

Effects of the time of drought occurrence within the growing season on growth and survival of *Pinus ponderosa* seedlings

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Abstract

Key message A drought event during spring produces a stronger and long lasting decrease in growth of ponderosa pine seedlings than a summer drought event. However, survival is not differentially affected.

Abstract Although there is certainty about the increasing frequency of extreme climatic events, the consequences of changing patterns of drought events within the growing season on the growth and survival of different species are much less certain. In particular, little knowledge is available on the differential effect on tree seedlings of a drought event at different times within the growing season. The objective of this study was to quantify the effect of a drought event imposed at different times over the growing season on the growth, survival and some related morphological and physiological variables of *Pinus ponderosa* seedlings from two seed sources. Four treatments were applied: control conditions; spring drought; summer drought and spring plus summer drought (SpSuD). A drought event in spring reduced stem growth and biomass accumulation in ponderosa pine seedlings during the occurrence of the drought and afterwards, even when plant

water status had recovered. The lack of growth recovery could not be associated with loss of stem hydraulic conductivity or reduction in stomatal conductance after drought. However, the spring drought did not differentially affect plant survival, as was the case with prolonged drought in the SpSuD treatment. The summer drought event had a significant but much smaller impact on plant growth. Our results suggest different consequences of a drought event in spring or in summer in ponderosa pine seedlings. This knowledge may be relevant to understand and predict tree seedlings responses to changing patterns of drought events within the growing season in the framework of climatic change.

Keywords Spring drought · Summer drought · Drought acclimation mechanisms · Ponderosa pine

Introduction

Human-induced climate change has the potential to alter the prevalence and severity of extreme climatic events such as heat waves, cold waves, storms, floods and droughts, which will probably increase in the future (Rusticucci 2005; Lehner et al. 2006; Hayhoe et al. 2007; Mundo et al. 2010). Differences in the degree and type of environmental stress, biomass allocation patterns, ontogenic changes in stress susceptibility, growth variability and genetic variability in drought resistance may differentially predispose seedlings and adult trees to remain alive or to die during severe drought (e.g. Niinemets 2010). In this regard, the development of adaptive strategies during early ontogeny seems to be a determining factor for survival in habitats with drought stress (Pratolongo et al. 2003). Though predicting changes in extreme drought events under a

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changing climate is difficult, understanding vulnerabilities to such changes is a critical part of estimating vulnerabilities and future climate change impacts on the environment and plant species (IPCC 2008).

Several studies have documented the influence of variable climatic conditions on the phenological stages of plants (e.g. Menzel and Fabian 1999; Kramer et al. 2000; Peñuelas and Filella 2001; Parmesan and Yohe 2003; Menzel et al. 2006), as well as the influence of a drought event at different times in the growing season on the growth of adult trees (e.g. Schweingruber 1996; Wimmer et al. 2002; Suarez et al. 2004; Mundo et al. 2010). However, information about the differential effect of the occurrence of a drought event at different times within the growing season on the tree-seedling stage is scarce.

Pinus ponderosa (Dougl.) Lawson is considered to be one of the most drought-tolerant native tree species in north-western USA, surviving in hot, dry sites where other tree species fail to establish (Daubenmire 1968). Characteristics of mature *P. ponderosa* trees and seedlings that contribute to increased survival on these sites are the maintenance of high water-use efficiency, osmotic adjustment of cell water, an extensive root system capable of utilizing deep soil water resources, high transpiration as a cooling mechanism protecting the plant from high soil temperature, wood anatomy plasticity leading to increased hydraulic conductivity in arid areas, and biomass allocation plasticity decreasing leaf area per unit of sapwood area in arid conditions compared to mesic sites (e.g. Daubenmire 1968, Jackson and Spomer 1979, DeLucia et al. 1988, DeLucia and Heckathorn 1989, DeLucia and Schlesinger 1991; Kolb and Robberecht 1996; Maherali and DeLucia 2001). But even *P. ponderosa* seedlings sustain high mortality from the combined effects of drought, high temperature and evaporative demand (Harrington and Kelsey 1979, Vance and Running 1985).

Managed forests of *P. ponderosa* provide several goods and services in North America. It is also the most widely planted forestry species in NW Patagonia, South America (approx. 77,000 planted hectares, Loguercio and Deccechis 2006), and the most highly recommended species for most forestry land with some environmental constraints in this region. The climate of NW Patagonia is highly variable, with summer drought. In this region, plants are exposed to highly unpredictable environmental conditions in time and space, which constrain their survival, regeneration and productivity (Paruelo et al. 1998).

Considering the ecological and commercial importance of the studied species as well as the lack of information about the general topic even in other tree species, the objective of this study was to quantify the effect of a drought event imposed at different times during the growing season on growth, survival and some related

morpho-physiological variables in *P. ponderosa* seedlings. We hypothesized that a spring drought event would have a greater effect on its growth than a summer drought event, since this species grows mainly during spring as a normal strategy to avoid summer drought stress. Since some studies (e.g. Cregg 1994; Olivas-García et al. 2000) have demonstrated that there is intraspecific variability in ponderosa pine seedlings responses to drought, two different seed sources were analyzed.

Materials and methods

Experimental design

The experiment was conducted in a hydroponic system in a greenhouse during the 2009–2010 Southern Hemisphere growing season (October–March) at Bariloche Agricultural Experimental Station of the National Institute of Agricultural Technology, Argentina (INTA; 41° 07' 26'' S; 71° 15' 08'' W; 787 m a.s.l.). Drought stress was simulated adding polyethylene glycol (PEG 6000) periodically, decreasing the water potential of the nutrient solution. The hydroponic system was built using 11-cm diameter and 3 m length PVC tubes connected to two recirculation pumps that flushed water from and to two 100 l water tanks. Ten 2-year-old *P. ponderosa* seedlings were placed in each PVC tube, 20 cm apart from each other. Each treatment (see below) had three true replicates (PVC tubes). Two local seed sources [El Bolsón (EB); 41° 58' S, 71° 33' W and Huinganco (H); 37° 09' S, 70° 37' W] were used, and analyzed as separate taxa in order to evaluate potential differences between sources. Each tube thus contained five plants from the EB seed source and five from the H seed source (15 plants from each source per treatment, distributed in three tubes). Plants were installed in the tubes in early September, and the experiment began on October 20, when the first dose of PEG was added to some treatments.

One of the tanks contained a nutrient solution (NPK 18-7-17, New Plant Argentina), and was connected to all tubes that received the control solution at each time. The second tank also contained the nutrient solution, but received increasing amounts of PEG 6000 during the growth season. The amount of PEG added was determined from preliminary experiments, in which we evaluated the relationship between the PEG solution and the plants' water potential. The target maximum stress level was defined as such leading to a complete stomatal closure of the seedlings throughout the whole day (around -2 MPa in pre-dawn; Olivas-García et al. 2000). This was reached gradually adding PEG at a rate of $14\text{--}28$ gl^{-1} of water per day every 3 days, with one water stress cycle in spring and

another in summer. Between these cycles, plants water status was allowed to recover by connecting their tubes to a nutrient solution without PEG (similar to the control solution).

Four water stress treatments were applied: (1) control (C), with nutrient solution during the whole growing season (October–March), (2) spring drought (SpD), (3) summer drought (SuD) and (4) spring plus summer drought (SpSuD). SpD plants were connected to the PEG solution from October 20 to December 14, and to the control solution afterwards. SuD plants were connected to the control solution during the spring and to the PEG solution in summer (from January 15 to March 8). SpSuD plants were connected to the PEG solution during spring and summer, with a period without PEG in the middle (from December 15 to January 14). The maximum accumulated PEG concentration at the end of each drought cycle was 252 and 302 gl^{-1} of solution in the spring and summer cycle, respectively. The different final concentrations were due to the fact that higher pre-dawn water potentials were observed, for a similar PEG concentration, in the second than in the first drought cycle (see “Results” section).

Plant response variables

Before the beginning of the experiment, destructive measurements of total biomass (TB) and its allocation to the different plant compartments (root, stem and leaves) were performed on 12 seedlings per source. It showed that the TB of H plants was almost double that of EB plants [8.2 (SD 2.6) and 4.5 (SD 1.8) grams per plant, respectively] but with similar allometric relationships ($p > 0.05$, data not shown).

Plant water potential (Ψ_{pd} , MPa) was measured periodically (every 15–20 days) in four seedlings (one fascicle per seedling) from each source and treatment at pre-dawn with a pressure chamber (PMS Instrument Company, Model 1003, USA). In addition, leaf relative water content (R) was recorded every 40 days in four seedlings per source and treatment. Fresh weight (FW, g), fresh weight at full hydration (HW, g) and dry weight (after 72 h oven-drying at 60 °C; DW, g) were determined in one fascicle per plant using a precision scale. Full hydration was attained putting the leaf in distilled water overnight. This was done after fresh weight (natural conditions at the sampling date) was measured. R was calculated as:

$$R = \frac{(FW - DW)}{(HW - DW)}$$

Stem basal diameter and plant height were measured every 15 days in all the seedlings with a caliper, and then used to estimate diameter and height growth rates. Seedling survival was also recorded on the same dates.

In the middle of the first drought cycle (late November) and at the end of the experiment (late March), destructive sampling of 4–5 seedlings per source and treatment was performed. On these dates, dry biomass of the different plant compartments was determined, as well as the following variables: Hüber ratio [leaf biomass (g)/stem cross-sectional area (cm^2)], aboveground: belowground biomass ratio; specific leaf area (SLA), Pressure–Volume (P – V) curves (see below) and stem specific hydraulic conductivity (k_s).

SLA was determined in needles of three fascicles per seedling ($n = 4$); their area was estimated following the methodology described in Gyenge et al. (2009). After this, needles were oven-dried at 60 °C to constant weight. SLA was calculated as the ratio between green leaf area (m^2) and dry leaf biomass (kg).

In order to assess adjustments in osmotic potential and cell wall elasticity in the different treatments, P – V curves were plotted ($n = 5$) by means of the free transpiration technique following Corcuera (2003). We focused our analysis on the following parameters estimated from P – V curves: osmotic potential at saturation (π_{sat} , MPa), water potential at the turgor loss point (Ψ_{tlp}), relative water content at turgor loss point (RWC_{tlp} , %) and maximum modulus of elasticity (e_{max} , MPa) of cell walls.

Specific hydraulic conductivity (k_s) of stem wood was measured in four seedlings per treatment and source following the pipette methodology described in Fernández et al. (2010). The stem segment (approximately 4 cm long) used to estimate k_s was cut below branch insertion. Stem segments were connected with a transparent hose to a graduated pipette placed 1 m above the segment, thus applying a water column pressure of 0.01 MPa (ΔP , see next equation). Sapwood cross-sectional area was estimated from the mean diameter of both segment ends. Conductivity normalized for sapwood area (k_s , $\text{m}^2 \text{s}^{-1} \text{MPa}^{-1}$) was thus calculated as:

$$k_s = \frac{Ql}{A_s \Delta P}$$

where Q volume flow rate ($\text{m}^3 \text{s}^{-1}$); l segment length (m); A_s sapwood area (m^2); and ΔP difference in pressure between the two ends of the segment (MPa).

Stomatal conductance (g_s , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) was measured on three dates when plants presented different water status (January 26, February 16, and March 8, 2010). Measurements were performed on four seedlings per treatment at about 10.00 a.m. (when maximum stomatal opening was expected) using a PMR-5 steady state porometer connected to a EGM-4 CO_2 analyzer (PP Systems Int. Inc., Amesbury, USA). All measurements were carried out in completely expanded needles of the upper third of the seedling at saturating photosynthetic active radiation (PAR).

Intrinsic water use efficiency of plants from the different sources and treatments ($n = 4$) was estimated at the end of the experiment from the proportion of the rare C^{13} isotope in leaves expanded during the experiment. δC^{13} was analyzed using an isotope ratio mass spectrometer (Finnigan MAT Delta S, USA) following the methodology of Hoefs and Schidlowski (1967) and Panarello (1987) at the Institute of Geochronology and Isotopic Geology (CONICET-National University of Buenos Aires, Argentina).

Wood anatomy, particularly the number of rows of cells and their lumen diameter formed during the growing season, was analyzed in stem wood samples taken at the end of the experiment. Five plants per treatment and source were analyzed. Microscopic slides were prepared following the usual methods of softening, cutting (hand-cut with a scalpel) and staining with safranin (diluted in 50 % water and 50 % alcohol). Cross-sectional preparations were observed by means of light microscopy (microscope Olympus BX 41). Digital photographs were taken with a camera (Olympus Evolt E-330 SLR) attached to the microscope. The number of cell rows in the last growth ring (i.e. the one produced during the experimental period) was counted at $40\times$ magnification in two to three preparations per plant. In one preparation per plant, 15 consecutive cells were selected at the central, medium and external portions of the last growth ring. The same procedure was applied in four positions around the external growth ring, thus selecting 60 cells per ring-zone per preparation ($60 \text{ cells} \times 3 \text{ ring-zones} = 180 \text{ measured cells per plant}$). Lumen diameter was measured in these cells (following radial direction) from photographs taken at $100\times$ magnification using Image J free-software (Image J 1.37v, National Institute of Health, USA). We applied this criterion in order to describe lumen diameter in the whole ring in which no clear transition between early- and late-wood was observed.

The mean conduit hydraulic diameter (d_H) was estimated on the basis of the conduit's contribution to wood hydraulic conductivity. According to the Hagen-Poiseuille formula, a conduit's hydraulic conductivity is proportional to the fourth power of its diameter. Each value of conduit diameter (d) was multiplied by its fourth power ($d \times d^4 = d^5$) to represent the conduit in proportion to its contribution to conductivity. The mean of this hydraulically weighted diameter distribution was the sum of all conduit diameters ($\sum d^5$) divided by the total number of conduits ($\sum d^4$, Sperry and Saliendra 1994).

Statistical analysis

Non-linear regression was applied to fit growth curves to each source and treatment using GraphPad Prism 5.04 software (GraphPad Software, San Diego, CA, USA). A

visual inspection of the data showed more or less symmetrical sigmoid growth patterns well described by a logistic equation. Its mathematical expression is:

$$y = K / (1 + be^{-ax})$$

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 accuracy of the fitted parameters was examined via the ratios between the standard error of estimation and the best-fitted values. We used an F test to compare the models between plant sources and treatments within sources (Zar 1999).

The following variables were compared with two-way ANOVA for detecting differences between treatments and plant sources: total biomass, aboveground : belowground ratio, Hüber ratio, SLA, P - V curves parameters, k_s , leaf R , g_s in each date, leaf δC^{13} , mean lumen diameter of tracheids and mean hydraulic diameter of tracheids. We considered the seedling source (at two levels, EB and H) and the treatment (with four levels: C, SpD, SpSuD, SuD) as factors. Assumptions of normality and homocedasticity were checked and when they were met, post-comparisons were performed with Tukey tests. When data did not fulfill statistical assumptions, we transformed the variables or we used non-parametric Mann-Whitney (Wilcoxon) two-sample tests or Kruskal-Wallis test. It is important to note that all morpho-physiological variables were measured in living plants at the date of measurement, maintaining the same n (as described above) throughout the experiment except for growth determinations. For this variable, which it was measured on all the plants of the experiment ($n = 15 \times 4 \text{ drought treatments} \times 2 \text{ seed sources} = 120 \text{ plants}$), n decreased with time due to some plants' mortality.

Results

Growth, biomass allocation and survival

Plant predawn water potential (Ψ_{pd}) measured throughout the experimental period reflected the two imposed drought cycles (Fig. 1). A mean minimum Ψ_{pd} of about -1.7 MPa was reached in mid-December and then in mid-March. As stated before, in the second cycle, although the addition of PEG was similar to that in the first cycle, more time was needed in order for the seedlings to attain similar Ψ_{pd} than during the spring. Some differences in Ψ_{pd} values were observed between plant sources in response to PEG addition. On some of the early measurement dates (November 4 and 24, 2009), stressed plants of EB origin had similar Ψ_{pd}

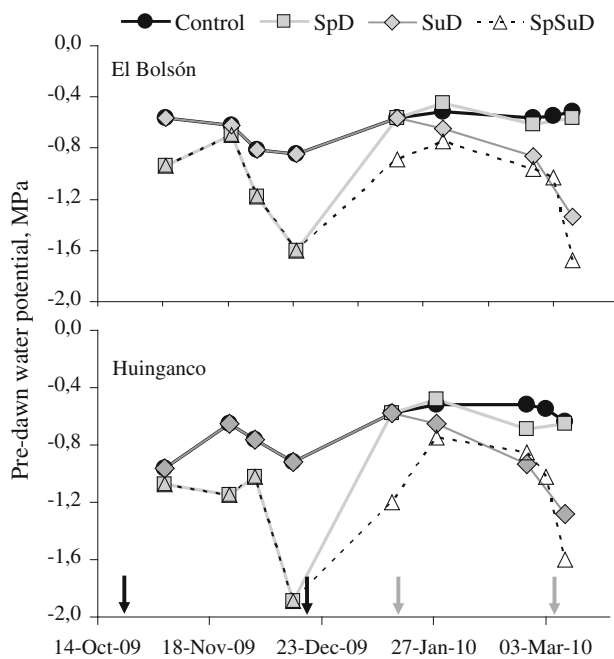


Fig. 1 Mean values of predawn water potential measured in ponderosa pine seedlings of each treatment throughout the growing season. Statistical differences between treatments and sources are discussed in the text. *Upper panel* El Bolsón source plants, *lower panel* Huinganco source plants. *SpD* spring drought; *SuD* summer drought; *SpSuD* spring plus summer drought. *Black arrows* indicate the beginning and end of PEG addition during the spring. *Grey arrows* indicate the beginning and end of PEG addition during the summer

values than control plants ($p > 0.05$) and higher than stressed plants of H source ($p < 0.05$; Fig. 1 showing mean values). Fifteen days after the beginning of the second drought stress cycle (January 28, 2010) only plants from the EB source in the SpD treatment had significantly higher Ψ_{pd} values than plants in SpSuD treatment ($p < 0.05$) even though plants from the H source were subjected to similar conditions (Fig. 1). On February 25, 2010, SpD plants from the H source only differed significantly from the SpSuD treatment ($p < 0.05$) but not from those of SuD ($p > 0.05$; Fig. 1 showing mean values), suggesting a lower capacity of water status recovery in this source than in EB plants.

From our results, three different types of plants differential Ψ_{pd} responses to PEG addition can be noted: (a) under the same amount of PEG addition, the Ψ_{pd} of plants from both sources was higher in summer than in spring; (b) under the same amount of PEG addition, we found differences in water potential between plants from the two sources; seedlings from the H source had lower water potential values on some dates than EB seedlings; and (c) under the same amount of PEG in summer, plants previously subjected to drought stress (but after a recovery period, like those in SpSuD treatment) had lower Ψ_{pd} than plants recently exposed to drought stress (SuD).

In spite of the fact that initial biomass measurements (September, just before plants were installed in the hydroponic system) indicated that H plants were larger than EB plants (see “Materials and methods” section), the first basal stem diameter and plant height determination carried out on October 19 (immediately prior to the first PEG addition), indicated that there was no difference between origins, suggesting a higher initial stem growth and/or acclimation capacity to hydroponic conditions in EB source than in H source. Mean (SD) initial basal diameter was 4.64 (0.77) and 4.99 (0.72) mm in EB and H plants, respectively. Mean (SD) initial plant height was 16.23 (3.20) and 16.68 (3.75) cm in EB and H plants, respectively.

Relative diameter and height growth, and TB production at the end of the first drought cycle (from mid-October to mid-December) showed significant differences between treatments (Fig. 2, left panels, showing relative growth data; $p < 0.05$), with no differences between sources in diameter growth ($p > 0.05$) but a higher height growth and TB in H than EB ($p < 0.05$ for both variables). However, no changes in biomass allocation were observed between treatments or sources ($p > 0.05$). Relative growth differences between treatments were even higher after the 30-day recovery period (Fig. 2, right panels). Since initial stem diameter and height were similar between sources, the same patterns were observed in terms of absolute growth (data not shown).

Considering the whole study period (October–March), stem basal diameter increased almost constantly under control conditions. A similar pattern was observed in the SuD treatment, except for the last two measurement dates (last month), in which there was no increase in stem diameter (Fig. 3). In spite of the fact that the water status recovered in the SpD treatment in the second experimental period (Fig. 1), diameter growth in this treatment was fairly similar to that in the SpSuD treatment, and both tended to be lower than in C and SuD (Fig. 3). Statistical analyses revealed that growth models were different in each treatment within each source, with significant differences between all treatments in K parameter (maximum growth) in the case of diameter growth, but no differences in maximum height growth between C and SuD or between SpD and SpSuD in plants from the EB source (Table 1). In addition, differences in height growth models between sources were found in all treatments, in which H plants presented higher height growth than EB plants (Fig. 4).

A similar trend was observed in total biomass, which presented significant differences between some treatments (C = SuD > SpD = SpSuD) and sources (H > EB) at the end of the experimental period (Table 2). At this moment, EB plants had higher relative aboveground allocation than H plants; however when each separate treatment was

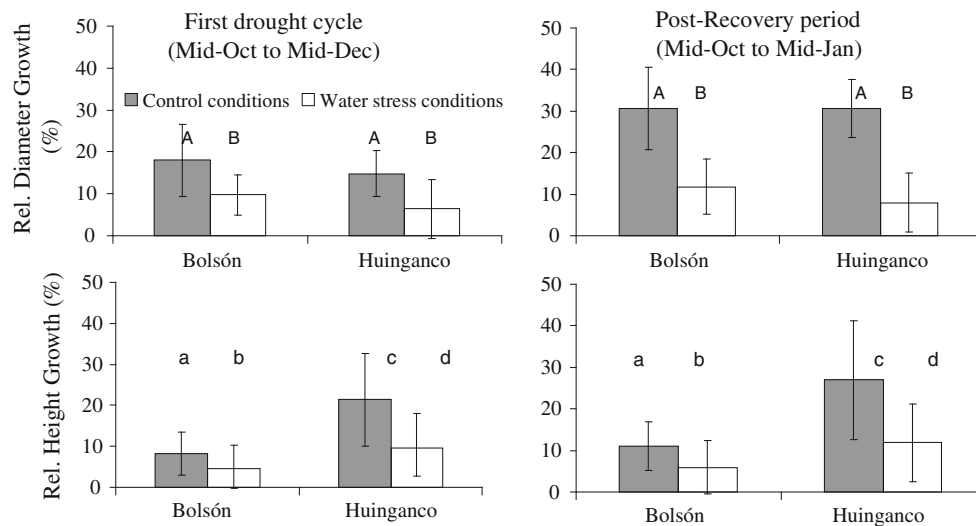


Fig. 2 Relative basal diameter growth (*upper panel*) and relative height growth (*lower panel*) measured in ponderosa pine seedlings from El Bolsón and Huinganco sources (Patagonia, Argentina) at the end of the first drought cycle (mid-December 2009, *left panels*) and a

month later, after a 30-day recovery period applied to previously drought-stressed plants (*right panels*). Different letters within each chart indicate significant differences (two-way ANOVA, and post hoc comparisons, $p < 0.05$)

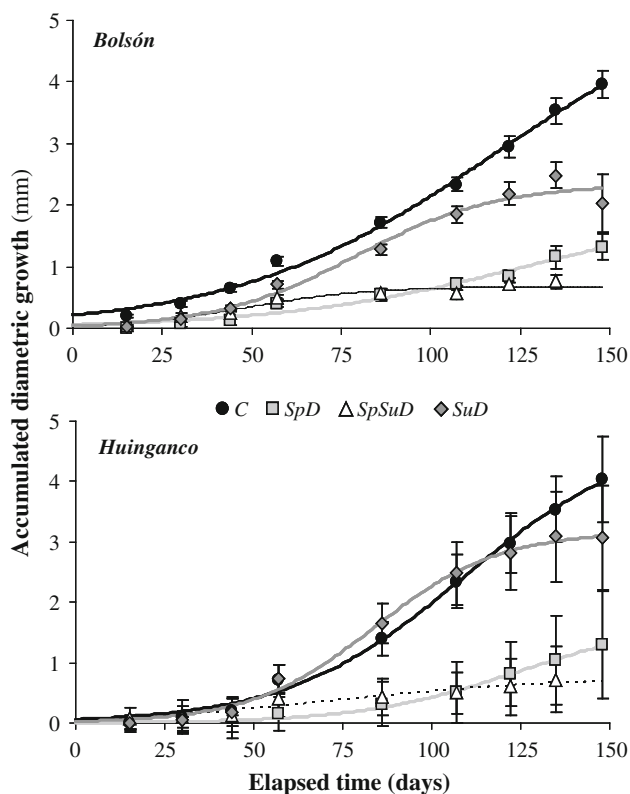


Fig. 3 Accumulated stem basal diameter growth (mm) since the beginning of the experiment (days) in ponderosa pine seedlings from El Bolsón (*upper panel*) and Huinganco sources (*lower panel*), Patagonia, Argentina. Circles, squares, triangles and diamonds indicate the mean values (\pm SE) of 15 individuals in each treatment. Lines represent the logistic models adjusted to each treatment

considered, no difference was observed between sources (Table 2). Regarding the treatment effect, seedlings in C and SpD treatments showed higher above: belowground biomass ratio than SpSuD and SuD treatments (Table 2). In addition, the Hüber ratio for seedlings from the EB source was similar in all treatments, whereas for H seedlings it was lower when they were growing under C and SuD than in SpD and SpSuD treatments (Table 2).

Survival was almost identical (about 90 %) in C, SpD and SuD treatments for both plant sources. By contrast, SpSuD treatment resulted in higher plant mortality of approximately 30 % (i.e. survival 70 %).

Morpho-physiological variables

In contrast to growth results, in general no differential leaf or wood morpho-physiological adjustments were observed in response to drought treatments, except for the stomatal control of gas exchange.

The only change in *SLA* was observed at the end of the experiment in the SpSuD treatment (Table 2), where plants presented higher *SLA* than in C conditions as a consequence of a lower biomass per leaf in SpSuD than in C.

Specific hydraulic conductivity was not affected by the treatments (Table 2), which was consistent with similar tracheids dimensions of the ring formed in the different treatments during the studied growing season (Table 3). However, considering plants source effect, H wood cells presented significantly larger lumen diameter (d) and hydraulically weighted lumen diameter (d_H) than EB plants

Table 1 Parameters of the logistic models fitted to growth data and goodness of fit value (R^2) for the factors Source (EB El Bolsón, H Huinganco), H Huinganco and Drought Treatment (control; SpD spring drought; SpSuD spring and summer drought; SuD summer drought)

Variable/parameter	Origin/treatment							
	EB-Control	EB-SpD	EB-SpSuD	EB-SuD	H-Control	H-SpD	H-SpSuD	H-SuD
Height								
<i>K</i>	47.5 ± 20.3	31.6 ± 43.2	17.6 ± 8.3	28.3 ± 2.5	65.7 ± 9.9	35.6 ± 24.2	27.3 ± 3.4	61.4 ± 7.0
<i>B</i>	11.9 ± 4.0	10.0 ± 11.7	12.4 ± 14.8	18.2 ± 8.2	14.3 ± 5.8	14.3 ± 7.0	6.0 ± 3.3	11.3 ± 3.9
<i>A</i>	0.023 ± 0.464	0.016 ± 0.011	0.036 ± 0.030	0.040 ± 0.009	0.033 ± 0.008	0.022 ± 0.010	0.038 ± 0.016	0.033 ± 0.008
R^2	0.464	0.246	0.114	0.603	0.549	0.349	0.320	0.590
Diameter								
<i>K</i>	5.7 ± 0.9	2.1 ± 1.2	0.7 ± 0.1	2.3 ± 0.2	4.8 ± 0.4	1.7 ± 0.7	0.7 ± 0.2	3.2 ± 0.1
<i>B</i>	25.7 ± 4.4	35.4 ± 16.6	17.2 ± 15.2	56.1 ± 46.6	80.5 ± 23.0	274.7 ± 428.5	11.5 ± 7.5	12.17 ± 64.1
<i>a</i>	0.027 ± 0.004	0.028 ± 0.009	0.059 ± 0.021	0.051 ± 0.012	0.040 ± 0.004	0.045 ± 0.019	0.033 ± 0.017	0.057 ± 0.007
R^2	0.883	0.520	0.426	0.581	0.938	0.520	0.282	0.883

Significance of differences between models and parameters are commented in the text

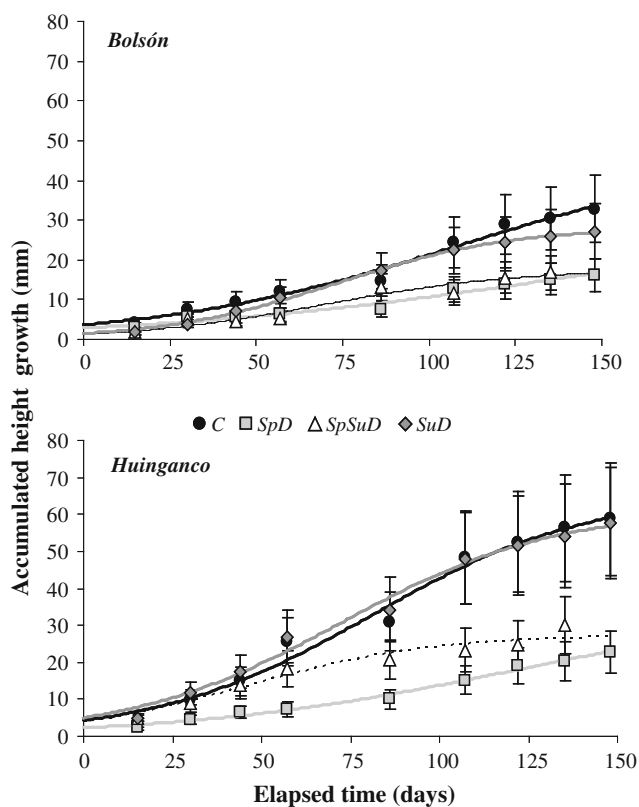


Fig. 4 Accumulated height growth (mm) since the beginning of the experiment (days) in ponderosa pine seedlings from El Bolsón (upper panel) and Huinganco sources (lower panel), Patagonia, Argentina. Circles, squares, triangles and diamonds indicate the mean values (\pm SE) of 15 individuals in each treatment. Lines in the graph represent the logistic models adjusted to each treatment

($p < 0.05$). All drought treatments had lower number of cell rows than in C plants, with no difference between sources (Table 3). The only significant difference between drought treatments was observed between SpSuD and SuD (Table 3).

Neither osmotic nor elastic adjustments ($p > 0.05$ comparing control and water stressed plants) were observed in response to the different drought treatments in the measurement dates, and no differences between plant sources ($p > 0.05$) were detected in almost all the estimated parameters. In late-November mean (SD) π_{sat} was -1.23 (0.6) MPa, mean (SD) e_{max} was 3.72 (3.48) MPa and mean (SD) Ψ_{tlp} was -2.06 (1.05) MPa. However, a source effect ($p = 0.03$) was observed in RWC_{tlp} , with EB seedlings presenting higher RWC_{tlp} (80 ± 1 %) than H plants (69 ± 1 %). At the end of the experiment, mean (SD) π_{sat} was -1.28 (0.4) MPa; mean (SD) e_{max} was 0.06 (0.07) MPa; mean (SD) Ψ_{tlp} was -2.01 (0.58) MPa, and mean (SD) RWC_{tlp} was 84 (6) %, with no differences between treatments and seed sources in neither parameter ($p > 0.05$ in all cases).

Relative water content (R) of the leaves was affected by source and treatment in mid-November, with interaction

Table 2 Mean \pm standard deviation values of total biomass (TB), Hüber ratio (Hüber), aboveground/belowground biomass ratio (*A/B*), specific leaf area (SLA), stem specific hydraulic conductivity (k_s) and δC^{13} in leaf tissue (δC^{13}) measured at the end of the experiment in ponderosa pine seedlings of control, spring drought (*SpD*), spring and summer drought (*SpSuD*), and summer drought (*SuD*) treatments

Origin/treatment	TB (g)	Hüber (kg m ⁻²)	<i>A/B</i>	SLA (m ² kg ⁻¹)	k_s (ml MPa ⁻¹ s ⁻¹ cm ⁻¹)	δC^{13} (%)
El Bolsón						
Control	23.2 \pm 7.8 aB	14.0 \pm 3.0 aA	1.6 \pm 0.3 aA	12.2 \pm 1.0 aA	0.73 \pm 0.14	-24.84 \pm 0.90
SpD	10.6 \pm 4.2 bB	13.0 \pm 3.0 aA	1.8 \pm 0.6 aA	14.9 \pm 2.2 aA	0.59 \pm 0.23	-24.44 \pm 1.36
SpSuD	11.2 \pm 6.4 bB	14.0 \pm 5.0 aA	1.2 \pm 0.3 bA	14.6 \pm 4.2 aA	1.17 \pm 0.61	-25.88 \pm 1.53
SuD	23.6 \pm 14.0 aB	20.0 \pm 4.0 aA	1.4 \pm 0.4 bA	12.1 \pm 1.2 aA	0.83 \pm 0.47	-25.38 \pm 0.33
Huinganco						
Control	30.8 \pm 7.0 aA	7.0 \pm 2.0 bB	1.1 \pm 0.2 aB	12.5 \pm 1.0 abA	1.03 \pm 0.51	-25.76 \pm 1.59
SpD	14.8 \pm 6.9 bA	17.0 \pm 1.3 aA	1.2 \pm 0.1 aB	12.0 \pm 2.2 bA	0.51 \pm 0.13	-24.10 \pm 2.00
SpSuD	14.8 \pm 5.8 bA	9.0 \pm 3.0 abA	1.0 \pm 0.2 bB	16.9 \pm 2.3 aA	0.68 \pm 0.33	-24.34 \pm 1.86
SuD	37.4 \pm 9.0 aA	5.0 \pm 1.0 bB	0.8 \pm 0.1 bB	14.4 \pm 2.6 abA	1.03 \pm 0.28	-25.44 \pm 1.27
p_{treat}	<0.01	0.031	<0.01	0.017	0.107	0.299
p_{origin}	<0.01	<0.01	<0.01	0.483	0.909	0.626
$p_{\text{treat} \times \text{origin}}$	0.258	<0.01	0.245	0.054	0.111	0.308

p_{treat} treatment effect, p_{ori} origin effect, $p_{\text{treat} \times \text{ori}}$ interaction effect

Different lowercase letters in each column indicate significant differences between treatments within each source. Different capital letters in each column indicate significant differences between sources within each treatment. No significant difference was observed in δC^{13} and k_s values between treatments and sources. Probability values of two-way ANOVA are also presented

Table 3 Mean and standard deviation (SD) values of anatomical variables measured in wood rings of ponderosa pine seedlings of different treatments and sources

Origin	Control			SpD			SuD			SpSuD		
	RW (μm)	<i>d</i> (μm)	d_H (μm)	RW (μm)	<i>d</i> (μm)	d_H (μm)	RW (μm)	<i>d</i> (μm)	d_H (μm)	RW (μm)	<i>d</i> (μm)	d_H (μm)
El Bolsón												
Mean	1748.5	9.7	13.26	645.5	9.1	12.51	947.1	9.7	12.67	484.7	10.0	13.79
SD	444.4	1.1	1.84	76.2	0.6	2.20	92.9	0.4	1.49	103.2	0.6	0.72
Huinganco												
Mean	1417.2	12.2	23.51	690.3	11.5	14.05	879.4	12.6	16.42	543.1	12.5	17.21
SD	163.7	1.5	14.70	155.6	1.4	1.50	189.4	2.7	3.97	203.7	3.5	5.97
Significance	RW	<i>d</i>	d_H									
p_{treat}	$p < 0.05$	$p > 0.05$	$p > 0.05$									
p_{ori}	$p > 0.05$	$p < 0.01$	$p < 0.05$									
$p_{\text{treat} \times \text{ori}}$	$p > 0.5$	$p > 0.05$	$p > 0.05$									

RW ring width, measured in digital photographs taken at 40 \times magnification; *d* lumen diameter; d_H hydraulically weighted lumen diameter, *SpD* spring drought; *SuD* summer drought; *SpSuD* spring and summer drought; p_{treat} treatment effect; p_{ori} origin effect; $p_{\text{treat} \times \text{ori}}$ interaction effect. Probability values of two-way ANOVA are also presented

between the two factors. H seedlings in C conditions had higher leaf *R* than EB plants (94 \pm 4 and 83 \pm 4 %, respectively; $p < 0.05$). In contrast, seedlings under drought stress (moderate at that time) showed similar *R* values for both sources (mean of both sources 86 \pm 3 %), thus resulting in no difference between treatments in EB plants ($p > 0.05$) but significant differences between control and stressed plants from the H source ($p < 0.05$). A month later (December 18, 2009) source and

treatment effects were observed with no interaction between them. Seedlings of EB source had higher *R* than those of H source (84 \pm 4 and 80 \pm 3 %, respectively, $p < 0.05$). For both sources, leaf *R* was higher in C conditions than in water-stressed seedlings (mean values for both sources 82 \pm 4 and 72 \pm 11, respectively; $p > 0.05$). However, on the remaining measurement dates, *R* showed no statistically significant difference between sources or treatments (general mean \pm SD 88 \pm 6 %). From

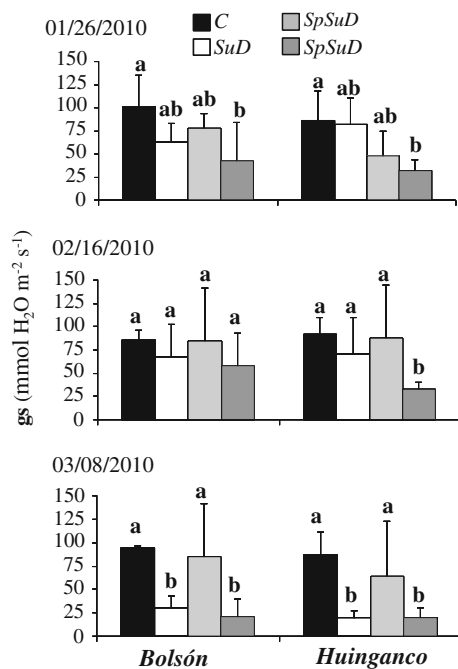


Fig. 5 Mean values (\pm SD) of stomatal conductance (g_s) measured in ponderosa pine seedlings of the different treatments and sources on January 26, February 16, and March 8, 2010. Different letters indicate statistical differences between treatments for each source. No statistical difference between sources was found on any of the dates considered

comparison of R with RWC_{tlp} and Ψ_{pd} with Ψ_{tlp} , it seems that leaf turgor was maintained during the whole experiment in all surviving plants of both seed sources in all the treatments.

Stomatal conductance (g_s) measured in January was significantly lower in SpSuD than in C plants (Fig. 5, upper panel) although the seedlings under water stress conditions (both SuD and SpSuD) did not show very negative water potential values (Fig. 1). Seedlings in SuD and SpD treatments showed intermediate g_s values between both SpSuD and C treatments (Fig. 5). By mid-February, although the hydroponic medium had a high concentration of PEG, the predawn water potential values were very similar to those recorded on the previous measurement date (a month earlier) (Fig. 1). This was also reflected in g_s values, which was similar between treatments, except in H plants of the SpSuD treatment (Fig. 5). Plants under SpSuD and SuD treatments (Fig. 1) only showed a significant decrease in g_s compared to plants under C and SpD treatments (Fig. 5) towards the end of the experiment (March), when their pre-dawn water potential reached relatively low values.

The few observed differences in g_s were not reflected in intrinsic water use efficiency, as suggested by δC_{13} . In this regard, all leaves presented similar concentration of the rare isotope (Table 2), with no source or treatment effects.

Discussion

The use of PEG for simulating water shortage has been applied in numerous studies because it can allow a progressive uniform application of drought treatment to different individuals. Its use in other pine species, such as *Pinus pinaster*, *Pinus canariensis* and *Pinus halepensis* (e.g. López et al. 2009; Calamassi et al. 2001), has proved to be useful in this type of experiments but attention must be paid to the rate and amount of PEG addition. In contrast, other studies have found severe toxic effects on plants, such as in *Pinus banksiana* and *Eucalyptus grandis* (Fan and Blake 1997; Tesón & Licata, INTA EEA Concordia, personal communication). In the case of *P. ponderosa* seedlings, we performed a preliminary experiment 1 year prior to the present study in order to determine their general response to PEG 6000 and the relationship between its concentration in the nutritional solution and plant water potential. We found a linear relationship between both variables (unpublished data), which was used for planning the amount and rate of PEG addition with a target minimum plant pre-dawn potential of about -2 MPa. In spite of the fact that the plants' water potential followed the theoretical value (based on the relationship with PEG concentration) quite well during the first drought cycle, similar amounts of PEG in the second drought cycle led to higher pre-dawn potential than expected, which resulted in a delay in the imposition of drought—compared to the first drought cycle— and the need for a higher PEG concentration at the end of the experimental period. These results highlight the difficulties in predicting plant response (in terms of water status) to PEG precisely, at least in rather long-term experiments such as the present 6-month study.

Beyond the methodological issue, which is interesting itself in the light of protocol development for screening drought-resistant genotypes in plant breeding programs, the main result of this study is the differential plant response—in terms of growth and biomass production—to a drought event imposed in spring or in summer. A drought event in spring led to a significant reduction in stem growth (diameter and height) as well as in plant biomass accumulation almost of the same magnitude as that observed in long-term drought stress during spring plus summer (SpSuD). It is important to note that SpD plants recovered their water status and g_s in the second part of the experiment, to similar levels as those of control plants. However, they did not recover their growth capacity. In this regard, in spite of the fact that under field conditions *P. ponderosa* grows mainly in spring—due to usual summer drought—in this experiment with high water availability during spring and summer, plants under control conditions maintained positive growth throughout the study period. Similarly to our results, a study carried out in adult ponderosa pines showed that a

severe drought event during spring affected carbon fixation (as determined by the Eddy-covariance method) during the rest of the growing season (Goldstein et al. 2000). Similar results were observed in a poplar plantation in China; its carbon fixation was severely and long-lastingly affected by a spring drought event and it was hardly affected by a late-growing season drought (Zhou et al. 2013). In the case of the ponderosa pine study (Goldstein et al. 2000), the authors attributed this effect to potential losses in hydraulic conductance due to xylem cavitation (although they did not measure this variable). In our study, no significant losses in stem k_s were observed, at least on the dates on which this variable was measured (middle of the first drought cycle and at the end of the experimental period). However, a trend of a k_s decrease in SpD and SpSuD treatments was observed in H plants, which deserves future research increasing the number of studied plants to reduce the standard deviation of data as well as the development of vulnerability to cavitation curves comparing both seed sources. In addition, with our data we cannot rule out the possibility of k_s losses followed by cavitation repair at the time of our measurements. However, it has been suggested that this process has limited magnitude in conifers branches and stems, in accordance with their low parenchyma proportion in xylem (e.g. Johnson et al. 2012; Meinzer and McCulloh 2013). At our knowledge, no particular studies are available quantifying cavitation repair in ponderosa pine. Therefore, in spite of we cannot definitively reject the possibility of any k_s decrease in some dates, the rigorous stomatal control of transpiration of ponderosa pine during drought conditions such as those experienced (e.g. Gyenge et al. 2012) enables us to suggest that this was not the case, but surviving seedlings were able to maintain most of their hydraulic integrity throughout the experiment. In addition, anatomical determinations indicated that d_H was similar between treatments, suggesting that theoretical k_s may be similar between different drought conditions. As a consequence, we cannot attribute the lack of growth recovery during summer in SpD plants to losses or reductions in stem k_s due to cavitation or decreased tracheid d . A study carried out in *Fagus sylvatica*, a drought-sensitive species, showed that in spite of the fact that water potential immediately recovered with irrigation after a severe drought period of 1 month, photosynthesis and g_s did not recover at the same rate (Gallé and Feller 2007). In that study, photosynthesis did completely recover a month later and g_s did not recover completely at all. In our study, g_s of SpD plants recovered at least a month after drought ceased (our first measurement of this variable), and therefore neither can a reduction in this variable be the cause of reduced growth during summer.

Similar tracheid size observed in the different treatments, even after two consecutive drought cycles, are in agreement with similar results in adult ponderosa pines

growing under field conditions in Patagonia, which were also subjected to severe water stress (Fernández et al. 2012). It seems that lumen size of xylem cells is a very conservative trait in this species, which—since it depends on the capacity of cell expansion due to turgor—must require an internal optimum maintenance of stem water status even during severe and quite long-lasting drought events. This result contrasts with those reported for other conifer species, in which lumen size clearly decreased when they were subjected to water shortage (e.g. Barij et al. 2007; Pasho et al. 2012). Stem water status was maintained in the different treatments of the present study except for some plants in the SpSuD treatment, which showed evident dehydration symptoms in their leaves and stem and died during the second period of the experiment. Hydraulic conductivity was not measured in these plants, and therefore the mean k_s value, as well as the other morpho-physiological variables, of that treatment corresponds to the plants that did actually survive. In this regard, it is important to note that spring drought led to a severe growth reduction, although survival was not affected as it was in the SpSuD treatment, which was the only treatment in which a differential mortality was observed.

The spring drought event affected stem diameter and height growth and TB accumulation. In addition, it affected the Hüber ratio, but only in H plants, and in an opposite trend to that expected based on allocation responses to drought (Maherali and DeLucia 2001; Mencuccini and Grace 1995). However, a drought event in this moment of the growing season did not induce changes in tracheids diameter, k_s , leaf tissue parameters derived from P - V curves, SLA , intrinsic water use efficiency ($\delta^{13}C$ of the leaves), and biomass allocation patterns. A similar response to drought, characterized by a decrease in growth and g_s , but with no other acclimation responses, has also been observed in other seed sources of ponderosa pine by Olivás-García et al. (2000).

In contrast to the effects produced by a spring drought event, a drought event in summer did not affect growth, but it resulted in a significant reduction in above:belowground biomass. This was observed in both SuD and SpSuD treatments. This expected response, based on the optimal partitioning theory (Bloom et al. 1985), is however hard to explain as an acclimation mechanism in response to the drought itself considering that it was not observed in the SpD treatment.

Considering the differences between seed sources, in spite of the fact that differences were observed in some of the studied traits (e.g. differential Ψ_{pd} on some dates, response of SLA and Hüber ratio in some treatments, height growth and xylem lumen diameter), on the whole, neither source had better performance under drought conditions in terms of relative growth and survival.

Conclusion

The experiment leading to the above discussed results was performed in hydroponics with drought imposition by PEG addition. This method allowed us to reach similar pre-dawn water potential values in different times of the growing season, making it possible to conclude about the effects of a similar magnitude drought on plant growth performance. However, the artificial conditions preclude us to directly extrapolate the results to field conditions. Further comparative experiments with PEG and soil drying are needed to elucidate whether the observed patterns also develop under field conditions, where roots development is expected to be different than in a liquid medium, as well as competition with other plants may be crucial determining plant responses to decrease soil water content. In this regard, the initial differences in plant size as well as the differential height growth observed between both seed sources could result in differential performance under field conditions, due to different soil exploration capacity and/or competitive ability between different size plants. These size-related differences were not important, or they led to an opposite than expected pattern in terms of acclimation to experimental conditions (e.g. differential response to PEG), in our study system. However, in spite of the limitation to extrapolate the results to field conditions, our results suggest drought events have different consequences according to whether they occur in spring or summer—even if plants are growing actively in summer and under higher evaporative demand than in spring—which deserves special attention for the prediction of tree seedling responses to water shortage due to climatic change.

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Conflict of interest The authors declare that they have no conflict of interest.

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