al. 2011). The SPI allows determining the duration, magnitude and intensity of droughts at different time scales. The SPI is calculated using precipitation data and it allows identifying the different times of response of hydrological and ecological systems to the precipitation deficit. The SPI was computed by fitting the precipitation series to a given probability distribution. Initially, the SPI was calculated following a Gamma distribution (McKee et al. 1993), but further analyses indicated that the Pearson III distribution was more robust (see Vicente-Serrano 2006; Quiring 2009). We obtained for each forest the SPI at time scales ranging from 1 to 48 months to be compared with radial growth. Values of SPI above and below zero correspond to wet and dry conditions, respectively. Details of the method to calculate the SPI can be found in Vicente-Serrano (2006).

Xylogenesis

On March 2010, ten dominant P. halepensis trees were randomly selected in site PU (see Table S1). They were tagged and their diameter at breast height (dbh) was measured. Then, wood micro-cores (15-mm long and 2-mm thick) were extracted biweekly from April until December using a Trephor puncher (Rossi et al. 2006). Consecutive wood samples were collected 5-10 cm apart from each other, following a spiral up the stem (mean sampling height was 1.3 m) to avoid disturbance reactions caused by cambium wounds. Micro-cores contained at least the previous five tree rings and the developing cambial zone. Samples were placed in Eppendorf tubes containing a fixative solution (formalin-ethanol-acetic acid, 5:90:5). Micro-cores were cut to obtain transversal sections for histological analyses (Antonova and Stasova 1993). Before cutting, each micro-core was vertically oriented by marking the fibre orientation with a pencil under a stereomicroscope at $20 \times$ magnification. We obtained from four to six sections per micro-core with 10-20 µm thicknesses using a sliding microtome (Anglia Scientific AS 200, Cambridge, UK). The sections were stained with 0.05 % cresyl violet. The mounted and fixed sections were examined under a light microscope (Olympus BH2; Olympus, Hamburg, Germany) within 10 min of staining at 100-500 magnification. Images were photographed with a digital camera (JVC 3.3 CCD GC-X3E; JVC, Yokohama, Japan) to verify cell counts and to distinguish EW and LW tracheids according to their lumen and cell wall thickness. To distinguish EW and LW tracheids, first we measured their lumens and double-wall thickness along five radial lines per tree using the November wood samples. Then, we followed Denne (1988) criterion, i.e., LW tracheids were regarded as those whose single cell-wall thickness multiplied by four were greater or equal to their lumen widths.

For each analysed wood sample, the following cell types were distinguished and counted along the radial files: cambial cells, radially enlarging tracheids, wall-thickening tracheids and mature tracheids (Antonova and Stasova 1993). The first cells with evident radial diameter increments, but still containing a protoplast enclosed by a thin

primary wall, were regarded as the first radially enlarging tracheids. The end of radial-cell expansion and the onset of secondary cell wall formation were defined by the appearance of pit borders and cell corner rounding. Tracheid lignification corresponded to a color change from violet to blue. The maturation zone ended when visible traces of cytoplasm were not observed within the tracheid lumen and also when tracheids exhibited completely blue cell walls. The onset of xylem formation was regarded as the date when at least five trees showed more than one row of cells in the cell-enlargement phase. The ending of xylogenesis was considered the date when all trees lacked tracheids corresponding to the wall thickening phase.

Dendrochronological methods

Dendrochronological methods were used to quantify the temporal patterns of EW and LW widths in the studied sites. Forests were selected based on: (1) the dominance of P. halepensis in the canopy over at least 1 ha of fully forested area, and (2) the occurrence of harsh environmental conditions potentially constraining tree growth such as steep slopes, shallow or rocky soils. At each of the nine sampling sites, we randomly selected 15-20 trees within a 1,500 m long and 100 m wide transect randomly located within the stand and measured their dbh (Table S1). Distance between trees was always greater than 10 m to avoid capturing local effects on tree growth due to spatial autocorrelation. Two radial cores per tree were extracted at 1.3 m height using a Pressler increment borer. The cores were prepared following standard dendrochronological methods (Fritts 2001). They were air-dried, mounted and sanded with sandpapers of progressively finer grains until EW and LW were clearly visible under a stereomicroscope. All samples were visually cross-dated and the EW and LW widths were measured to a precision of 0.001 mm (accuracy \pm 0.0003 mm), using a LINTAB measuring device (Rinntech, Heidelberg, Germany). We distinguished EW and LW based on the cross-sectional area of tracheids and the thickness of their walls and following previous dendrochronological and xylogenesis studies on P. halepensis (De Luis et al. 2007; Camarero et al. 2010). The visual cross-dating was evaluated using the COFECHA program (Holmes 1983).

To retain the high-frequency variability of growth, each EW or LW series was double-detrended using a negative exponential function and a spline function with a 50 % frequency response of 32 years. Detrending and standardization involved transforming the measured values into dimensionless indices by dividing the raw values by the expected ones given by the negative exponential and spline functions. Autoregressive modelling was carried out on each series to remove the temporal autocorrelation. The indexed pre-whitened residual series of all trees within each site were then averaged using a biweight robust mean to obtain mean site residual chronologies of ring width, which were used in subsequent analyses. We used the program ARSTAN to obtain the residual site chronologies (Cook 1985).

The characteristics of the chronologies were evaluated for the common period 1970-2005 using several dendrochronological statistics (Briffa and Cook 1990): the mean width and standard deviation (SD) of the EW and LW raw width series; the first-order autocorrelation (AC1) of these raw series, which measures the year-to-year persistence; the mean sensitivity (MSx) of the residual series, which quantifies the relative change in EW or LW width among consecutive years; the mean correlation (Rbar) among individual series within each site; and the expressed population signal (EPS) of residual series, which indicates to what extent the sample size is representative of a theoretical infinite population. The common period 1970–2005 was selected as the interval for which all chronologies showed EPS values above the 0.85 threshold for this interval and were considered being well replicated (Wigley et al. 1984).

Statistical analyses

The climate-growth relationships were quantified using the residual EW and LW chronologies of each site and the detrended climate series of monthly mean temperatures, mean minimum and maximum temperatures and total precipitation. Correlations were computed over the common period 1970-2005 using a 15-month window from August of the year prior to growth (year t - 1) until October of the year of tree-ring formation (year t). According to the previous studies, this window encloses the most influential period for radial growth of P. halepensis (De Luis et al. 2007; Camarero et al. 2010). We also obtained the percentage of EW and LW growth variability explained by climate (minimum, average, maximum monthly temperature and monthly precipitation) in each site (R^2 adj, adjusted R^2) through multiple linear regressions, based on a stepwise forward selection of significant variables ($P \le 0.05$), calculated between EW and LW indices and the local monthly climatic data.

To quantify the impact of drought severity on tree growth at different time scales, we performed superposed epoch analysis (SEA) (Haurwitz and Brier 1981) and correlation analyses with SPI based on the Pearson's coefficient. SEA was used to detect the drought impacts on EW and LW formation by calculating deviations in EW or LW width 2 years after selected droughts. According to SPI data, we selected four dry years (1967, 1986, 1994 and 2002) with values close to SPI = -1, i.e., experiencing mild to severe drought. We assessed the significance of mean tree growth response to these extremely dry years (Orwig and Abrams 1997). We used the software *Resampling Stats* to select 10,000 random sets of 3 years from each EW or LW width series and to estimate the confidence intervals (P < 0.05) for the growth deviations (Bruce 1991).

Before assessing the correlations between SPI and EW or LW width indices, the trend in each SPI time series was removed by fitting a linear tendency in each monthly series at the different time scales (for details see Pasho et al. 2011a). This step avoided that possible SPI trends could disrupt potential relationships. Then, correlation analyses were performed for the period 1970–2005 between EW and LW residual chronologies representing forest growth at sampled locations and detrended monthly series of 1 to 48 months SPI representing drought severity. The threshold for significant correlations was set at $P \leq 0.05$ for all correlation analyses.

The periods of maximum intra-annual growth were identified by transforming the cumulative number of EW and LW tracheids into daily rates of tracheid production (cells day⁻¹). This was achieved by dividing the difference of the total number of tracheids formed in successive dates (ΔN) by the time interval (Δt) between both dates (Camarero et al. 1998).

Results

Climatic trends

The annual climatic series showed opposite trends for annual mean temperature (increasing) and annual precipitation (decreasing) since 1950, suggesting an aridification trend in the study area during the last half of the twentieth century (see Fig. S1, Supporting Information). The mean annual temperature rose significantly ($P \le 0.05$) between +0.01 and +0.03 °C in all sites while the annual precipitation declined from -0.75 to -2.88 mm year⁻¹, although this decrease was not significant. Warm and dry conditions prevailed in the 1960 s, 1980 s and 1990 s, whereas low temperatures were recorded in the early 1970 s. Minimum annual precipitation values were detected in the period 1991–1995 for most sites but similar low water records also occurred before (e.g., 1985).

Growth patterns

The mean width values of EW varied among sites more than those of LW (EW, 0.68–1.91 mm; LW, 0.18–0.54 mm) (Table 1). The average values of AC1 and MSx were higher for the EW (AC1 = 0.65, MSx = 0.44) than for the LW (AC1 = 0.53, MSx = 0.43) chronologies. Similar results were obtained for Rbar and EPS, considering EW (Rbar = 0.70, EPS = 0.93) and LW (Rbar = 0.56, EPS = 0.89) width series. Thus, EW chronologies showed a greater year-to-year persistence (AC1), a higher relative change between consecutive years (MSx) and a higher common signal (Rbar, EPS) than LW ones. The within-site correlation between EW and LW series varied from 0.38 (site PL) to 0.75 (site CS) and it was always significant ($P \le 0.05$). On an average, 32 % of LW width variability was related to EW width variability.

We observed similar high-frequency growth patterns for EW and LW width series with growth reductions in 1955, 1958, 1962, 1981, 1986, 1994, 2000 and 2005 (Fig. 1). The periods characterized by substantial increase in EW and LW width corresponded to the years 1954, 1959, 1977, 1988, 1997 and 2004. These sharp growth decreases and increases coincided with dry (SPI < 0) and wet conditions (SPI > 0), respectively.

Climate-growth relationships

On average the proportion of growth variance explained by climate was generally higher for EW (62.7 %) than for LW (45.3 %) and varied among sites (Table 1). Mean correlation coefficients revealed positive responses of EW growth to current spring and summer precipitation (April, May and June) and negative ones to temperature variables, particularly to mean maximum temperatures in May, June and July (Fig. 2). Wet conditions and high minimum temperatures in the previous December and the current January favored EW development whereas LW growth was also enhanced by high minimum temperatures in January.

Intra-annual dynamics of wood formation

Climatic conditions during 2010 were within the range of the local long-term climatology based on the data from a nearby meteorological station with mean annual temperature of 15.3 °C (long-term mean = 15.0 °C) and total precipitation of 267 mm (long-term mean = 327 mm) (see Fig. S3A in the Supporting Information). Considering monthly values in 2010, April and July temperatures and April rainfall amount were above long-term mean values, whereas May precipitation was below climate normal periods. Xylem formation had already started in March and xylogenesis was active until November (Fig. 3). The cambial activity followed a unimodal pattern, with two major peaks (May-June and mid July-August) of tracheid formation corresponding respectively to the enlargement and wall-thickening phase (Fig. 3). These two phases match with the periods of maximum EW and LW formation in that order (Fig. 4). The rate of EW tracheid production was higher (0.38 cells day^{-1}) than that of LW

Table 1 Dendrochronological statistics of earlywood (EW) and latewood (LW) P. halepensis chronologies for the common period 1970-2005

Site	No. trees (No. radii)	Period	Correlation EW–LW	Earlywood					Latewood						
				MW ± SD (mm)	AC1	MSx	Rbar	EPS	<i>R</i> ² adj (%)	MW ± SD (mm)	AC1	MSx	Rbar	EPS	<i>R</i> ² adj (%)
VM	17 (30)	1959–2009	0.61	1.39 ± 0.81	0.59	0.44	0.80	0.99	80.30	0.39 ± 0.21	0.52	0.47	0.56	0.95	72.45
CV	13 (23)	1928-2009	0.47	1.75 ± 0.94	0.75	0.38	0.47	0.89	82.85	0.54 ± 0.33	0.71	0.34	0.50	0.86	64.06
CS	13 (23)	1900-2009	0.75	0.68 ± 0.37	0.41	0.47	0.64	0.97	40.04	0.18 ± 0.10	0.39	0.44	0.62	0.91	18.33
VA	17 (31)	1925-2009	0.74	1.28 ± 0.75	0.62	0.43	0.68	0.92	54.01	0.37 ± 0.22	0.58	0.43	0.51	0.86	41.92
TA	14 (27)	1927-2006	0.57	1.91 ± 1.22	0.59	0.50	0.78	0.97	44.68	0.43 ± 0.24	0.35	0.48	0.52	0.85	24.60
PU	15 (22)	1943-2009	0.58	0.85 ± 0.43	0.60	0.38	0.69	0.85	50.10	0.30 ± 0.16	0.56	0.40	0.54	0.88	29.10
MA	15 (28)	1963-2009	0.60	1.49 ± 1.27	0.75	0.53	0.81	0.94	64.51	0.38 ± 0.26	0.53	0.46	0.69	0.93	46.54
PL	15 (30)	1961-2009	0.38	1.35 ± 1.08	0.73	0.49	0.79	0.94	70.67	0.39 ± 0.25	0.53	0.44	0.61	0.92	50.87
MP	14 (29)	1965-2009	0.52	1.53 ± 1.26	0.81	0.37	0.68	0.93	77.61	0.38 ± 0.22	0.61	0.37	0.49	0.90	59.49
Mean	-	-	0.58	1.36 ± 0.86	0.65	0.44	0.70	0.93	62.75	0.37 ± 0.22	0.53	0.43	0.56	0.89	45.26

Statistics: EW-LW Pearson correlation coefficient calculated between the residual earlywood and latewood chronologies for each site. Raw-data series: MW mean width, SD standard deviation of width, ACI first-order autocorrelation. Residual series: MSx mean sensitivity, Rbar mean interseries correlation, EPS expressed population signal, R^2adj adjusted R^2 obtained relating monthly climatic variables and EW, LW residual chronologies through stepwise linear regressions. The last line provides the mean values for all sites, considering statistics calculated for raw ring-width data (MW, SD, AC1) and residual chronologies (MSx, Rbar and EPS)

(0.22 cells day⁻¹). The EW tracheid formation started in late March and finished in September in most trees whereas the first LW tracheids were formed in July and the last ones were observed in November (Fig. 3).

Drought-growth relationships

The SEA revealed differences in the annual deviations of EW and LW widths in response to drought. Significant decreases in EW width were observed in five out of nine sites during the year of drought whereas only one site showed a significant reduction in LW width 1 year after the drought (Table 2). The mean deviations in EW and LW widths during the drought year were -0.48 and -0.31, respectively, whereas they showed similar responses up to 2 years after the drought.

The correlations between EW and LW growth indices and the different SPI time scales reached maximum values at similar time scales (10-14 months), despite significant correlations were found up to 48 (EW) or 35 (LW) months (Fig. 4). The strongest responses of EW to drought intensity (r = 0.70-0.72) were observed in July, whereas LW responded strongly (r = 0.54) to SPI September values. We also detected certain variability among sites in the EW and LW growth responses to drought (Fig. S2). The strongest response of EW growth to drought was found for the sites VM, CV, CS, VA, whereas the sites TA, PU, MA, PL and MP were less affected by drought. The same pattern was also observed with respect to LW growth. The months when we detected the highest responses of EW and LW width to drought at inter-annual scales coincided with those in which we observed low production rates of EW (July)

and LW (August–September) tracheids at intra-annual scales (Fig. 4). Finally, the maximum production rates of EW (May–June) and LW (mid July–August) tracheids occurred approximately one month before the strongest impact of drought on inter-annual EW and LW growth series was detected.

Discussion

In this study, we evaluated the impact of climatic drivers and drought stress on earlywood (EW) and latewood (LW) formation at inter- and intra-annual scales in P. halepensis forests from semi-arid areas. Climatic factors and drought highly impacted both components of the tree ring in the short and long terms, particularly EW production. At the intra-annual scale, the EW production was enhanced by relatively high rainfall amounts in April, May and June, whereas the production of radially enlarging tracheids, which are closely linked to the radial-growth rate (Larson 1994), was sharply reduced during the dry period (July-August) (see Fig. S3B in the Supporting Information). Interestingly, the LW tracheid production was intensified as a response to high summer temperatures and low water availability. These climatic conditions are linked to the cessation of EW tracheid production and may trigger the formation of LW tracheids and enhance cell-wall lignification as reported previously (von Wilpert 1991; Jyske et al. 2010).

In drought-stressed *P.halepensis* forests, water availability constrains the rate of EW tracheid production while temperature can be considered as the main climatic driver

Fig. 1 Residual chronologies of P. halepensis earlywood (EW) and latewood (LW) width (gray lines) for the studied sites and their overall means (lines with empty circles). Black lines with triangles show the evolution of the drought index (SPI, mean of all sites) at time scales of 12 months in July and September, the scale at which EW and LW growth series responded more strongly to the SPI drought index. Note that positive and negative SPI values indicate wet (high EW and LW indices) and dry conditions (low EW and LW indices), respectively. Selected dry years (1967, 1986, 1994 and 2002) for the study of growth deviations are *encircled* in the *upper graph*



determining the onset and ending of tracheid production (Camarero et al. 2010). The low cambial activity of *P. halepensis* in summer appears to be triggered by high temperatures and the decrease of precipitation, i.e. low soil water availability (Camarero et al. 2010). However, the ability to maintain tracheid production, although at a reduced rate, during the dry summer months (Serre-Bachet 1992) is most likely due to the high hydraulic activity and the deep root system of *P. halepensis* which enables it to make use of deep soil water reserves (Borghetti et al. 1998). The continuation of LW production also in late November is most likely favoured by wet conditions and mild temperatures in autumn as found in a previous study (Camarero et al. 2010).

The decrease in EW and LW production rates in July and August–September respectively, indicates that *P. halepensis* reduces tracheids formation when precipitation deficit surpasses a functional threshold. As a matter of fact, this relationship was also observed in the responses of EW and LW growth to long SPI time scales indicating that there may be a synchronized response of intra- and inter-

annual growth in this species to drought conditions. This response to drought stress might be linked to the physiological and growth adjustments of this species to cope with the scarcity of soil water, including a decline in photosynthesis rates, carbon uptake, needle elongation and wood formation as shown previously (Borghetti et al. 1998; Camarero et al. 2010).

At the inter-annual scale, the regression analyses indicate that EW and LW series are responding to the limitations imposed by the climatic factors as shown by the relatively high values of adjusted R^2 , particularly in the case of EW series. Comparing EW and LW chronologies, the former showed a greater year-to-year variability (MSx) and a higher tree-to-tree common variance (Rbar) than the later did. However, the EW and LW series presented a strong within-ring correlation indicating a dependence of LW formation on previous EW growth. In general, the EW and LW chronologies of the nine sampled sites showed a strong common signal related to climatic factors, mostly in response to spring and early summer rainfall amounts. This is particularly the case of EW, whose formation in **Fig. 2** Mean (\pm SE) correlation coefficients calculated between earlywood and latewood width chronologies of the nine study sites and monthly climatic variables (mean temperature, mean maximum and minimum temperatures, and total precipitation). Growth is related with climate data from the previous August to current October of EW and LW formation. The significance level ($P \le 0.05$) is indicated by *dashed horizontal lines*



P. halepensis sites from semi-arid areas usually peaks in that period. This means that water availability in the period when the maximum rates of EW tracheid formation occur is among the most critical factors for radial growth with cascading effects on water conduction and carbon uptake. An improved production of EW tracheids with wide lumens in response to wet spring conditions may result in an increase of hydraulic conductivity and photosynthesis leading to the synthesis of more carbohydrates for LW formation in summer and autumn (Camarero et al. 2010).

The positive (negative) correlation between EW growth and April, May and June precipitation (maximum temperatures) suggest that this growth component of the ring may be very susceptible to water deficits during the early part of the growing season. High temperatures increase water deficit which affects photosynthetic and tracheid division rates, constraining radial growth (Hsiao 1973; Linares and Tiscar 2010; Vicente-Serrano et al. 2010a). The high sensitivity of EW to maximum July temperatures indicates that growth decline of the study species may be caused by warming-induced drought stress. On the other hand, the positive association of EW growth with December and January minimum temperature and precipitation suggests that warm minimum (night) temperatures and wet conditions in winter can favour an earlier and longer growing season for *P. halepensis* as reported previously (De Luis et al. 2007; Camarero et al. 2010). The only explanation we found for the significant positive influence of January minimum temperatures on LW development is based on a direct improvement of EW



Fig. 3 Number of cells of *P. halepensis* according to their development phase (cambial cells, radially enlarging tracheids, wall-thickening tracheids and mature tracheids—earlywood and latewood tracheids are shown as bars with different fill types) formed during the year 2010 in the site PU. The image shows the earlywood (EW) and latewood (LW) of a cross-section of a ring (total width = 1.22 mm) from a wood sample taken in mid November. Data are means (n = 10 trees)

formation leading to an indirect LW enhanced growth. This indicates that the LW growth in *P. halepensis* is not only determined by weather conditions during the wall thickening process, mainly occurring in summer and autumn (Camarero et al. 2010), but it may be also modulated by the previous EW growth rates as shown by the high correlation between them.

The strong sensitivity of EW and LW growth to cumulative drought stress in July and September, respectively, indicates that summed (10-14 months) precipitation amounts during those periods are crucial for the development of both components of the tree ring. The EW growth appeared to depend more on water availability than LW did, showing two distinct patterns in terms of growth sensitivity to drought in P. halepensis. These patterns varied among sites indicating changing growth responses of P. halepensis EW and LW growth to different rainfall regimes at local scales. For instance, there was variability in terms of the EW and LW responses to drought when comparing the less xeric sites (TA, PU, MA, PL and MP) vs. the most xeric ones (VM, CV, CS, VA) showing a more intense and prolonged impact of drought on the formation of both wood types in the later as compared to the former sites. The higher impact of drought on EW growth as compared to the LW was also confirmed by the SEA analysis which clearly indicated significant EW growth reduction in most sites during the year of drought occurrence, a pattern that was not observed in the case of LW. The growth of both components during the 2 years after selected droughts did not significantly decrease, most likely due to soil water recharge after the drought event in those sites. There was an exception to this in site VA, where drought negatively impacted LW production 1 year after the drought possibly because of lagged effects on carbohydrates synthesis and growth or due to a poor waterholding capacity of soils.

P. halepensis is considered as a drought-avoiding species (Ferrio et al. 2003), but its inter-annual growth is very sensitive to lasting and severe droughts as our results support. Moreover, the vulnerability to drought may be aggravated by the soil types (e.g., gypsum) which may additionally intensify the negative effects of drought on P. halepensis growth. In the study area, a pronounced decline in winter and spring precipitation has also been observed during the late twentieth century (González-Hidalgo et al. 2009) which has negatively influenced the EW formation, leading to denser wood, i.e. with less EW in relative terms, and thus causing a reduction in hydraulic conductivity but increasing the resistance to droughtinduced xylem cavitation (Eilmann et al. 2011). Even if drought-stressed pines are able to form tracheids with wider lumens than non-stressed pines as it was shown by Eilmann et al. (2011), the production of less EW will undoubtedly induce a decrease in hydraulic conductivity and, it is plausible that this will lead to local growth declines in the long term and a reduction in carbon uptake at regional scales.

Fig. 4 Mean correlation coefficients of earlywood and latewood width chronologies and the SPI drought index calculated at different time scales (1–48 months) from January up to December for the nine study sites. *Bold lines* frame significant correlations ($P \le 0.05$). The *right area figure* shows the calculated daily rates of earlywood and latewood tracheids production in *P. halepensis* during the year 2010 (see also Fig. 3)



Table 2 Annual earlywood and
latewood width deviations
observed up to 2 years after the
occurrence of a severe drought
(year 0) (deviations were
calculated in response to
selected severe droughts (1967,
1986, 1994 and 2002; see
Fig. 1); significant ($P \le 0.05$)
values are in bold)

Site	Earlywood			Latewood				
	Year 0	Year 1	Year 2	Year 0	Year 1	Year 2		
VM	-0.56	-0.18	0.28	-0.25	-0.12	-0.06		
CV	-0.34	-0.24	0.16	-0.30	-0.05	0.24		
CS	-0.46	-0.04	0.23	-0.37	0.05	0.18		
VA	-0.35	-0.21	-0.14	-0.28	-0.52	0.17		
TA	-0.74	-0.42	0.01	-0.28	-0.18	-0.01		
PU	-0.20	-0.26	0.09	-0.11	-0.26	-0.08		
MA	-0.41	0.35	0.03	-0.35	-0.07	0.02		
PL	-0.85	-0.43	-0.28	-0.44	-0.20	0.08		
MP	-0.45	-0.15	-0.25	-0.38	-0.26	0.23		
Mean	-0.48	-0.18	0.04	-0.31	-0.18	0.09		

We conclude that despite P. halepensis is considered among the most drought-resistant Circum-Mediterranean conifers, its seasonal wood formation appears to be driven negatively by mid-term cumulative drought stress in semiarid areas. In the short term, these influences were associated with a reduction of tracheid production, whereas in the long term they caused declining EW and LW formation. Therefore, under expected decrease of winter and spring water availability in a warmer future world as predicted for continental areas in the Western Mediterranean Basin (IPCC 2007), the studied forests may show a more intense reduction in the production of EW tracheids than in the case of LW ones. This selective reduction in EW formation may lead to several cascading effects, namely a decline of radial growth (including LW formation) a reduction in hydraulic conductivity and, indirectly, a hampered carbon uptake under semi-arid conditions.

Acknowledgments Edmond Pasho thanks the financial support of the Albanian Ministry of Education and Science. This work has been supported by the research projects CGL2008-01189/BTE, CGL2008-04847-C02-01/BOS, CGL2011-27574-CO2-02, CGL2008-05112-C02-01/BOS CGL2011-27536 and CGL2011-26654 financed by the Spanish Commission of Science and Technology and FEDER, and EUROGEOSS (FP7-ENV-2008-1-226487) and ACQWA (FP7-ENV-2007-1-212250) financed by the VII Framework Program of the European Commission. JJC thanks the support of ARAID. We thank the support in the field of J.M. Gil and V. Pérez Fortea and the Spanish "Agencia Estatal de Meteorología" for providing the meteorological data.

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Supporting Information



Figure S1. Long-term trends in (A) mean annual temperature and (B) total annual precipitation in the studied sites. The statistics corresponding to the fitted linear regressions are also displayed. See sites' codes in Table S1.



Figure S2. Pearson Correlation coefficients obtained relating earlywood (left) and latewood (right) width indices *vs.* the monthly drought index (SPI) calculated at cumulative scales (1-48 months) from January up to December in the nine study sites. Bold lines frame significant correlations ($P \le 0.05$).



Figure S3. A. Climatic conditions (mean monthly temperature and total precipitation) at the site PU during the year 2010 as compared with historical data (period 1951-2011, boxplots showing the median values and outliers) from the Zaragoza-Aeropuerto meteorological station (latitude 41° 39' N, longitude 1° 00' W, elevation 263 m a.s.l.) located at ca. 24 km. **B.** Climatic diagram of Zaragoza-Aeropuerto station based on 1951-2011 data.

Site (code)	UTM X (m)	UTM Y (m)	Elevation (m)	Dbh (cm)	Temperature (ºC) ^a	Precipitation (mm) ^a
Valmadrid (VM)	672553.01	4590958.15	648	15.4 ± 0.4	12.2	537
Castejón de Valdejasa - Zuera (CV)	669056.80	4650279.64	565	18.8 ± 0.7	12.3	590
Castejón de Valdejasa (CS)	673445.71	4640263.00	498	22.4 ± 0.4	13.2	607
Valareña (VA)	636953.56	4661620.32	520	19.4 ± 0.4	13.3	635
Alcubierre-San Caprasio (TA)	707892.44	4624573.14	738	20.0 ± 0.5	12.4	650
Alcubierre- Peñaflor (PU)	707340.29	4632588.29	560	15.4 ± 0.5	15.0	327
Mirador de la Atalaya (MA)	695720.99	4445857.40	907	20.4 ± 0.3	11.0	1004
Pileto (PL)	695732.69	4446320.55	963	19.7 ± 0.2	10.6	1004
Mas de Puntar (MP)	698042.03	4453076.62	967	17.5 ± 0.3	10.3	1056

 Table S1. Characteristics of the nine study sites.

^aClimatic data are annual values and they were obtained from meteorological stations located at similar elevation and at less than 25 km from the closest sampling site. Station temperature data were corrected as a function of the elevation differences with sampling sites using a lapse rate of -0.6^o for each altitudinal increment of 100 m.