Ecophysiological basis of wood formation in ponderosa pine: Linking water flux patterns with wood microdensity variables

Alejandro Martinez-Meier [a,*], María Elena Fernández [b,c], Guillermina Dalla-Salda [a], Javier Gyenge [b,c], Julián Licata [b,d], Philippe Rozenberg [e]

[a] INTA, EEA Bariloche, Grupo de Ecología Forestal, San Carlos de Bariloche, Río Negro, Argentina
[b] CONICET, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina
[c] INTA, EEA Balcarce – Oficina Tandil, Tandil, Buenos Aires, Argentina
[d] INTA, EEA Concordia, Concordia, Entre Ríos, Argentina
[e] INRA, UR0588 Unité d'Amélioration Génétique et Physiologie Forestières, Orléans, France

ABSTRACT

Climate and stands management have an effect on the process of wood formation via resource availability. To improve knowledge of wood quantity and physical structure, increment cores of ponderosa pine were taken in high- and low-density stands growing in Patagonia, Argentina. Microdensity profiles were performed on trees for which sapflow density (Q_s) data were available over two consecutive years (2004 and 2005, dry and wet years, respectively). Conventional and non-conventional microdensity variables were analyzed in the segment of the microdensity profile where Q_s was measured. Trees in the low-density stand showed a greater capacity for water transport than trees in the high-density stand, even if no constraints in the soil water availability were confirmed, as was the case for the wet year. Minimum and earlywood density were significantly higher in the high-density stand in several analyzed years. These differences were not reflected in mean density of the whole radial profile. The structural changes in physical wood properties in relation to silvicultural treatments appear to affect plant water status, even in the wet growing season: greater wood density in the first part of the tree-ring may contribute to the observed Q_s decrease found in the high-density stand. This fact may lead trees to lack the coordinated mechanisms of response to drought observed in more xeric sites. The increase of wood density in the first part of the tree-ring in ponderosa pine could be not conducive to acclimation, exposing trees to an increasing "dilemma" facing drought.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Wood formation – in terms of quantity and physical structure – may be affected by environmental conditions, including climate, resource availability and silviculture, as well as cambial age (Zobel and Sprague, 1995). Different wood structures can lead to differences in water conduction capacity, which in turn would affect plant responses to environmental conditions (Hacke and Sperry, 2001). Structural changes in wood as a consequence of different silvicultural schemes could have an impact on tree resilience to adverse climatic conditions during the growing season.

The adaptive meaning of wood in relation to drought resistance has been studied across different species (Hacke et al., 2001; Domec and Gartner, 2002; Sperry et al., 2008). Several authors

(Stratton et al., 2000; Hacke et al., 2001; Bucci et al., 2004) describe the relevant role of the relation between cell lumen and wall thickness in the xylem, which could help trees to preserve their hydraulic system (Sperry et al., 2006; Pittermann et al., 2006). Paradigm-changing studies such as those of Pockman and Sperry (2000) and Maherali et al. (2004) have shown that resistance to cavitation is a key trait related to species distribution across aridity gradients. However, this does not mean that high resistance to cavitation is a necessary trait to overcome arid conditions. Some species have alternative mechanisms to a high resistance to cavitation xylem, e.g., maximizing water conduction capacity under dry conditions in order to avoid high xylem tensions. This appears to be the mechanism developed by *Pinus ponderosa (Dougl. ex Laws)*, a North-American tree species capable of occupying very dry environments (Barnard et al., 2011; Domec and Gartner, 2003; Waring et al., 1982). Studies in native populations indicate that the capacity of ponderosa pine to cope with drought is not directly related with changing its resistance to cavitation, which in turn is

rather low in relation to other Pinaceae (Martínez Vilalta et al., 2004), but with changes in hydraulic conductance at the whole tree level (Maherali and DeLucia, 2000).

An increase in hydraulic conductance in xeric environments compared to wetter areas has been observed as a result of changes in Huber ratio (leaf area/sapwood area) and specific hydraulic conductivity of wood (Maherali and DeLucia, 2000, 2001; Tyree and Ewers, 1991). Efficiency of xylem conductivity seems to be related to larger tracheid lumen diameters (Maherali and DeLucia, 2000) and lower wood density (Bouffier et al., 2003). The aforementioned studies have examined different populations growing in different sites under different environmental conditions. However, a lack of knowledge still exists concerning how constraints in water availability affect wood structure in this species, and how such structural adjustments can, in turn, affect functional traits more closely related to an adaptive response.

In previous studies, Fernández et al. (2012) and Gyenge et al. (2012) suggested that wood anatomy is a highly conservative trait in this species, in spite of the different water status of the plants arising from different intra-specific competition levels. In this study, we aim to infer the relationship between ring wood density and environmental conditions, extending the analysis to different years (tree-rings) and silvicultural treatments (high- and low-density stands), by means of X-ray microdensity profiles. Increment cores for wood density analyses were taken from the same trees where sapflow density patterns (Q_s) were measured during two growing seasons, in order to improve our knowledge about how trees react to management (silviculture) and climate, taking into account the water status of the trees. The implications of the physical structure of wood on trees' xylem hydraulic properties are discussed taking into account the mechanism allowing ponderosa pine to cope with drought.

2. Material and methods

2.1. Study site and plant material

The studied plant material came from two different stands (one high- and one low-density stand) of ponderosa pine located in Meliquina Valley (40°29′S–71°13′W, Patagonia, Argentina), planted in 1978 and 1979, respectively. The small differences in cambial age were considered to have negligible effects over microdensity variables of the studied tree-rings. The high-density stand was planted at 3 m × 3 m (1100 trees ha^{-1}). The low-density stand was installed at an initial density of 500 trees ha^{-1} (4 m × 5 m) and then thinned to 350 trees ha^{-1} five years before increment cores were taken. At the thinning time, canopies were still open. Thus, it is assumed that the low-density stand trees grew with null or very low level of intra-specific competition throughout their lifespan. Ten trees were sampled in the high-density stand and 13 trees were sampled in the low-density stand. These trees had complete series of Q_s data available for two consecutive growing seasons: 2004–2005 and 2005–2006. As has been well described in Licata et al. (2008), these growing seasons may be considered dry (70 mm) and wet (225 mm) respectively, representing ±1 standard error of the mean precipitation value from November to April, according to a 23 year-long climate time series obtained from a meteorological station belonging to the National Meteorological Service of Argentina located 70 km south of the study site.

The tree-rings are identified in this study with the first calendar year corresponding to the period of wood formation (i.e., the growth ring formed in the Southern Hemisphere growing season 2004–2005 is named 2004).

2.2. Environmental variables: soil water content and potential evapotranspiration (PET)

Soil water content was continuously measured in the study plots during 2004 and 2005 (dry and wet years, respectively) with multiple sensors of frequency domain capacitance probes (Sentek EnviroSMART, Sentek Pty Ltd., Adelaide, Australia) (Licata et al., 2008). Soil water content data were recorded every 15 s (sec.), and 30 min (min.) averages were stored in a CR10X Campbell Scientific datalogger. The Sentek measurement system employs long probes, which are inserted into 5.5 cm diameter PVC casings. The casings, in turn, are installed in holes augured into the soil. Probes were 2 m long, with six sensors per probe at 10, 20, 40, 80, 140, and 180 cm deep. From this data, daily average soil water content was determined for each sensor (four probes in each canopy treatment in 2004 and three probes in each treatment in 2005). For the purpose of this study, daily averages for the whole soil profile (0–180 cm depth) from each treatment were used. For details about soil water content in each soil layer, see Licata et al. (2008). One composite soil sample from each site was used to determine standard parameters of water retention curves (soil water moisture at 0.03 and 1.5 MPa) for 0–40, 40–80 and 80–120 cm soil layers at the INTA EEA Bariloche Soil Laboratory (Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Bariloche). Water retention parameters were acquired using the methodology described by Stackman (1980) and Hillel (1984).

The FAO Penman–Monteith method was used to calculate evapotranspiration reference values (ET$_{ref}$) following the procedure detailed in FAO Irrigation and Drainage Paper 56 (Allen et al., 1998) for daily climatic data. ET$_{ref}$ represents the maximum PET of a hypothetical reference crop with no water deficiency, growing under the same meteorological conditions as measured in the field site. Meteorological data were acquired from a meteorological station (Campbell Scientific; Logan, UT, USA) installed seven km from the study plots. As mentioned above, climatic data (23 years of monthly precipitation averages) were obtained from a meteorological station belonging to the National Meteorological Service of Argentina located at the Bariloche airport (70 km south of the study site).

2.3. Sapflow density

Sapflow density (Q_s) is the amount of water that passes through a standardized wood area (sapwood area in this case) per unit of time. It is a physiological variable that can be continuously measured and logged by different methods, and may serve as the key variable needed to estimate whole plant transpiration, canopy conductance and carbon fixation. In this study, Q_s was continuously measured by Granier's method (Granier, 1987) during the dry (2004) and wet (2005) growing seasons, in trees that were subsequently sampled to obtain wood microdensity profiles (10 trees in the high-density stand and 13 in the low-density stand).

Two cm-long sensors were installed in the outermost part of the xylem at 1.4 m height, on the southern side of the stems. All sensors were replaced between the two measurement years. A ring of thermal and radiation insulation (Mexpol®, 9 mm) was placed around the stems, covering the sensors and at least 20 cm above and below them, to avoid any thermal gradient effects on the stems. Sensor output was measured every 15 s and averaged every 30 min with a Campbell CR10X data-logger and an AM416 multiplexer. The data were analyzed on a daily time-step and were transformed into sapflow density on a sapwood area basis (Q_s) following Granier (1987). Daily transpiration estimated from these data, taking into account the decrease in sapflow density along the wood radial profile, can be consulted in Licata et al. (2008).

For the purpose of this study, the maximum sapflow density of the radial profile, that is, the Q_s of the outermost part of the stem xylem, was used to analyze the relationship of plant water status and wood microdensity (see below).

In order to separate the effects of soil water content from those of PET on Q_s, relative changes in this variable were compared to relative changes in PET according to the following index:
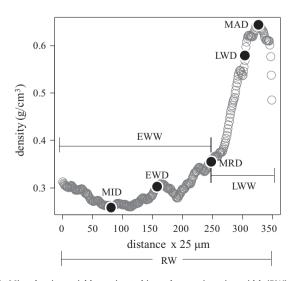
$$RCI = PET_{rel} - Q_{srel}$$

where RCI is the Relative Change Index, PET_{rel} is the maximum PET of the corresponding growing season minus the PET in each day; Q_{srel} is the maximum Q_s in the corresponding treatment and growing season minus the Q_s in each treatment and day. A positive RCI value indicates that Q_s has fallen proportionately more than the evaporative demand, suggesting additional hydraulic limitations to water movement. A negative RCI value indicates that Q_s decreased proportionately less than the evaporative demand.

2.4. Wood microdensity

Microdensity variables were obtained from increment cores (one 5.15 mm sample per tree) taken where the Q_s sensors were previously placed. Twenty-three samples (10 in the high- and 13 in the low-density stand) were then processed, interpreted and analyzed using indirect X-ray densitometry (Polge, 1966) and the resulting X-ray films were scanned. The digital images were processed with WinDENDRO software (Guay et al., 1992), obtaining a final spatial resolution of 25 μm.

The complete microdensity profiles were used to estimate mean stem density and diameter growth for each tree following the methodology described in Rozenberg et al. (1997). Microdensity variables were computed yearly, using a function written in R language (R Development Core Team, 2014). We described tree-rings using nine microdensity variables (Fig. 1): ring width (RW), mean ring density (MRD), earlywood width (EWW), minimum ring density (MID), earlywood density (EWD), earlywood proportion (EWP), latewood width (LWW), maximum ring density (MAD) and latewood density (LWD).

Fig. 1. Microdensity variables estimated in each year-ring: ring width (RW), mean ring density (MRD), earlywood width (EWW), minimum ring density (MID), earlywood density (EWD), latewood width (LWW), maximum ring density (MAD) and latewood density (LWD). Earlywood and latewood densities were taken as the average density of their respective ring portion. Earlywood proportion (EWP), not shown, is calculated as the proportion of earlywood width (EWW) over the total ring width (RW).

While it is known that ponderosa pine typically can have sapwood width deeper than two cm, in this work we focused on the relationship between Q_s and microdensity in the wood section in which the two-cm-long sensors were inserted. For this reason a two-cm-long segment from the microdensity profile of each tree was examined (Fig. 2a). This segment accurately describes the microdensity variation in the small wood portion in which the sensor was inserted. Because this segment does not contain a fixed number of complete rings, we followed the method proposed by Rozenberg et al. (1999) and Ruiz Diaz Britez et al. (2014) to compute microdensity variables. The segment was vertically divided into high- and low-density parts using a moving density criterion (density threshold). The density threshold values ranged from 0.25 g/cm³ to 0.80 g/cm³ with a 0.05 g/cm³ step. Then, for the high- and low-density parts of the microdensity segment, non-conventional microdensity variables were computed for each of the density threshold values: the width of the segment under the density threshold ($SegWidthUnder_{th}$), the mean density of the part under the density threshold value ($MeanDenUnder_{th}$) and the mean density of the part over the density threshold ($MeanDenOver_{th}$) (Fig. 2b). Additionally, minimum, mean and maximum Q_s for each tree in each growing season were computed. The area under the curve of the daily Q_s variation over the course of the growing season was calculated and used as an indication of the cumulative Q_s of each tree during the growing season (Fig. 2c).

2.5. Statistical analysis

A descriptive procedure was used to compare soil water content during the two growing seasons in each treatment. Daily differences in Q_s between the treatments (high-density stand vs. low-density stand) were tested for significance by t-test ($p < 0.05$) in each of the growing seasons. A similar t-test ($p < 0.05$) procedure was used for mean stem density (estimated by weighted mean tree-ring wood density by the corresponding basal area), diameter growth and all of the tree-ring microdensity variables (see Fig. 1). The sampling unit used was each tree corresponding to a high- or low-density stand.
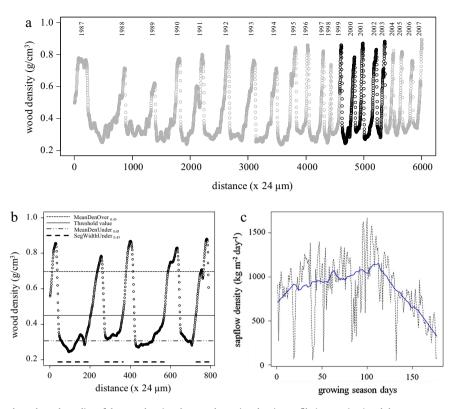
The Q_s sensors were installed during late winter 2003 and 2004. Given their length, they would have sensed both the previous and following years, which was considered in the analyses. We compared the microdensity variables of the 1999 tree-ring (corresponding to the beginning of the two-cm-long segment in the high-density stand) with those of the 2006 tree-ring.

Statistical analyses were performed using R software (R Core Team, 2014). Relationships between Q_s variables (minimum, maximum, mean and area) and microdensity variables of the 2 cm segment where the sapflow sensors were installed were calculated using the Pearson correlation coefficient by means of the cor.test function in R (R Core Team, 2014).

3. Results

3.1. Environmental variables

According to differences in precipitation levels registered during the growing seasons, soil water content varied between years and treatments. Fig. 3(panels a and b) shows the soil water moisture at 1.5 MPa for each type of soil (horizontal black line for high-density stand and horizontal grey line for low-density stand). In both treatments this value was reached at a similar date during the dry growing season but at different dates during the wet growing season. During the dry year (2004), the soil profile reached the water moisture at 1.5 MPa during the first week of January in both treatments and from then on, soil water content did not increase

Fig. 2. (a) Wood microdensity values along the radius of the tree showing the complete microdensity profile (grey points) and the segment corresponding to the two-cm-long sensors (black points) where Q_s was measured. This segment corresponds to the Q_s measurement taken during the 2004 growing season, (b) two-cm-long segment of the microdensity profile showing MeanDenOver$_{th}$, MeanDenUnder$_{th}$ and SegWidthUnder$_{th}$ (see text for acronym definitions) for a threshold value of 0.45 g/cm^3, and (c) daily Q_s variation (dotted line) for a given year in a given tree and a smoothed curve (in blue) showing the temporal variation in Q_s throughout the growing season. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(except for a very short period in the high-density stand after a rain event registered on January 20th, 2005) (Fig. 3a). During the subsequent growing season soil water content reached 1.5 MPa water moisture in mid-February in the low-density stand and almost a month later in the high-density stand (Fig. 3b).

While soil water content was very different between years and treatments, PET was similar between seasons (Fig. 3c), with similar maximum values recorded from January to February and minimum values from mid-March. Mean PET (and standard deviation) of the September 28th-April 20th period, most of the growing season in the study site (Gyenge, 2005), was 4.3 (±1.8) and 4.6 (±1.7) mm day^{-1} for the 2004 and 2005 growing seasons, respectively.

3.2. Sapflow density patterns

The average daily Q_s was higher in the low-density stand (Fig. 4a and b). In the dry year, significant differences in daily Q_s samples between sites increased toward the end of the growing season (Fig. 4c). These differences between stand density treatments began to be significant from December 15th on (late spring: period of maximum growth according to Gyenge, 2005). In 2005, mean Q_s values from both treatment sites were not significantly different for 80 days during the growing season. However, they were higher in the low-density stand than in the high-density stand for at least 65 days of the same growing season (Fig. 4d).
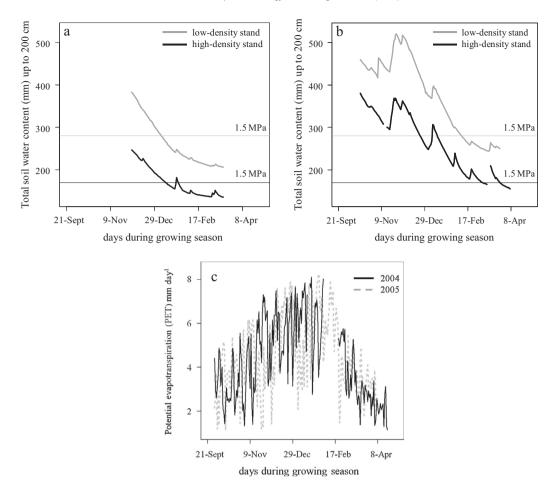
Considering the temporal changes in RCI during the first part of the dry year (until early January), the trees in both treatments showed similar negative values (Fig. 5a). Later, the trees in the high-density stand showed mostly positive values increasing toward the end of the season. This means that Q_s dropped faster than PET in the high-density stand, indicating that these trees

suffered greater soil water constraints than those in the low-density stand. Meanwhile, in the wet year (Fig. 5b) trees from both treatments showed similar RCI trends throughout the whole season. This would indicate that tree growth was not limited by soil water content in neither of the treatments.
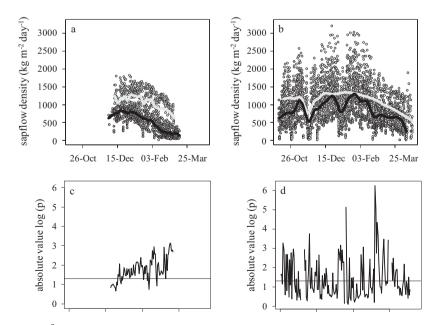
3.3. Wood microdensity patterns

The trees in the low-density stand were significantly different from the trees in the high-density stand in terms of stem diameter (mean and standard deviation: 28.5 cm ± 2.6 and 22.7 cm ± 6.5 respectively), but not in mean stem density (0.43 g/cm^3 ± 0.05 and 0.42 g/cm^3 ± 0.02, respectively). The conventional microdensity variables followed the general patterns described for the whole radial profile (Fig. 6). RW, as well as its components EWW and LWW, were significantly higher in the low-density stand during all of the analyzed years (Fig. 6a, only RW is shown), while MRD was significantly higher in the high-density stand only in 2001 and 2006 (Fig. 6b). The tree-rings formed during 1999, 2000, 2001, 2004 and 2006 (that is, the year following the wet 2005 year) presented significantly higher MID and EWD (not significant for EWD in 1999 and 2000) in the high-density stand (Fig. 6c and d). EWP was always similar between treatments (Fig. 6e), as were LWD and MAD (Fig. 6f, only LWD is shown).
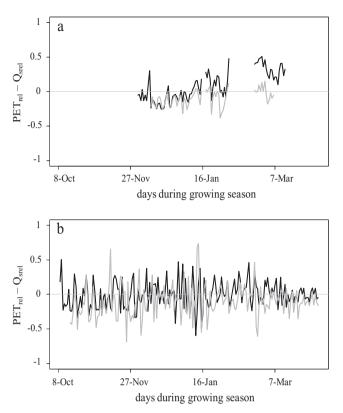
Significant correlations ($p < 0.05$) were found between Q_s and the non-conventional microdensity variables defined by the different density thresholds in the two-cm segments of the microdensity profile. Moderate positive relationships were found between SegWidthUnder$_{0.25}$, minimum Q_s and mean Q_s. However, for the density threshold values from 0.55 to 0.65, an increment of SegWidthUnder$_{th}$ was negatively correlated with mean Q_s and

Fig. 3. Daily average of soil water availability (n = 4 probes in 2004, n = 3 probes in 2005) and PET in Meliquina Valley, Patagonia Argentina: (a) Soil water content (mm in the whole soil profile, up to 200 cm) during the 2004 growing season in the low- (grey line) and high-density (black line) ponderosa pine stands, (b) soil water content during the 2005 growing season in the low and high-density stands and (c) potential PET (mm day^{-1}) during the 2004 (black line) and 2005 (grey line) growing seasons. For practical reasons, growing seasons were named after the first year of wood formation in the season.

Fig. 4. Daily Q_s (kg water per unit area (m^2) of active xylem per day), individual variation (black and grey circles ponderosa pine trees in high and low-density stands, respectively) and mean values (black and grey solid lines for trees in high and low-density stands, respectively) during (a) the 2004 growing season and (b) the 2005 growing season. In (c) and (d) the solid line represents the log 10 of the probability of the treatment effect in the *t*-test analysis for the 2004 growing season (c) and the 2005 growing season (d). The horizontal line represents the 5% significance level. The values above this line indicate the days with significant differences between the treatments.

Fig. 5. Mean relative potential evapotranspiration minus daily sapflow density in high- (black line) and low-density (grey line) stands during the 2004 (a) and 2005 (b) growing seasons: dry and wet years, respectively.

maximum Q_s, as well as with the integral of Q_s (Q_s area) for the growing season (Table 1). On the other hand, for the density threshold values between 0.40 and 0.60, a decrease of MeanDenUnder$_{th}$ resulted in increased minimum Q_s. Also, MeanDenOver$_{th}$ (between 0.25 and 0.35) was positively correlated with mean Q_s, maximum Q_s and Q_s area. These correlations indicate that low-density wood in the low-density part of the ring (generally the first part of the ring) and high-density in the high-density part of the ring (generally the last portion of the ring) are associated with high Q_s (Table 1).

4. Discussion

Significant differences in ponderosa pine wood production under different management systems have been described by many authors (Jaakkola et al., 2005; Zobel and van Buijtenen, 1989) in native forests (McDowell et al., 2006) and in plantations, in its natural distribution area (Wei et al., 2014; Powers and Reynolds, 1999) as well as in Patagonia (Fernández et al., 2012, Gyenge et al., 2010, 2012). However, there are fewer studies relating growth rates with wood density in ponderosa pine, and, according to our knowledge, no study has documented the relationship between these variables and continuously-measured Q_s, which is a direct measure of the hydraulic activity of the plant. In the present study, significant differences were observed in growth and Q_s between the two stand density treatments, with little impact on mean stem wood density. However, at a more detailed scale of analysis facilitated by the microdensity technique, wood density differences were detected under particular environmental circumstances.
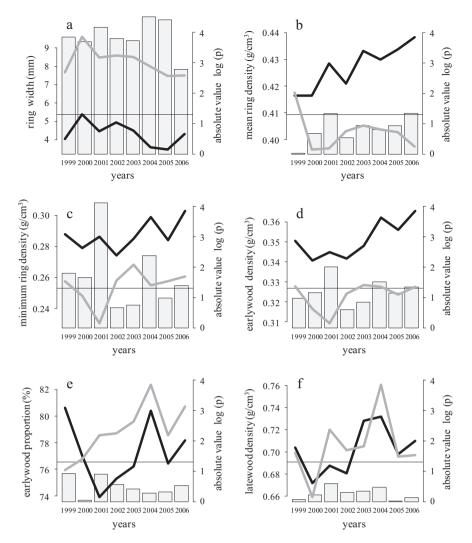
Ponderosa pine trees growing in Patagonia Argentina have a tree-ring mainly formed by earlywood (EWP > 70%, Fernández

et al., 2012). The same result was found in trees studied here. EWP was over 75% in both treatments, along the entire microdensity profile (from the 1991-data not shown-onwards). Wood formed in the high-density stand had higher MID and EWD. These observed differences were not necessarily reflected in an increase in mean stem density. However, it is necessary to take into account: (1) the reduced number of sampled trees that could affect the power of the statistical analysis, and/or (2) that the intraspecific level of tree competition was not strong enough to result in an increment of EWD so as to find significant differences at the tree level. This would be the case if after the 2006 growing year EWD and MRD had continuously increased along the profile. On the other hand, mean stem density is an integrative variable that could hide the inter-annual tree-ring variations found in this study.

For ponderosa pine, the pre-dawn water potential has been observed to drop exponentially as soil water content declines under 1.5 MPa (Gyenge et al., 2012). Therefore, soil water content should be considered a key environmental parameter. Nevertheless it does not seem, by itself, to be a factor of stress. This variable should be assessed in the context of tree competition levels and interpreted in conjunction with the general water status of the tree. We could establish that in the high-density stand Q_s decreased more during the dry growing season than in the low-density stand. The positive value of RCI allows us to determine a water constraint related to soil water deficit rather than to PET: an increase in the significant differences between treatments for Q_s means over the course of the growing season was observed during the dry year, due to a strong soil water deficit. On the other hand, even in the wet year, Q_s was lower in the high- than in the low-density stand, showing approximately the same amount of days with and without significant differences between treatments. These findings coincide with other reported results (Gyenge et al., 2003; Fernández et al., 2012) for a more xeric site in Patagonia, Argentina.

Stand density management improved individual growth, operating via physiological and structural traits. The trees in the low-density stand showed a greater capacity for water transport than the trees in the high-density stand, even if there were no apparent constraints on soil water availability, as was the case for the wet year. This greater hydraulic efficiency could be related to the lower density of the first part of the growth ring in the low-density stand. This decreased density could be associated with wood anatomy containing a higher cell lumen/cell wall ratio, and thus being more permeable for water transport.

During the dry growing season, trees in the low-density stand had higher Q_s and produced a significantly lower MID and EWD. However, despite having low values of EWD in the low-density stand, they were not statistically different from the high-density stand in 2003, which should have had an influence on the 2004 Q_s together with the wood formed in 2004. In the high-density stand, the two cm-long portion of wood in which the 2004 Q_s was measured corresponded to the rings formed between 1999 and 2003. The structural changes toward higher wood density detected in the high-density stand in the first part of the ring can explain the significant differences detected in Q_s between stand density treatments. This is true not only for the dry growing season, but also for the wet period, given the relevant role that EWD may have in terms of its relationship with wood hydraulic conductivity (k_s). In this regard, k_s depends on tracheids size. The Hagen Poiseuille law states that the radius of the tracheid or vessel has an influence that is exponential to the fourth power on k_s. Thus, even very little differences in cell-lumen radius could have significant consequences in terms of k_s. Additionally, Bouffier et al. (2003) described a negative relationship between wood density and xylem hydraulic conductivity in ponderosa pine. Moreover, Maherali and DeLucia (2001) compared desert and mountain

Fig. 6. Wood microdensity variables estimated in the ponderosa pine trees growing in the high-density (black solid line) and the low-density (grey solid line) stands for i variable (wood microdensity) in year j (years analyzed: from 1999 to 2006). The solid grey bars represent the $\log 10$ of the probability of the treatment effect in the t-test analysis of the microdensity variables. The horizontal line represents the 5% significance level. Only ring width (RW), mean ring density (MRD), minimum ring density (MID), earlywood density (EWD), earlywood proportion (EWP) and latewood density (LWD) are shown. Similar results as for RW were found for earlywood width (EWW) and latewood width (LWW). Maximum ring density (MAD) showed similar trends to LWD.

Table 1

Pearson correlation coefficient and associated probability between non-conventional microdensity variables (computed in the segment of the microdensity profile corresponding to the wood part where Q_s was measured) and the corresponding Q_s variable. Only SegWidthUnder$_{th}$, MeanDenUnder$_{th}$, and MeanDenOver$_{th}$ variables which associated probability levels were <0.05 are shown. SegWidthUnder$_{th}$ = $_{th}$segment width under each threshold density, MeanDenUnder$_{th}$ = mean density of the $_{th}$segment under the threshold density; MeanDenOver$_{th}$ = mean density of the $_{th}$segment over the threshold density, Q_s minimum, Q_s mean and Q_s maximum = the minimum, mean and maximum Q_s, tree in each growing season respectively, and Q_s area = the area of the Q_s curve during each growing season.

	Q_s							
	Q_s minimum		Q_s mean		Q_s maximum		Q_s area	
SegWidthUnder$_{0.25}$	0.44	(0.025)	0.39	(0.046)				
SegWidthUnder$_{0.55}$			−0.39	(0.050)	−0.43	(0.030)		
SegWidthUnder$_{0.60}$			−0.43	(0.027)	−0.46	(0.017)	−0.40	(0.045)
SegWidthUnder$_{0.65}$			−0.39	(0.048)	−0.41	(0.036)		
MeanDenUnder$_{0.40}$	−0.40	(0.040)						
MeanDenUnder$_{0.45}$	−0.42	(0.033)						
MeanDenUnder$_{0.50}$	−0.43	(0.028)						
MeanDenUnder$_{0.55}$	−0.43	(0.029)						
MeanDenUnder$_{0.60}$	−0.42	(0.032)						
MeanDenOver$_{0.25}$					0.42	(0.035)		
MeanDenOver$_{0.30}$			0.42	(0.034)	0.40	(0.043)		
MeanDenOver$_{0.35}$			0.43	(0.027)	0.42	(0.030)	0.41	(0.040)

native populations of this species and found a higher k_s associated with larger lumen diameters of xylem tracheids in desert than in mountain populations.

Several authors have suggested that ponderosa pines have coordinated adjustments in their hydraulic xylem architecture (xylem efficiency and water storage capacity, Huber ratio, leaf area) as well

as a highly plastic growth response that may serve to avoid embolism along a gradient of increasing aridity (Barnard et al., 2011; Domec et al., 2004; Domec and Gartner, 2003; Panek and Goldstein, 2001; Maherali and DeLucia, 2000; Fernandez et al., 2012). These functional adjustments could be the result of an adaptive response of the individuals allowing the species to occupy a wide range of climates including those with frequent water deficit. With regard to Patagonia, Licata et al. (2008) and Gyenge et al. (2012) did not find differences in the leaf area/sapwood area ratio (Huber ratio) between high- and low-density stands of ponderosa pine growing in this region. In this work we sampled in the same high- and low-density stands as in Licata et al. (2008). Stand structure (trees ha^{-1}, radial growth and basal area) describes a marked difference between silvicultural treatments. However, as shown in Licata et al. (2008) the Huber ratio was very similar (0.15 and 0.12 m^2 cm^{-2} for high- and low-density stands, respectively). Therefore, we infer that the observed differences in the Q_s values between high- and low-density stands for both dry and wet growing seasons are mostly the result of a change in wood structure rather than in plant evaporative area (leaf area).

Numerous studies have shown that ponderosa pine prevents high xylem tensions (Barnard et al., 2011; Domec and Gartner, 2003; Waring et al., 1982, Martínez Vilalta et al., 2004; Bouffier et al., 2003; Maherali and DeLucia, 2000) due to the coordination of different mechanisms leading to an increment in hydraulic efficiency (avoidance strategy). Here we found that ponderosa pine responded to water constraints resulting from high intra-specific competition with an MID and EWD increase in the high-density (more stressed) stand. These mechanisms may resemble those found in other conifers such as Norway spruce (Rosner et al., 2014) and Douglas-fir (Dalla-Salda et al., 2011, 2014). In these tolerant species, higher wood density would imply a higher resistance to drought. In the case of the studied ponderosa pines, we hypothesize that a higher EWD could be not conductive to drought acclimation, exposing trees to an increasing dilemma as they face more recurrent and intense droughts and/or an increase in intra-specific competition, affecting the productivity and compromising their survival under severe drought events.

The question that arises from our study is: which comes first, the chicken or the egg? The wood density influence on Q_s or the Q_s influence on the (newly-formed) wood density? In the high-density stand, a dry year, reflected in marked differences in Q_s between treatments, resulted in a tree-ring with higher EWD and MID. In fact, these differences could explain, at least in part, the differences in Q_s in the following wet year, since the Q_s would be driven by the wood formed during the previous years (at least at the beginning of the wet growing season, until the already-formed earlywood takes over). On the other hand, if the inter-annual variation in resource availability due to climate has some effect on the physical properties of the wood formed during the growing season, a wet year should result in similar EWD in both treatments, and that is effectively what we found. This result means that Q_s in year x depends on the water availability in year x and on wood density of the previously formed rings (in year x − 1, year x − 2, etc). So, as was corroborated in the high-density stand during the dry year, constraints in resource availability during the growing season would determine a wood density increase (at least in the earlywood portion) for the current year tree-ring, leading to differences in Q_s in the following years, even without water constraints. Our results provide evidence to support this hypothesis. The studied relationships between wood density and Q_s for the two-cm segment suggest a functional relationship between variables. The increased Q_s must be due to the most conductive portion of the wood, that is, the portion of lowest density. What contributes to the Q_s increase is not only an increasing amount of this conductive portion, but also the fact that this portion has a lower density

(negative correlations between MeanDenUnder$_{th}$ and Q_s). As denser portions of the growth-ring were incorporated in the analytical procedure and the density threshold increased, the relationship between the amount of low-density wood and Q_s changed from positive to negative. An unexpected positive relationship arose between the denser portion of the ring, described by MeanDenOver$_{th}$ variables and Q_s. However, we interpret that these correlations do not reflect functional relationships. Instead they may be the consequence of the negative correlation observed between mean density of the first and second portions of the ring ($r = -0.75$, $p < 0.001$, between MeanDenOver$_{0.40}$ and SegWidthUnder$_{0.65}$, or $r = -0.72$, $p < 0.001$, between MeanDenOver$_{0.45}$ and SegWidthUnder$_{0.65}$, results not shown). These negative correlations suggest that trees with lower density in the first part of the ring (and therefore, potentially high Q_s) have for some reason higher density in the final portion, thus explaining the positive relationship between MeanDenOver$_{th}$ and Q_s. The observed within-ring correlations need to be tested in a greater number of samples, adding other sites and years, and incorporating previous rings, not only those corresponding to the two-cm segment of the sensors.

In relation to the latewood portion of the ring, we found differences in terms of the quantity of latewood formed during the growing season (LWW) similar to what we found for the earlywood portion (EWW), although no significant differences were found for LWD, nor for MAD. However, this increment of the LWW should not be considered a signal of a longer growing season (toward the end of summer/beginning of autumn). Note that microdensity profiles are represented in the x-axis by distance units, not by time, so it is not possible to establish a priori if the observed differences found in RW are in relation to a longer growing season, a greater velocity in growth and/or a greater turgor and expansion of tracheids.

Differences in the amount of latewood, as well as in late season Q_s between treatments give rise to the question of potential consequences on carbohydrate reserves. The effects of drought conditions during the 2004 growing season should have consequences on the initial growth (earlywood variables) of the following year (year x + 1). A possible consequence would be a lower ring width in 2005 in relation to the ring formed in 2004, even if there was high soil water availability and Q_s. In fact, the increment in RW was lower due to a decrease in EWW in the 2005 season than in 2004, with a relative increase in LWW. This could be considered evidence supporting the hypothesis of reserves affecting mainly the next year's initial growth, whereas the rest of the ring is formed with carbohydrates created during the current growing season (Michelot et al., 2012).

Considering these findings, ecological sustainability of forest plantations could be threatened in the context of new climate scenarios. Ponderosa pine is the most planted tree species in Patagonia, representing 75% of the region's area forested with introduced species (Loguercio and Deccechis, 2006). The low plantation density scheme – 350 trees ha^{-1} at 24 years old – could significantly reduce the rotation age due to increased tree growth, improving the economic turnover, one of the major concerns for forestry development in the Patagonia region. Higher growth rates of these trees can counterbalance the decrease in the amount of trees per unit area, maintaining stands level productivity (Gyenge et al., 2010). In addition, summer drought events have been recorded more frequently during the last 20 years in Patagonia (Mundo et al., 2010). In this study we analyzed the impact that water availability (mediated by climate and intra-specific competition) has on the development of this species in terms of quantity and physical characteristics of wood (here estimated through microdensity parameters). Our findings are in agreement with results previously reported coming from other

growth conditions using a different methodological approach (analysis of wood anatomy) (Fernández et al., 2012). Although it is not within the scope of the present study, it should be mentioned that the lower EWD formed under a scheme of low-density stands will be desirable for impregnations, finishing and absorption of chemical substances that improve wood hardness, mechanical properties, and dimension stability, making a wide variety of uses of ponderosa wood possible for solid final products.

Finally, we should take into account the genetic basis of ponderosa pines introduced in Patagonia. The selected materials were mainly oriented to high productivity, and therefore the great adaptability to drought present in some populations in its natural range could be absent in Patagonia. A review of the introduced genetic origins as well as the design of site-specific recommendations within the potential area of ponderosa pine implantation, are recommended in order to define a general strategy to face the new environmental conditions predicted for the Patagonia region.

5. Conclusions

Resource availability, mediated by tree competition, can be manipulated via stand density management. Faced by limitations in water availability, ponderosa pine trees growing under high intra-specific competition are affected in their hydraulic and structural traits. Earlywood density increases in response to more stressful conditions such as those experienced in high-density stands. Considering the known strategy of ponderosa pine for coping with drought, characterized by an increase in hydraulic conductance to decrease xylem tensions, this structural trait change could be interpreted as non-conductive to acclimation with potential consequences on the whole hydraulic integrity of the tree. Given the limited number of sampled trees in this study, complementary research is necessary in similar or more stressful conditions, to elucidate this hypothesis.

Acknowledgements

References

Allen, R., Pereira, L., Raes, D., Smith, M., 1998. Crop evapotranspiration – guidelines for computing crop water requirements. FAO Irrigation and drainage, Paper 56. <http://www.fao.org/docrep/X0490E/x0490e00.htm#Contents>.

Barnard, D., Meinzer, F., Lachenbruch, B., McCulloh, K., Johnson, D., Woodruff, D., 2011. Climate-related trends in sapwood biophysical properties in two conifers: avoidance of hydraulic dysfunction through coordinated adjustments in xylem efficiency, safety and capacitance. Plant, Cell Environ. 34, 643–654.

Bouffier, L., Gartner, B.L., Domec, J.-C., 2003. Wood density and hydraulic properties of ponderosa pine from the Willamette valley vs. the Cascade mountains. Wood Fiber Sci. 35 (2), 217–233.

Bucci, S., Goldstein, G., Meinzer, F., Scholz, F., Franco, A., Bustamante, M., 2004. Functional convergence in hydraulic architecture and water relations of tropical savanna trees: from leaf to whole plant. Tree Physiol. 24, 891–899.

Dalla-Salda, G., Martinez-Meier, A., Cochard, H., Rozenberg, P., 2011. Genetic variation of xylem hydraulic properties shows that wood density is involved in adaptation to drought in Douglas-fir (Pseudotsuga Menziesii (Mirb.)). Ann. Forest. http://dx.doi.org/10.1007/s13595-011-0091-1.

Dalla-Salda, G., Fernández, M.E., Sergent, A.-S., Rozenberg, P., Badel, E., Martinez-Meier, A., 2014. Dynamics of cavitation in a Douglas-fir tree-ring: transition-wood, the lord of the ring? J. Plant Hydraul. 1, e-0005.

Domec, J.C., Gartner, B., 2002. How do water storage differ in coniferous earlywood and latewood? J. Exp. Bot. 53, 2369–2379.

Domec, J.C., Gartner, B.L., 2003. Relationship between growth rates and xylem hydraulic characteristics in young mature and old-growth ponderosa pine trees. Plant, Cell Environ. 26 (3), 471–483.

Domec, J.C., Warren, J.M., Meinzer, F.C., Brooks, J.R., Coulombe, R., 2004. Native root xylem embolism and stomatal closure in stands of Douglas-fir and ponderosa pine: mitigation by hydraulic redistribution. Oecologia 141 (1), 7–16.

Fernandez, M.E., Gyenge, J., Urquiza, M., Varela, S., 2012. Adaptability to climate change in forestry species: drought effects on growth and wood anatomy of ponderosa pines growing at different competition levels. Forest Syst. 21 (1), 162–173.

Granier, A., 1987. Evaluation of transpiration in a Douglas-fir stand by means of sapflow measurements. Tree Physiol. 3, 309–320.

Guay, R., Gagnon, R., Morin, H., 1992. A new automatic and interactive tree ring measurement system based on a line scan camera. Forest Chron. 68, 138–141.

Gyenge J., 2005. Uso de agua y resistencia a la sequía de pino ponderosa y ciprés de la cordillera. ThD Thesis, Universidad Nacional del Comahue, Centro Regional Universitario Bariloche, San Carlos de Bariloche, Argentina, 222 page.

Gyenge, J., Fernández, M.E., Schlichter, T., 2003. Water relations of ponderosa pines in Patagonia Argentina: implications on local water resources and individual growth. Trees Struct. Func. 17, 417–423.

Gyenge, J., Fernández, M.E., Schlichter, T., 2010. Effect of stand density and pruning on growth of ponderosa pines in NW Patagonia, Argentina. Agrofor. Syst. 78, 233–241.

Gyenge, J., Fernández, M.E., Varela, S., 2012. Short- and long-term responses to seasonal drought in ponderosa pines growing at different plantation densities in Patagonia, South America. Trees 26 (6), 1905–1917. http://dx.doi.org/10.1007/s00468-012-0759-7.

Hacke, U., Sperry, J.S., 2001. Functional and ecological xylem anatomy. Perspect. Plant Ecol. Evol. Syst. 4 (2), 97–115. http://dx.doi.org/10.1078/1433-8319-00017.

Hacke, U., Sperry, J., Pockman, W., Davis, S., Mc Culloh, K., 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia 126, 457–461.

Hillel D., 1984. L'eau el le soil. Principes et processus physiques. Ed Cabay. 288 pp.

Jaakkola, T., Mâkinen, H., Sarén, M.-P., Saranpââ, P., 2005. Does thinning intensity affect the tracheid dimensions of Norway spruce? Can. J. Forestry Res. 35, 2685–2697.

Licata, J., Gyenge, J., Fernandez, M.E., Schlichter, T., Bond, B., 2008. Increased water use by ponderosa pine plantations in northwestern Patagonia, Argentina compared with native forest vegetation. For. Ecol. Manage. 255 (3), 753–764.

Loguercio, G., Deccechis, F., 2006. Forestaciones en la Patagonia andina: potencial y desarrollo alcanzado. Patagonia Forestal (March 2006), 4–6.

Maherali, H., DeLucia, E.H., 2000. Xylem conductivity and vulnerability to cavitation of ponderosa pine growing in contrasting climates. Tree Physiol. 20 (13), 859–867.

Maherali, H., Delucia, E.H., 2001. Influence of climate-driven shifts in biomass allocation on water transport and storage in ponderosa pine. Oecologia 481–491. http://dx.doi.org/10.1007/s004420100758.

Maherali, H., Pockman, W.T., Jackson, R.B., 2004. Adaptive variation in the vulnerability of woody plants to xylem cavitation. Ecology 85, 2184–2199.

Martínez Vilalta, J., Sala, A., Piñol, J., 2004. The hydraulic architecture of Pinaceae – a review. Plant Ecol. 171, 3–13.

McDowell, N., Adams, H., Bailey, J., Hess, M., Folb, T., 2006. Homeostatic maintenance of ponderosa pine gas exchange in response to stand density changes. Ecol. Appl. 16, 1164–1182.

Michelot, A., Simard, S., Rathgeber, C., Dufrêne, E., Damesin, C., 2012. Comparing the intra-annual wood formation of three European species (Fagus sylvatica, Quercus petraea and Pinus sylvestris) as related to leaf phenology and non-structural carbohydrate dynamics. Tree Physiol. 32, 1033–1043. http://dx.doi.org/10.1093/treephys/tps052.

Mundo, I., El Mujtar, V., Perdomo, M., Gallo, L., Villalba, R., Barrera, M., 2010. Austrocedrus chilensis growth decline in relation to drought events in northern Patagonia, Argentina. Trees 24, 561–570.

Panek, J.A., Goldstein, A.H., 2001. Response of stomatal conductance to drought in ponderosa pine: implications for carbon and ozone uptake. Tree Physiol. 21, 335–342.

Pittermann, J., Sperry, J., Wheeler, J., Hacke, U., Sikkema, E., 2006. Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. Plant, Cell Environ. 29, 1618–1628.

Pockman, W.T., Sperry, J.S., 2000. Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. Am. J. Bot. 87, 1287–1299.

Polge, H., 1966. Establissement des courbes de variations de la densité du bois par exploration densitométrique de radiographies d'échantillons prélevés à la tarière sur des arbres vivants. Application dans les domaines technologiques et physiologiques. PhD thesis, Université de Nancy, Nancy 215 pages.

Powers, R.F., Reynolds, P.E., 1999. Ten-years response of ponderosa pine plantations to repeated vegetation and nutrient control along an environmental gradient. Can. J. For. Res. 29, 1027–1038.

R Development Core Team, 2014. R: A language and environment for statistical computing, R Foundation for Statistical Computing. Vienna, Austria. Available in: <http://www.Rproject.org>.

Rosner, S., Andreassen, K., Børja, I., Dalsgaard, L., Evans, R., Karlsson, B., Solberg, S., 2014. Wood density as a screening trait for drought sensitivity in Norway spruce 1. Can. J. For. Res. 44, 154–161.

Rozenberg, P., Franc, A., Commère, P., Schermann, N., Bastien, J-C., 1997. Height growth, wood density and dry fibre weight of four 33 year-old Douglas-fir provenances. In: Proceedings of CTIA/IUFRO International Wood Quality Workshop. Timber Management Towards Wood Quality and End-Product Value, 18–22 August, Vancouver, Canada, pp. 83–90.

Rozenberg, P., Franc, A., Mamdy, C., Launay, J., Schermann, N., Bastien, J.-C., 1999. Genetic control of stiffness of standing Douglas fir; from the standing stem to the standardised wood sample, relationships between modulus of elasticity and wood density parameters. Part II. Ann. Forest Sci. 56 (2), 145–154.

Ruiz Diaz Britez, M., Sergent, A.-S., Martinez Meier, A., Bréda, N., Rozenberg, P., 2014. Wood density proxies of adaptive traits linked with resistance to drought in Douglas fir (Pseudotsuga menziesii (Mirb.) Franco). Trees. http://dx.doi.org/10.1007/s00468-014-1003-4.

Sperry, J., Hacke, U., Pittermann, J., 2006. Size and function in conifer tracheids and angiosperms vessels. Am. J. Bot. 93, 1490–1500.

Sperry, J., Meinzer, F., McCulloh, K., 2008. Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. Plant, Cell Environ. 31, 632–645.

Stackman, W.P., 1980. Measuring soil moisture. Drainage principles and applications, vol. III. Wageningen, pp. 221–251.

Stratton, L., Goldstein, G., Meinzer, F., 2000. Stem water storage capacity and efficiency of water transport: their functional significance in a Hawaiian dry forest. Plant, Cell Environ. 23, 99–106.

Tyree, M.T., Ewers, F.W., 1991. Tansley review 34. The hydraulic architecture of trees and other woody plants. New Phytol. 119, 345–360.

Waring, R.H., Schroeder, P.E., Oren, R., 1982. Application of the pipe model theory to predict canopy leaf area. Can. J. For. Res. 12, 556–560.

Wei, L., Marshall, J., Zhang, J., Zhou, H., Powers, R., 2014. 3-PG simulations of young ponderosa pine plantations under varied management intensity: why do they grow so differently? For. Ecol. Manage. 313, 69–82.

Zobel, B., Sprague, J., 1995. Juvenile Wood in Forest trees. Springer Series in Wood Science. Springer-Verlag, Berlin Heidelberg New York.

Zobel, B., van Buijtenen, J., 1989. Wood Variation Its Causes and Control. Springer-Verlag Berlin and Heidelberg GmbH & Co. K.