Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/forestsystems
doi: http://dx.doi.org/10.5424/fs/2112211-12586

Forest Systems 2012 21(1), 000-000
ISSN: 1131-7965
eISSN: 2171-9845

Adaptability to climate change in forestry species: drought effects on growth and wood anatomy of ponderosa pines growing at different competition levels

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Abstract

More stressful conditions are expected due to climatic change in several regions, including Patagonia, South-America. In this region, there are no studies about the impact of severe drought events on growth and wood characteristics of the most planted forestry species, *Pinus ponderosa* (Doug. ex-Laws). The objective of this study was to quantify the effect of a severe drought event on annual stem growth and functional wood anatomy of pines growing at different plantation densities aiming to understand how management practices can help to increase their adaptability to climate change. Growth magnitude and period, specific hydraulic conductivity, and anatomical traits (early- and latewood proportion, lumen diameter, cell-wall thickness, tracheid length and bordered pit dimensions) were measured in the ring 2008-2009, which was formed during drought conditions. This drought event decreased annual stem growth by 30-38% and 58-65% respect to previous mean growth, in open vs. closed stand trees, respectively, indicating a higher sensitivity of the latter, which is opposite to reports from the same species growing in managed native forests in USA. Some wood anatomical variables did differ in more water stressed trees (lower cell wall thickness of earlywood cells and higher proportion of small-lumen cells in latewood), which in turn did not affect wood function (hydraulic conductivity and resistance to implosion). Other anatomical variables (tracheid length, pit dimensions, early- and latewood proportion, lumen diameter of earlywood cells) did not differ between tree sizes and plantation density. The results suggest that severe drought affects differentially the amount but not the function and quality of formed wood in ponderosa pine growing at different competition levels.

Key words: functional wood anatomy; *Pinus ponderosa;* Patagonia; drought resistance; stand density effects.

Resumen

Adaptabilidad al cambio climático en especies forestales: efectos de la sequía sobre el crecimiento y la anatomía de la madera de pino ponderosa creciendo bajo distintos niveles de competencia

Los pronósticos de cambio climático indican condiciones de mayores niveles de estrés ambiental para muchas regiones del mundo, incluyendo la Patagonia, Sudamérica. En esta región no existen antecedentes acerca del impacto de eventos de sequía severa sobre el crecimiento y la anatomía funcional de la madera de *Pinus ponderosa* (Doug. ex-Laws), la especie forestal más plantada. El objetivo de este estudio fue cuantificar el efecto de un evento de este tipo sobre el crecimiento anual en diámetro y la anatomía de la madera de pinos creciendo a densidades de plantación bien diferenciadas, con la intención de entender cómo las prácticas de manejo pueden ayudar a incrementar la adaptabilidad al cambio climático. Se midieron las siguientes variables en el anillo de crecimiento 2008-2009: magnitud y período de crecimiento, conductividad hidráulica específica, proporción de madera temprana y tardía, y morfometría de los elementos de conducción: diámetro de lúmen, grosor de pared y longitud de traqueidas, y dimensiones de las punteaduras entre traqueidas. Este evento de sequía redujo la magnitud del crecimiento en un 30-38% y un 58-65% con respecto a los años previos en árboles creciendo en baja y alta competencia intraespecífica, respectivamente. Esto indica una mayor sensibilidad de los segundos, mientras se había encontrado una tendencia opuesta en bosques nativos de esta especie gestionados en EEUU. Se observaron diferencias en algunas variables anatómicas de los árboles más estresados (menor grsor de pared en tracejidas del leño temprano y células con lúmenes más pequeños en el leño tardío), que sin embargo no tuvieron un efecto en la función de la madera (conductividad hidráulica y resistencia a la implosión). Otras variables anatómicas no difirieron

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Received: 13-06-11. Accepted: 12-12-11.

entre ambas densidades de plantación ni tamaño de árboles analizados (largo de traqueidas, dimensiones de punteaduras, proporción de madera temprana y tardía, diámetro de lúmenes de madera temprana). Los resultados sugieren que los eventos de sequía extrema afectan diferencialmente la cantidad pero no la función y calidad de la madera formada en pino ponderosa creciendo bajo distintos niveles de competencia.

Palabras clave: anatomía funcional de la madera; *Pinus ponderosa;* Patagonia; resistencia a la sequía; efecto de la densidad de plantación.

Introduction

In the context of Global Change, climatic forecasts predict more stressful conditions than current or historical ones for several areas of the world, namely higher average temperature and more variable precipitation condition along successive years (IPCC, 2007). This is the case of NW Patagonia (South-America), where climate change has been just noticed in terms of a higher frequency of drought events during the last two decades. The climatic trends corresponding to this region is presented and analyzed in the Final Report about Vulnerability of Patagonia to Climatic Change (Fundación Torcuato Di Tella, 2006). This detailed study shows that there is positive trend in mean annual temperature of NW Patagonia (Western Neuquén and Chubut provinces) of about 0.18°C per decade between years 1961-2000, mainly explained by the increase in spring and summer temperature. Other studies cited in the same report indicate a mean increase of 1°C for this region in the period 1964-2004 (Caffera, 2005). More even marked is the negative trend (–34 to –2 mm month^{-1} decade^{-1} from the beginning of xx century) in mean annual precipitation of NW Patagonia, contrasting with the positive trend in the E and NE part of this region (Fundación Torcuato Di Tella, 2006). Dendrochronological studies performed in this region have also demonstrated that 10 severe drought events occurred in the last 20 years, whereas only 6 events happened during the previous 80 years (Mundo *et al.*, 2010). This climatic phenomenon threatens the sustainability of natural and managed forest systems in the region. In this regard, growth response to drought of conspicuous species of Andean forests has been studied (Villalba *et al.*, 1997; Villalba and Veblen, 1998; Daniels and Veblen, 2004; Masiokas and Villaba, 2004; Suarez *et al.*, 2004; Mundo *et al.*, 2010), but no studies have been carried out evaluating the influence of severe drought events on forestry productive systems based on exotic fast growing species.

Pinus ponderosa (Doug. ex-Laws) is the most planted species in N.W. Patagonia occupying 80% of the forested land (approx. 77,000 ha. in NW Patagonia, Loguercio and Deccechis, 2006). This species is planted in the ecotone area between native Andean forests and the Patagonian steppe, reaching good mean annual volume increments (18.5 m^3 ha^{-1} year^{-1}, Andenmatten *et al.*, 2002). Drought response of this species growing in managed natural forests in Arizona (USA) showed that trees growing in thinned very low-density forests (basal area (BA): 7 m^2 ha^{-1}) are more sensitive to drought events than trees growing in unthinned forests (BA: 45 m^2 ha^{-1}), and that the former are more resilient after drought events (McDowell *et al.*, 2006). Genetic, environmental and management conditions of native USA ponderosa pine forests are however different from those of South-American populations where this species is exotic and commercially planted. In this regard, higher volumes for Patagonian stands have been observed than those attained by highly productive California ponderosa pine plantations of the same age, dominant height, and plantation density (Gonda, 1998). Patagonian stands support very high stocking and they exhibit negligible mortality despite their very high basal areas and relative densities by North American standards (Gonda, 1998). Accordingly, water use was more than twice for a similar stand leaf area index in Patagonian plantations than in North American ponderosa pine forests (Gyenge, 2005). All these observations demonstrate that although Patagonian plantations are installed at similar latitudes and general climatic conditions than in the Northern Hemisphere, there exist other biotic and/or abiotic factors determining the observed productivity differences between systems.

Drought influences not only growth rates but also the anatomy of wood (e.g. Martínez Meier *et al.*, 2008). In general, water deficit leads to a decrease in tracheid/vessel lumen diameter by affecting the expansion capacity of the cells due to turgor decrease (e.g. Abe and Nakai, 1999). In addition, cell wall thickness can be affected if carbon fixation (or allocation to this compartment) is severely constrained by drought (Antonova and Stasova, 1997). The occurrence of severe drought during spring time can lead to the formation of false rings or other types of intra-annual density fluctuations (Rigling *et al.*, 2002; Campelo *et al.*, 2006). When drought, or water deficit due to competition, occurs in early summer, latewood cell formation can initiate before than under wetter conditions, affecting the proportion of earlywood to latewood in the growth ring. However, this proportion may not change if growth period is also shortened (Cregg *et al.*, 1988). Moreover, severe drought can drastically interrupt growth leading to rings with no latewood at all.

The combination of some or all of these phenomena may occur within a species making it difficult to generalize or predict the effect of different environmental (including management) conditions on wood formation. Wood anatomy is important from both the productive (since it determines wood density) and functional points of view, e.g. determining wood hydraulic conductivity and the vulnerability of xylem to cavitation (Sperry *et al.*, 2006).

Based on this background, the objective of this study was to quantify the effect of a severe drought event on annual stem growth of ponderosa pine trees growing at two much contrasted plantation densities, aiming to understand how management practices can help to increase adaptability of these systems to climate change. In addition, anatomical and functional characteristics of the wood formed during this very dry growing season were analyzed. Based on previous research on ponderosa pine and other conifer species, our hypotheses were:

a) The higher sensitivity to drought of open stand trees leads to a higher relative decrease in annual wood production in these trees compared to those of high density stands. Under drought conditions, however, the absolute growth will still be higher in open stand than in closed stand trees reflecting a better performance (higher carbon fixation) of the former.

b) Severe drought events have an impact on wood anatomy of water-stressed pines, leading to the formation of tracheids with small lumen diameters. Since wood hydraulic conductivity (ks) is directly influenced by this tracheid trait, pines growing under closed stand conditions will produce wood with a lower ks than pines growing in the open stand.

Materials and Methods

The study was performed during the 2008-2009 growing season in a *Pinus ponderosa* (Doug. ex-Laws) plantation located in "La Veranada" ranch, 15 km away from Bariloche city, Rio Negro province (41°13'53"S, 71°11'40" W), N.W. Patagonia, Argentina. The stand was planted 25 years before, and all trees had the same age. The climate of the region is characterized by cold and wet winters, and hot and dry summers. While mean precipitation from September to April (growing season) was 323 mm for the period 1990-2000, approximately one third of this value (114.6 mm) fell during 2008-2009 growing season. In contrast, autumn-winter rainfall was higher than the mean historical value (770 mm vs 480 mm in season 2008-2009 vs decade 1990-2000, these last data from National System of Weather Information of Argentina).

Two contrasted plantation density areas were sampled within a plantation of 30 ha. These areas were about 80 m apart one from each other; and were labeled as "Open stand" (500 pines/ ha) and "Closed stand" (2,500 pines/ha), respectively. Differences in the number of trees per unit area were due to different initial planting density and not due to thinning or differential mortality. Aspect (SW), slope and soil characteristics were similar between areas. Soil depth was approx. 80 cm, where a clay pan appears. Soil texture was sandy-loam (clay: 7%; silt: 28%; very fine, fine and medium sand: 55%; coarse and very coarse sand: 10%). One composite soil sample from the different soil depths was used to determine water retention curves standard parameters (field capacity (FC), 0.03 MPa, and permanent wilting point (PWP), 15 MPa) at the Soil Laboratory of the EEA Bariloche INTA (National Institute for Agricultural Technology, Argentina). Water retention parameters were acquired using the methodology described by Baver *et al.* (1972).

Ten trees were selected in each competition level area for plant sampling: five of the largest (dominant canopy) and five of the smallest (suppressed within the closed stand). Largest and smallest trees of each stand were selected in order to determine potential differential effects of plantation density on different size (social position) trees within a stand. These 20 trees (10 in each plantation density) were intensively studied during three consecutive growing seasons (2007-2008, 2008-2009 and 2009-2010), measuring variables reported in this paper as well as other variables analyzed in another paper in preparation (such as sapflow density, canopy conductance, etc.).

Initial structure of both contrasted density stands is reported in Table 1. A great variation in diameter at breast height (DBH) was observed reflecting the existent variation in growth rates between stand conditions

Table 1. Stand structure of contrasted competition level areas selected in "La Veranada" ranch, Río Negro province, NW Patagonia, Argentina

	Open stand	**Closed stand**
Stand density (trees/ha)	519	2,500
Basal area (m^2/ha)	38.1	79.7
DBH (cm)***	29.7 ± 7.7	19.5 ± 6.3
DBH Largest (cm)**	34.7 ± 2.8	24.8 ± 3.3
DBH Smallest trees (cm)**	24.4 ± 3.3	13.8 ± 2.7
Height (m)*	11.8 ± 0.8	13.2 ± 1,1
Leaf Area Index	6.38	6.38

ANOVA ($\alpha = 0.05$). ***$P < 0.01$. **$P < 0.05$. *$P = 0.058$. Values are mean ± standard deviation.

(plantation density and tree size) at the beginning of this research. DBH was different in 10 cm between stands, and from largest to smallest trees within each stand; and very similar DBH figures were observed between the smallest trees of the open stand and the largest trees of the closed stand, reflecting the average growth of those trees was similar before performing this study. Despite the great difference in plant density between the selected stands, leaf area index (LAI; measured as in Gyenge *et al.*, 2009), for ponderosa pines growing in a more humid stand of NW Patagonia) was identical between them (Table 1). Estimation of LAI from spherical photographs also indicated that both stands have the same LAI (data not shown).

The following environmental variables, and plant growth and wood properties of the selected trees, were measured in both contrasted plant density areas:

Climatic variables: rainfall, air temperature, air relative humidity and wind speed were recorded and logged by an automatic meteorological station (HOBO Weather Station, Onset Computer Corp., USA) located 100 m away from the plantation, in an open area.

Soil water content: gravimetrically determined, from the soil surface to 80-100 cm depth. Samples were periodically taken with a soil auger every 20 cm depth in five points within each stand.

Tree water status: leaf water potential was measured in four to five trees (one fascicle with three leaves of each tree) of each stand at pre-dawn with a pressure chamber (PMS Instrument Company, Model 1003, USA). Water potential was measured four times as drought progressed along the growing season.

Growth rate: DBH was measured every 15 days on the selected trees. A flagging tape was fixed at the precise height of the first measurement, so measurement error due to misplacing on next measurements was avoided. A logistic model was adjusted to illustrate the relationship of cumulated growth and time for each considered group of trees in every stand density area, i.e.:

$$y = K / (1 + b\, e^{-a\,x})$$

Where: y is the accumulated growth (mm), x is the time, K is the maximum accumulated growth, b is accumulated growth at time = 0, and a is a parameter related to time at the inflexion point.

The derivative of the logistic function was applied in order to estimate the daily growth rate (mm/day) during different periods of the growing season:

$$y' = K\, b\, a\, e^{-a\,x} / [(1 + b\, e^{-a\,x})^2]$$

Data were adjusted by regression models using Table Curve 2D v. 2.02 software (Jandel Scientific, AISN Software, UK).

Wood hydraulic conductivity: wood samples including the last growth ring were taken at DBH with a hand-made device in May 2009. This device (a sharp-edged sampler) allowed us to extract a cylindrical portion of wood (approx. 10 cm length and 2 cm diameter) in the same direction of water circulation (longitudinal cut). Previous studies in another site in NW Patagonia (Gyenge, 2005) indicated that ponderosa pine diameter and height growth ends in February/March and January/February, respectively. Therefore, we considered that wood sampling in May (late autumn) allows an appropriate characterization of wood formation in the immediately previous growing season. Wood samples were immediately kept into water within glass vials and taken to the laboratory. We re-cut wood pieces to 2.5 cm long segments under water with the width of the corresponding 2008-2009 growth ring (two rings in the case of the very smallest trees of the closed stand). Specific hydraulic conductivity (ks) of the growth ring was measured as in Spicer and Gartner (1998) and Fernández *et al.* (2010). The method consisted of a double ended pressure chamber inside which wood samples are introduced with a modified pressure sleeve to seal samples sides and only allowing longitudinal flow.

Wood anatomy: microscopic slides were prepared following the usual methods of softening, cutting (hand-cut with a scalpel) and staining with safranine (diluted in 50% water and 50% alcohol). Wood properties were measured in samples obtained from the wood used for ks determinations. Five cross sectional preparations from each tree were observed by means of light microscopy (microscope Olympus BX 41). Transition from one type of wood to the other was quite abrupt in the samples, with latewood cells presenting clear wider cell walls than early- and transition wood. Transition wood was included within earlywood. Additionally, we followed Mork's index criterion (Denne, 1988) to accurately determine latewood (cells with double wall thickness greater or equal to its lumen diameter).

Digital photographs were taken with a camera (Olympus Evolt E-330 SLR) attached to the microscope. Lumen diameter distribution and cell wall thickness of earlywood cells were automatically determined with Cell-Profiler free-software (Carpenter *et al.*, 2006, www.cellprofiler.org). Lumen diameter (d_l) was estimated as $d_l = 2xy/(x + y)$, where x and y are the short and long perpendicular diameters of the tracheid, respectively (Lewis and Boose, 1995). Five hundred to 1000 earlywood cells were measured in each sample.

Cell-Profiler could not be used with latewood cells because contrast was not enough good in this type of tissue; so at least 50 latewood cells of each wood sample were measured in the obtained digital photographs using Image J free-software (Image J 1.37v, National Institute of Health, USA). Size variability was much lower in latewood than in earlywood, so a much lower number of cells was required to analyze for representative figures.

The same procedure was applied to measure the dimensions of bordered pits of earlywood cells. The following dimensions were measured in these structures with 400X magnification: pit aperture, torus diameter, membrane (margo) diameter, and maximum pit diameter.

Conduit wall reinforcement, as a measure of safety against cell implosion due to tension, was estimated from mean values of early and latewood cells of each tree as:

$$\text{Safety factor} = (2\, t_w/d_l)^2$$

where: t_w is the cell wall thickness and d_l is lumen diameter (Hacke *et al.* 2001).

Observation and measurement of tracheid length was carried out following Jeffrey technique (e.g. D'Ambrogio de Argüeso, 1986). Two cm-long wood chips from the 2008-2009 growth ring of each individual tree were collected and then subdivided into smaller chips (approx. 0.5 cm x 1 cm each). Air was removed boiling these chips and then allowing them to cool repeatedly. Maceration was carried out using a 1:1 v:v solution of aqueous 10% nitric acid and aqueous 10% chromic acid, during one week at 60°C. Tracheid length was measured in 50 tracheids per individual at 4X magnification with the help of a graduate eyepiece of 0.1 mm.

Lumen diameter distribution was estimated for each tree group (smallest and largest trees of open and closed stands) for earlywood and latewood tracheids, and then for the whole ring weighting the proportion of tracheids in each diameter range by the proportion of latewood/earlywood in each individual tree. Hydraulic contribution of each lumen diameter range (from 5 μm to 60 μm) was estimated as in Cai and Tyree (2010):

$$\text{Hydraulic weighted proportion} = N_i\, d_{li}^4/\Sigma\, N_i\, d_{li}^4$$

where N_i is the number of tracheids in diameter range i and d_{li} is the mean value of the diameter range i (5, 15, 25, 35, 45 and 55 μm).

All variables were compared between situations using one-way ANOVA after checking that assumptions of normality and homocedasticity were met. Post-comparisons were performed with Tukey tests.

Results

Environmental conditions and growth

Soil water content decreased continuously from October to mid-January, after which it remained constant around a 5% dry weight until the beginning of April, that is, below the PWP of the soil (8.1% DW) (Fig. 1A). No differences were observed in this variable between plantation densities along the growing season. However, we found differences in tree pre-dawn water potential between open and closed stands, at least from January onwards. This variable decreased during drought in both density stands, but minimum pre-dawn water potential was significantly higher for open-stand trees (≈ –1.5 MPa) than in closed-stand ones (≈ –2 MPa) (Fig. 1B). No differences were observed in the water status of smallest and largest trees of each stand, thus a unique mean is presented for each stand density.

Growth in diameter clearly differed between both density stands and considered tree sizes (Fig. 2A), whereas larger trees accumulated 13 mm·year^{-1} in the open stand, smallest ones barely accounted for 3 mm·year^{-1} in the close stand. Cumulative growth was significantly different among all tree groups (ANOVA, Tukey tests, $\alpha = 0.05$). Basal area growth was also significantly different between groups (ANOVA, Tukey tests, $\alpha = 0.05$). Mean (standard deviation) of basal area growth were: 80.8 (5.6), 35.6 (5.9), 24.3 (7.8) and 6.4 (2.8) cm^2 year^{-1} for largest and

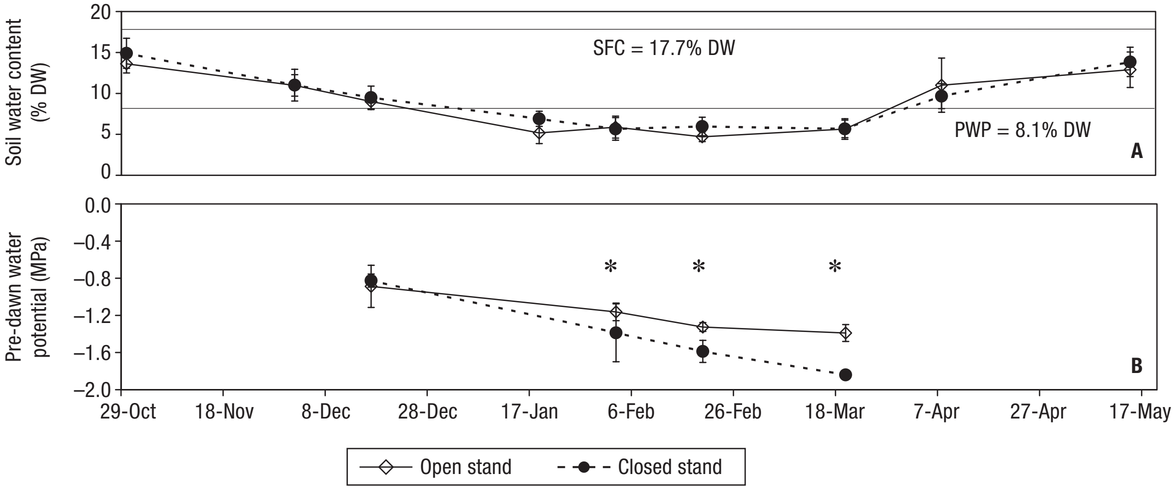

Figure 1 A. Gravimetric soil water content (% dry weight) in open and closed stands (mean ± SD, n = 5). Each mean value corresponds to the whole soil profile (80-100 cm depth). **B.** Evolution in time of pre-dawn water potential (mean ± SD) in both stands (n = 4–5). Asterisks identify significant differences between density areas at a certain date (T test, α = 0.05).

smallest trees of the open and the closed stand, respectively. When daily growth rates were estimated (Fig. 2B), it was clear that largest trees of the open stand differed from all other groups not only in the maximum growth rate but also in the growth period. From logistic model estimations (which may obscure little differences between groups) it appears that all groups of trees began growing at a similar date, but the largest trees of the open, as well as the smallest trees of the closed stand, kept a positive growth for a longer period. In the case of the suppressed trees of the closed stand, general adjustment of the logistic model was low ($R^2 = 0.45$ against $R^2 = 0.75$, 0.84 and 0.86 for dominant trees of the closed stand, suppressed and dominant trees of the open stand, respectively).

When 2008-2009 seasonal growth was compared to the mean growth rate of each tree before that growing season (last 17 years, corresponding to the age at DBH at the beginning of the study), it results that all trees of the open stand presented a lower decrease in their growth rate (30-38%) than those of the closed stand (58-65%). There were no significant differences between large and small trees within each stand (Fig. 3).

Functional wood anatomy

Early- and latewood proportion were similar between plantation density conditions. Mean latewood proportion was 26 % (SD: 5%; minimum value: 21%; maxi-

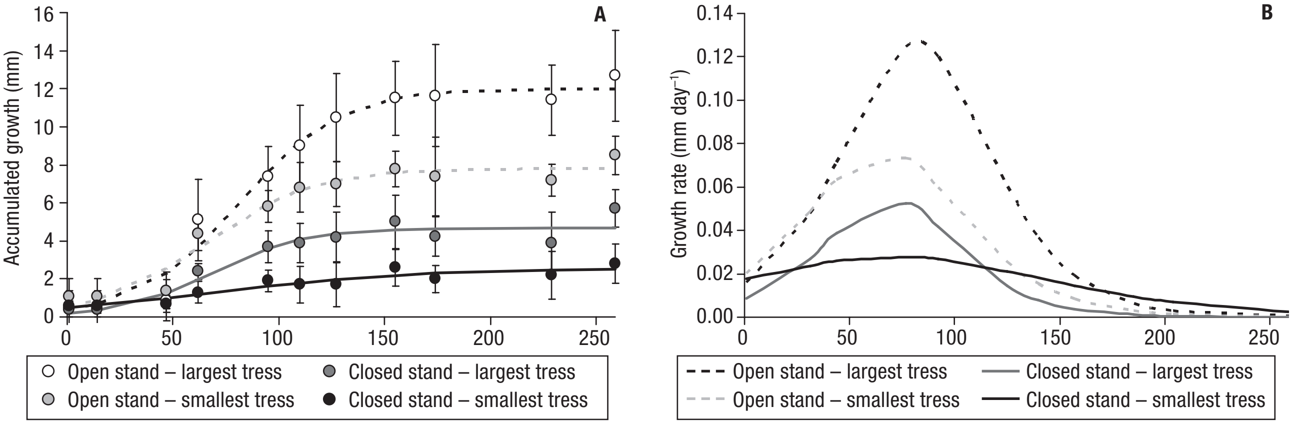

Figure 2 A. Accumulated growth (mean ± SD) measured in each stand during 2008-2009 (n = 5). **B.** Daily growth rate estimated from upper figure (logistic model).

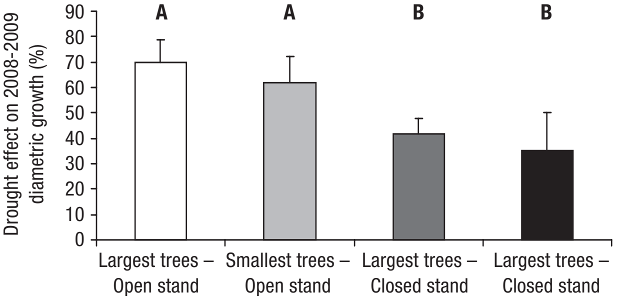

Figure 3. Diametric growth for 2008-2009 as a percentage of average diametric growth along previous 17 years. Letters (A, B) identify homogenous groups by Tukey's test ($p < 0.05$).

mum value: 34%) and 29 % (SD: 8%; minimum value: 19%; maximum value: 47%) in trees of the open and closed stands, respectively. No relationship was observed between latewood proportion and growth (ring width).

Earlywood tracheids presented similar mean lumen diameter in both density stands and all tree sizes (Table 2). In contrast, open stand trees presented significant higher values of cell wall thickness compared to the smallest trees of the closed stand (Table 2), which in turn did not result in significant differences in safety factors between tree groups (Table 2). Not only mean lumen diameter was similar but also the diameter distribution was very similar in all individuals (ANOVA, $p < 0.05$, data not shown) with similar proportions of tracheids with small and large lumen areas. The highest proportion (around 35%) of tracheids in earlywood had a mean lumen diameter in the range of 20-30 µm. However, tracheids with lumen diameter between 35-60 µm where those contributing to more than 80% of theoretical conductivity of the whole ring (Fig. 4).

Considering latewood cells, significant differences were observed in lumen diameter comparing smallest and largest trees within each stand, and between stands (Table 2). Smallest trees within each stand presented smaller lumen diameter than largest trees.

Table 2. Cell dimensions of early- and latewood and bordered pit dimensions of earlywood cells, corresponding to largest and smallest trees of open and closed stands of ponderosa pine growing in NW Patagonia

		Open stand		Closed stand	
		Largest	Smallest	Largest	Smallest
Earlywood cell dimensions	Lumen diameter	24.88 a (2.36)	23.76 a (2.39)	23.04 a (1.10)	22.81 a (3.02)
	Cell-wall thickness	4.83 a (0.32)	4.61 ab (0.36)	4.42 abc (0.34)	3.91 c (0.42)
	Safety factor	0.15 a (0.04)	0.16 a (0.04)	0.15 a (0.03)	0.12 a (0.05)
Latewood cell dimensions	Lumen diameter	14.07 a (0.81)	11.16 b (0.72)	12.68 ab (2.22)	9.47 c (1.30)
	Cell-wall thickness	7.10 a (0.89)	7.00 a (0.66)	6.81 a (1.20)	6.09 a (1.09)
	Safety factor	1.05 a (0.36)	1.59 a (0.28)	1.26 a (0.58)	1.84 a (0.94
Whole-ring	Tracheid length	2.35 a (0.27)	2.44 a (0.34)	2.27 a (0.19)	2.22 a (0.13)
Earlywood bordered pit dimensions	Pit aperture	5.35 a (1.15)		5.29 a (0.18)	
	Torus diameter	9.80 a (1.58)		9.57 a (0.83)	
	Margo diameter	17.00 a (2.49)		16.69 a (1.16)	
	Pit maximum diam.	20.89 a (2.88)		19.72 a (0.62)	

All values are mean ± standard deviation ($n = 5$). Different letters within each row indicate significant differences between tree groups (ANOVA, Tukey tests; $p < 0.05$). Values, except safety factor and tracheid length, are in µm. Tracheid length is expressed in mm.

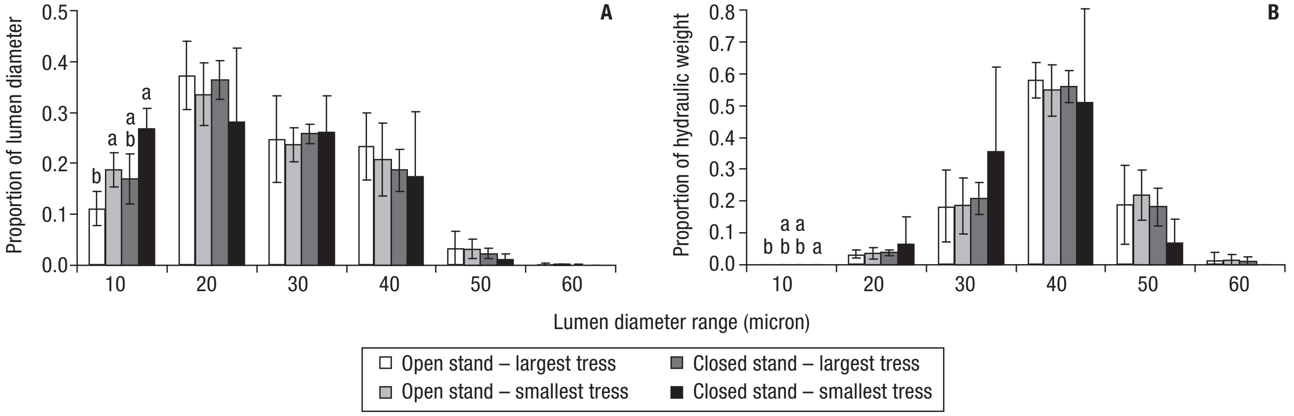

Figure 4 A. Distribution of cells lumen diameter in the ring 2008-2009 of largest and smallest ponderosa pine trees growing in open and closed stands (n = 5). **B.** Distribution of hydraulic contribution (weight) of each lumen diameter range to whole ring hydraulic conductivity. Significant differences, indicated by different letters, were only observed in the lowest diameter range (10 µm) (ANOVA, Tukey tests, $p < 0.05$).

However, cell wall thickness was similar in all tree groups as was also the safety factor (Table 2). Lumen diameter of latewood cells (5-25 µm) was in the range of the smallest cells of earlywood, thus its theoretical contribution to whole-ring hydraulic conductivity was almost negligible. In this regard, small-lumen tracheids of latewood explain the significant differences observed in the proportion of smallest tracheids in the whole ring (Fig. 4), and also the significant differences in hydraulic weight observed in smallest trees of the closed stand (Fig. 4). However, those tracheids contribute to 0.05% (proportion: 0.0005, not visible in the figure) of whole ring hydraulic conductivity of smallest trees of the closed stand, whereas their contribution is 0.01-0.02% of ks in the other tree groups.

No significant differences were observed between open and closed stand trees in any of the measured dimensions of bordered pits of earlywood cells, nor were observed in tracheid length as well (Table 2).

Specific hydraulic conductivity (ks) was similar in trees with a ring width between 2 and 5.5 mm (corresponding to annual diameter growth of 4 to 11 mm), and increased in trees with very low growth rates (less than 2 mm per year) (Fig. 5). These trees did not differ in maximum lumen diameters nor pit dimensions than the rest of the trees (data not shown). A general similar pattern was observed measuring ks of the previous growth ring (Fig. 5), suggesting that those data were not due to a measurement error, or to the method itself being not robust enough for very small rings. Since we cannot separate potential methodological problems in these small-rings samples, we will not discuss these particular results.

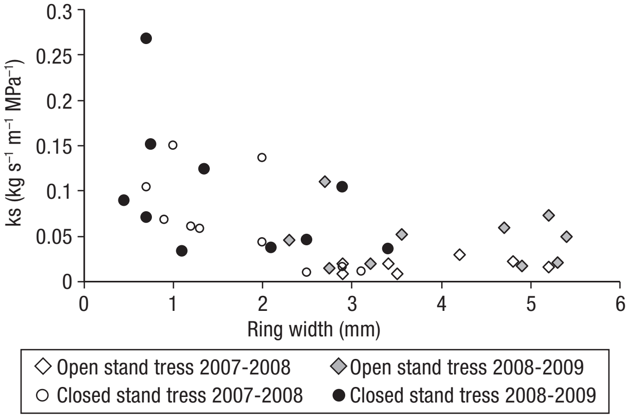

Figure 5. Xylem-specific hydraulic conductivity (ks) measured in the growth rings of season 2007-2008 and 2008-2009 (two growth rings measured each time in those trees with annual radial growth lower than 1 mm), as a function of ring width.

Discussion

Trees growing in the same site can experience very different stress intensities depending on the competition level. In the studied dry growing season, largest trees of the open stand increased their DBH in 13 (SD: 0.55) mm, whereas the smallest trees of the closed stand increased their DBH in 3 (SD: 0.22) mm (including trees with negligible stem growth), a great difference that should be attributed to both a high competitive level for nutrients and water, and a suppressed position in the stand to receive light. Differences in mean diameter growth of about 10 mm between ponderosa pines growing at different density stands have been also reported in another site in N.W. Patagonia (Gyenge *et al.,* 2010).

The observed differences in annual growth rate between trees were due to both differences in maximum growth rates and length of growth period. The largest trees of the open stand could grow during late summer, when all other trees were not growing (Fig. 2B). As was mentioned earlier, the model applied to the suppressed trees of the closed stand indicates that these trees were still growing during late summer, but it appears that this is an artifact of the model which presented a low R^2 due to the very low growth rates (around zero). New research including electronic dendrometric results may be necessary to elucidate the actual differences in growth period between individuals.

On the hand, trees of the open stand were less sensitive to drought than closed stand ones. This result is contrary to what was observed by McDowell *et al.* (2006). Those authors showed that ponderosa pines growing in open stands in Arizona, EEUU, were bigger and more sensitive to drought than those growing slowly in high competitive much closed stands. They proposed that those structures generated to lead high growth during wet periods (e.g. high leaf area, high hydraulic conductance) could in turn be detrimental under a drought event. They also proposed that trees growing under limiting conditions (due to high intraspecific competition) could not take advantage of wet periods because of chronic stressful conditions, but at the same time, could be less sensitive to a drought period. The maximum BA in Arizona stands was around 45 m^2 ha^{-1} at which mean tree BA increment was around 2 cm^2 $year^{-1}$, whereas the stand with the lowest BA (7 m^2 ha^{-1}) had a mean BA increment around 28 cm^2 $year^{-1}$ (McDowell *et al.,* 2006). Maximum BA in our study (the closed stand) was around 80 m^2 ha^{-1}, where tree BA increment was around 15 cm^2 $year^{-1}$. Moreover, at a similar BA of our open stand (around 38 m^2 ha^{-1}), BA increment in Arizona stands was 5 cm^{-2} $year^{-1}$ whereas in Patagonia was 58 cm^2 $year^{-1}$. These differences of stocking and basal area increment highlight the marked differences in resources availability for growth in both geographical locations, which may be responsible, at least in part, of the different sensibility to drought observed in open vs. closed stands between them.

The observed differences in growth rate reduction (i.e. sensitivity to drought) could be the result of significant differences in pre-dawn water potential of trees growing in the different stands, despite their similar soil water content. This has been observed in several studies describing the effect of intra-specific competition in *Pinus* species (eg. Kolb *et al.*, 1998; Warren *et al.*, 2001). However, it is quite novel considering the previously described behavior of ponderosa pines in other site in NW Patagonia (Meliquina Valley; Gyenge *et al.*, 2003), where pre-dawn water potential differences between plantation density conditions were never detected even during dry seasons. Comparing the study site with that at Meliquina Valley, average rainfall is similar between sites (Meliquina Valley: mean precipitation around 700 mm), but soil depth is very different (Meliquina site has deep soils, over 2 m in depth, Licata *et al.*, 2008), suggesting that this soil variable could be crucial in determining drought responses of ponderosa pines in Patagonia. Trees of the closed stand reached pre-dawn water potential values of –2 MPa in late summer, a threshold value for this species (e.g. Kolb *et al.*, 1998; Maherali and DeLucia, 2001). This means that ponderosa pines cannot open their stomata during the whole day if this value is reached at predawn. Potential differences in carbon fixation resulting from different stomatal conductance could result not only in the measured differences in stem growth, but also in carbohydrates stock, which may affect the growth of the next season, thus affecting the resilience of the trees. In this regard, McDowell *et al.* (2006) found that more sensitive trees (those with higher mean growth rate) were also more resilient after drought; while we expect so for those less sensitive trees of the open stand.

In contrast to growth rate, drought almost did not differentially affect functional wood anatomy of ponderosa pines. This is particularly relevant if we consider that ponderosa pine capacity to cope with drought is not directly related with plasticity for modifying its vulnerability to cavitation, but with the change of 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 (proportion between leaf area and sapwood area) and specific hydraulic conductivity (ks) of wood (Maherali and DeLucia, 2000). Those authors found that ks of branches was higher in pines growing in a drier than in a wetter site due to higher lumen diameters of the tracheids. Therefore, the increase in total conductance could help plants in drier environments to avoid high tension in the xylem. We supposed that an episodic drought event does not lead to the same sign in the wood formed during a dry season compared to trees growing in xeric areas, where the whole tree is better adapted to that condition. We expected that pines may experience a higher water stress instead leading to the formation of cells with lower lumen diameters, thus decreasing ks. Partially according to our expectation, significant differences were detected in lumen diameter of latewood tracheids, being smaller in the smallest trees of each stand. However, the hydraulic contribution of these small-lumen tracheids is expected to be very low (less than 0.05% of ks). In addition, some significant differences were observed in cell wall thickness of earlywood tracheids, being higher in open stand trees than in the smallest trees of the closed stand. However, as was also the case in latewood cells, no significant differences were observed in tracheids safety factor between tree groups. For these reasons, we expect that the observed changes in wood anatomy in response to higher water stress in some individuals have no significant impact on wood function (hydraulic conductivity and implosion resistance). Moreover, no differences were observed in the dimensions of the bordered pits between individuals of both studied stands. Provided bordered pits were of same dimension, we could not expect a differential vulnerability of the xylem to cavitation between trees of both stands.

Maherali and DeLucia (2000) found that tracheid length is subjected to environmental control in ponderosa pines when they compared montane vs. desert trees. In this study, no differences were observed in tracheid length between treatments. Figures were similar to those reported by Maherali and DeLucia (2000) for their largest trees of montane sites in USA, as well similar to those found in juvenile wood portions of a ponderosa pine growing in N.W. Patagonia (Zingoni *et al.*, 2005). As other anatomical traits just discussed, tracheid length resulted quite conservative across plant density stands and tree sizes.

Finally, growth rings formed in season 2008-2009 presented the same proportion of early and latewood, as it was described for other *Pinus* species (*P. taeda,* Cregg *et al.*, 1988; *P. brutia,* Guller, 2007) in response to different competition levels. In contrast, results from *P. pinaster* indicate that this species can modify the proportion of early- and latewood depending on growing conditions (Gaspar *et al.*, 2009). A similar proportion of latewood in both treatments could be the result of an earlier beginning in latewood formation in the closed stand, which may be linked to an earlier closure of annual diametric growth, as was reported in *P. taeda* (Cregg *et al.*, 1988). This is supported by the earlier water stress signs observed in closed stand trees compared to open stand trees, as well as by the longer growth period measured in some trees (largest ones) of the latter.

Conclusions

Differential effect of severe drought has been observed on diametric growth of ponderosa pines growing under different plantation densities, as well in a few wood anatomical variables. However, in this case, the observed changes are expected to have no impact on wood function (hydraulic conductivity and implosion resistance). The reduction of intra-specific competition through thinning could suppose a good practice for decreasing sensitivity to drought (and possibly increase resilience). Pines of the closed stand not only presented lower diameter growth but also were more severely affected by a drought event, result which is opposite to what has been previously reported for the same species growing in managed native forests in USA.

Acknowledgements

We gratefully acknowledge Martins family, owners of "La Veranada" Ranch, at which we carried out all field measurements, and Guillermina Dalla Salda and three anonymous reviewers for her valuable comments on the manuscript. We also thank Laura Borrelli (INTA EEA Bariloche) for borrowing laboratory equipment and providing her advice for tracheid length determinations. This work was supported by the National Institute for Agricultural Technology (INTA) of Argentina [grant PNFOR 2213].

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