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## Response of *Pinus pinaster* Ait. provenances at early age to water supply. I. Water relation parameters

Manuel Fernández, Luis Gil, José A. Pardos\*

Unidad de Anatomía, Fisiología y Genética, ETS de Ingenieros de Montes, Ciudad Universitaria s/n,  
Universidad Politécnica de Madrid, 28040 Madrid, Spain

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**Abstract** – The seasonal evolution of tissue water relations was assessed in 1-year-old seedlings of four *Pinus pinaster* Ait. provenances growing in a nursery and subjected to two water supply regimes. Seedlings were also submitted to water stress cycles in a controlled environment chamber. Water relation parameters were deduced from pressure-volume curves. Significant differences were found between water supply regimes and measurement dates and sometimes among provenances. For the lowest water availability treatment, osmotic potential at full turgor decreased by 0.4 MPa in some provenances, whereas well-watered seedlings showed almost no osmotic adjustment. Provenances originating from hotter sites demonstrated a larger and more rapid acclimation to water stress conditions than provenances from colder sites. Osmotic adjustment, as an initial or short-term reaction, together with longer-term changes in cellular elasticity, are both observed in *P. pinaster* in response to water shortage. These physiological adaptations complement known morphological adaptations to drought stress in this species. With caution, assessment of these parameters in young seedlings can be used as a tool for early selection and prediction of future performance under conditions of water limitations. (© Inra/Elsevier, Paris.)

**maritime pine / early selection / water relation parameter**

**Résumé** – Réponse au stress hydrique des provenances de *Pinus pinaster* Ait. à un âge précoce. I. Paramètres hydriques. L'évolution saisonnière des relations hydriques a été déterminée chez quatre provenances de semis d'un an de *Pinus pinaster* Ait, installées en pépinière et soumises à deux régimes d'arrosage. Des semis étaient aussi soumis à des cycles de stress hydrique dans une chambre climatisée. Les paramètres des relations hydriques ont été déduits de courbes pression-volume. Des différences significatives ont été trouvées entre les différents types d'arrosage et aussi entre dates de mesure et provenances. En ce qui concerne le traitement correspondant au stress hydrique le plus important, on a constaté que le potentiel osmotique à pleine turgescence diminuait de 0,4 MPa chez certaines provenances alors qu'il n'y avait pratiquement pas d'ajustement osmotique chez les semis bien arrosés. Les provenances originaires des stations les plus chaudes ont montré une acclimatation plus grande et plus rapide aux conditions de sécheresse que les provenances des stations plus froides. En réponse à la sécheresse il a été observé chez *Pinus pinaster* un ajustement osmotique, réaction à court terme, avec un changement à long terme de l'élasticité cellulaire. Ces adaptations physiologiques complètent des connaissances déjà acquises sur les adaptations morphologiques à la sécheresse chez ces espèces. Avec précaution, la détermination de ces paramètres chez de jeunes semis peut être utilisée comme un outil pour une sélection précoce et la prédiction des performances futures en situation de limitation en eau. (© Inra/Elsevier, Paris.)

**pin maritime / sélection précoce / paramètres de relation hydrique**

\* Correspondence and reprints  
jpardos@montes.upm.es

## 1. INTRODUCTION

*Pinus pinaster* is widely distributed in the Mediterranean basin. Natural populations as well as plantations occupy more than 1.4 million ha in Spain. New plantations are being established in the Iberian Peninsula and more are planned for the near future [10]. However, water supply affects survival and growth in some plantations especially if appropriate provenances are not used. Limited research has shown the presence of some differences in response to water stress between provenances [18, 37]. Nguyen and Lamant [30] observed differences in osmotic adjustment between provenances; however, more research is needed [26].

The historic necessity to complete a breeding cycle in order to select and propagate high yielding trees may be shortened through early selection [11]. This not only reduces the waiting time but allows the selection intensity to be increased and even leads to a higher heritability because of the lower environmental variation [19]. In fact, for many species early selection revealed the existence of genetic differences in growth rate and the occurrence, in some genotypes, of a better adaptation and a higher yield under water stress conditions [8]. A common experimental approach consists of submitting plants to a range of water supply regimes, and to evaluate morphological, physiological and genetic parameters in order to establish a ranking regarding the taxons (species, provenances, genotypes) under study [23].

Exposure to drought induces some acclimation; however, plants need to detect small decreases in soil moisture content and react quickly to avoid harmful dehydration [33]. This response is likely under moderate genetic control [29].

The parameters deduced from pressure-volume curves (osmotic potential at full turgor and at turgor loss, relative water content at turgor loss, bulk elasticity modulus, apoplastic water) provide some information on a plant's capacity (such as osmoregulation, cellular elasticity, cel-

lular water relations) to maintain growth and to avoid damage due to water stress [6].

The present work analyses the responses of several ecologically distant provenances of *P. pinaster* to water availability in terms of tissue water relation parameters. Seedlings are subjected to a range of water supply regimes under nursery and growth chamber conditions, in order to establish criteria for early selection and suitability for afforestation on droughty sites.

## 2. MATERIALS AND METHODS

During April 1994, seeds from the three Iberian provenances (Oria [Or], Arenas de San Pedro [Ar] and San Leonardo de Yagüe [SL]) and two open pollinated families of one French provenance (Landes [Ld]) were collected (*figure 1, table I*) and germinated on moist perlite at 20 °C and 14 h photoperiod. After germination, seedlings were taken to open air under translucent cover and sown in containers filled with 230 mL of sand:black peat mixture (2:1 v/v). A weather station recorded air temperatures (*figure 2*).

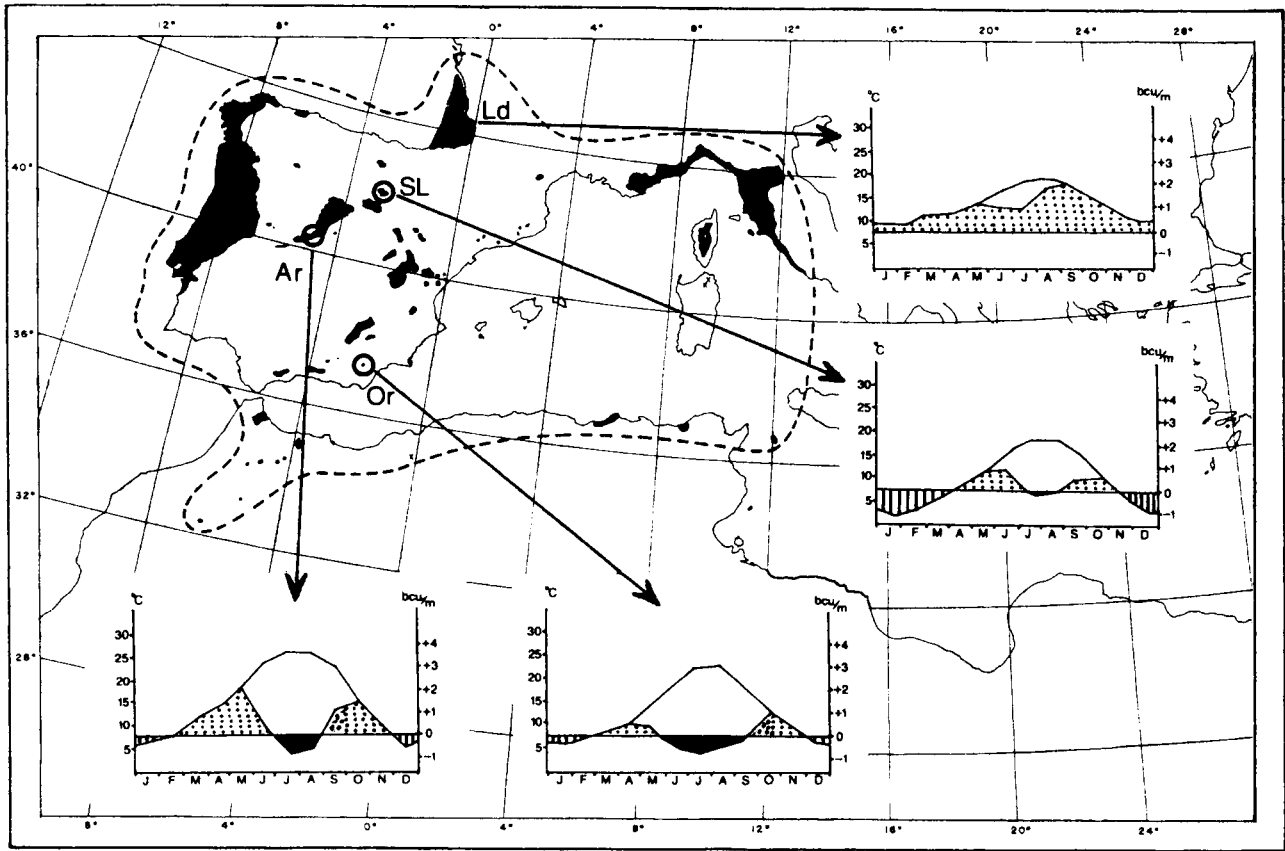
All seedlings were watered twice a week for 2 months. A fungicide (Captan 0.1 %) was systematically sprayed on the plants. After 2 months, two different water supply regimes were applied: once a week (R1) and every 2nd week (R2) to field capacity. The experimental design consisted of 12 completely randomised blocks with 15 plants per block, provenance and water supply regime – altogether 1 440 seedlings.

Three times (June, 2nd week; July, 3rd week; and September, 2nd week), four plants per provenance and water supply regime were removed just before watering and used for the pressure-volume analysis. Water potential was measured using a pressure chamber (PMS Instruments Co. Corvallis, OR, USA) according to Ritchie and Hinckley [34]. Pressure-volume curves were constructed following the technique of Koide et al. [22]. In brief, the construction of pressure-volume curves was

**Table I.** Ecological characteristics of *Pinus pinaster* provenance regions.

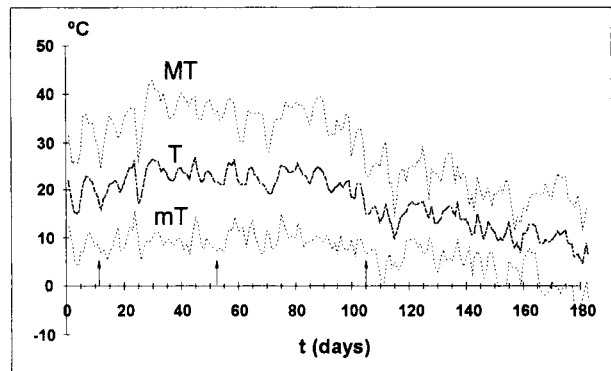
	Area (ha)	Altitude (m)	T (°C)	P (mm)	Latitude	Longitude	DBI (bcu/m)	Phytoclimate
Or	1 632	1 150	15.8	357	37° 30'N	2° 20'W	-1.00	IV <sub>1</sub>
Ar	34 760	750	13.4	1190	40° 07'N	4° 17'W	-1.38	VI(IV) <sub>2</sub> / IV <sub>4</sub>
SL	21 464	1 200	8.7	641	41°43'N	2°27'W	-0.08	VI(IV) <sub>1</sub> / VI(IV) <sub>2</sub>
Ld	1 000 000	40	12.0	833	44°00'N	1°00'W	0.00	VI(V)

T: annual mean temperature; P: annual mean precipitation; Phytoclimate [3]; DBI: dry bioclimatic intensity [28].



**Figure 1.** Natural range of *Pinus pinaster*, location of the provenances (Oria [Or], Arenas de San Pedro [Ar], San Leonrado de Yagüe [SL], Landes [Ld]) and bioclimatic diagrams according to [28]. Bioclimatic intensities: ◻ free = favourable growing conditions for plants, ▨ dry = summer dormancy and ▩ cold = winter dormancy.

as follows: Five-cm long shoot segments from the apex of the plants were removed, their basal ends were placed into distilled water and were allowed to rehydrated for 12 h in closed tubes in a cool dark humid chamber. As a result, a water potential value between  $-0.02$  to  $-0.05$  MPa was achieved. At this point, the shoot segments were allowed to dry under ambient conditions in the laboratory (at a nearly constant temperature of  $20$  °C). Then, at intervals, fresh weight and water potential were measured. Curves with oversaturation points were less than 5 % of the samples; in these cases the points in the plateau region were omitted and the curves were corrected according to Kubiske and Abrams [24]. The following parameters were then calculated: osmotic potential at full turgor ( $\Psi\pi_{100}$ ) and at turgor loss ( $\Psi\pi_0$ ) and the osmotic amplitude for turgor maintenance ( $\Delta\Psi\pi = \Psi\pi_{100} - \Psi\pi_0$ ), relative water content at turgor loss (RWC0),



**Figure 2.** Maximum (MT), minimum (mT) and mean (T) daily temperatures (°C) throughout the experiment (1 June to 30 November 1994). Arrows indicate the days of measurements.

apoplastic water at full turgor to dry weight ratio (Wap/DW), maximum elasticity modulus ( $\epsilon_{\max}$ ) and weight at full turgor to dry weight ratio (TW/DW).

At the same time, height (H), dry weight (DW) after 48 h at 70 °C, projected needle area (PNA), specific leaf area (SLA,  $\text{m}^2_{\text{needles}}/\text{g}_{\text{needles}}$ ), predawn and midday water potentials ( $\Psi_{\text{pd}}$ ,  $\Psi_{\text{n}}$ ) and gas exchange parameters (net photosynthetic and transpiration rates [A, E] and stomatal conductance to water vapour [gw]) were recorded, immediately before the next irrigation, on ten plants per provenance and water supply regime. Projected needle area was measured with a leaf area meter (Delta T Devices Cambridge, UK). A, E and gw were measured with a portable infrared gas analyser (LCA-4, ADC, Hoddesdon, England) between 1200 and 1400 hours, and expressed and analysed on a projected needle surface basis.

On 1 May 1995, 18 seedlings of each Iberian provenance were taken to a growth chamber and watered twice a week until 16 June. Chamber conditions were 22 °C, 65 % relative humidity (RH) and 200  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  maximum photosynthetic active radiation (PAR) during the light period (14 h) and 17 °C, 75 % RH in the dark. Plants were submitted to consecutive cycles of drought, each cycle ending as soon as predawn water potential was between  $-1.2$  and  $-1.5$  MPa. The plants were then watered again to field capacity and a new cycle was begun. Three times (19 June, 21 July and 7 September) four plants per treatment were again removed and pressure-volume curves were constructed.

Variance analysis using a BMDP2V statistic package (BMOP Statistical Software Inc., Cork, Ireland) was applied to the data in order to discriminate among provenances, watering treatments, measurement dates and blocks. The Tukey HSD (Honest Significant Difference) for means comparison was applied whenever differences were significant ( $P < 0.05$ ).

### 3. RESULTS

#### 3.1. Plants at the nursery

The block effect was not statistically significant for any water relation or gas exchange parameter ( $P > 0.20$ ), so this was excluded from the statistical analysis presented henceforth.

Tables II and III illustrate the mean values of water potential and other morphological and gas exchange parameters. Water potential and gas exchange rate values were not significantly different among provenances; however, provenances showed differences in growth and SLA. Arenas, Oria and Landas provenances stand out because of their growth for the R1 treatment. For R2, the Landas families lost the potential of biomass production they showed under high water availability. Survival rate was higher than 97 % for all provenances for the R1 treatment and in the range of 67–80 %, according to provenance, for the R2 treatment; the largest mortality

**Table II.** Mean value for each provenance (Or, Ar, SL, Ld) and water supply regime (R1, R2). Height increment ( $\Delta H = H_{\text{september}} - H_{\text{june}}$ , mm), dry weight increment ( $\Delta DW = DW_{\text{september}} - DW_{\text{june}}$ , g), projected needle area increment ( $\Delta PNA = PNA_{\text{september}} - PNA_{\text{june}}$ ,  $\text{cm}^2$ ) and mean specific leaf area (SLA,  $\text{m}^2_{\text{needles}}/\text{g}_{\text{needles}}$ ) from June to September. Means with the same letter do not differ significantly (Tukey's HSD test,  $P = 0.05$ ).

	$\Delta H$	$\Delta DW$	$\Delta PNA$	SLA
Provenance				
Or	57.1 a	0.327 b	10.9 ab	7.56 a
Ar	78.2 b	0.352 b	11.7 b	8.05 a
SL	58.0 a	0.236 a	8.6 a	8.81 b
Ld	81.5 c	0.331 b	11.7 b	9.23 b
Water treatment				
R1	79.8	0.362	13.2	8.65
R2	57.7	0.262	8.3	8.18
P value				
Provenance (P)	***	***	***	***
Water treatment (WT)	***	***	***	**
P $\times$ WT	**	*	*	n.s.

**Table III.** Mean value  $\pm$  standard error of predawn water potential ( $\Psi_{pd}$ , MPa), midday water potential ( $\Psi_n$ , MPa), net photosynthetic rate (A,  $\mu\text{molCO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), net transpiration rate (E,  $\text{mmolH}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and stomatal conductance ( $\text{mmolH}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ).

Water treatment	Date	$\Psi_{pd}$	$\Psi_n$	A	E	gw
R1	June	$-0.54 \pm 0.01$	$-0.89 \pm 0.01$	$9.2 \pm 0.4$	$2.2 \pm 0.1$	$100 \pm 6$
	July	$-0.49 \pm 0.01$	$-1.05 \pm 0.02$	$6.3 \pm 0.3$	$2.0 \pm 0.1$	$68 \pm 5$
	September	$-0.62 \pm 0.02$	$-1.05 \pm 0.01$	$10.1 \pm 0.4$	$2.8 \pm 0.1$	$144 \pm 8$
R2	June	$-0.65 \pm 0.01$	$-0.99 \pm 0.01$	$3.2 \pm 0.4$	$1.3 \pm 0.1$	$28 \pm 3$
	July	$-2.50 \pm 0.02$	$-2.87 \pm 0.02$	$-0.2 \pm 0.5$	$0.1 \pm 0.1$	$1 \pm 2$
	September	$-0.75 \pm 0.01$	$-1.61 \pm 0.02$	$1.5 \pm 0.4$	$0.7 \pm 0.1$	$10 \pm 1$

Differences between water supply treatments and dates were significantly different for these five parameters ( $P < 0.001$ ).

**Table IV.** Mean value  $\pm$  standard error of parameters deduced from pressure-volume curves for provenances altogether and for every water supply regime and measurement date. Osmotic potential at full turgor ( $\Psi\pi_{100}$ , MPa), osmotic potential at turgor loss ( $\Psi\pi_0$ , MPa), relative water content at turgor loss (RWC0, %), maximum elasticity modulus ( $\epsilon_{\text{max}}$ , MPa), full turgor weight to dry weight ratio (TW/DW) and apoplastic water to dry weight ratio (Wap/DW).

	$\Psi\pi_{100}$	$\Psi\pi_0$	RWC0	$\epsilon_{\text{max}}$	TW/DW	Wap/DW
Provenance						
Or	-1.37 b	-1.91 b	81.3 a	5.71 b	3.69 a	1.05 a
Ar	-1.24 a	-1.76 a	84.9 b	5.08 ab	4.11 a	1.34 b
SL	-1.14 a	-1.71 a	83.9 b	4.35 a	4.35 ab	1.64 c
Ld	-1.18 a	-1.73 a	83.9 b	6.31 b	4.47 b	1.51 bc
Water treatment						
R1	-1.19	-1.60	83.8	6.23	4.49	1.38
R2	-1.27	-1.95	83.2	4.49	3.82	1.39
Date						
June	-1.17 a	-1.60 a	84.0 a	6.21 b	4.44 b	1.53 b
July	-1.26 b	-1.94 c	83.4 a	4.48 a	3.92 a	1.51 b
September	-1.26 b	-1.79 b	83.2 a	5.40 ab	4.10 a	1.13 a

**Table V.** Significance level ( $P$ ) for every parameter according to variance analysis.

	Provenance (P)	Main effects		$P \times WT$	Interactions		
		Water treatment (WT)	Date (D)		$P \times D$	$WT \times D$	$P \times WT \times D$
$\Psi\pi_{100}$	***	***	***	***	***	***	***
$\Psi\pi_0$	***	***	***	***	***	***	***
RWC0	***	n.s.	n.s.	n.s.	n.s.	n.s.	*
$\epsilon_{\text{max}}$	**	***	***	n.s.	n.s.	**	n.s.
TW/DW	***	***	***	n.s.	n.s.	***	*
Wap/DW	***	n.s.	***	n.s.	n.s.	***	n.s.

n.s.: not significant ( $P > 0.05$ ); \*:  $P \leq 0.05$ ; \*\*:  $P \leq 0.01$ ; \*\*\*:  $P \leq 0.001$ .

(16 %) occurred during July, the period of highest water stress.

Table IV shows the mean values for the majority of water relation parameters, and table V presents the levels of significance, taking into account the effect of provenance, water supply treatment, measurement date and their interaction. The differences between provenances or between dates with regard to water relation parameters derived from pressure-volume curves were greater for the R2 than for the R1 treatment, with the exception of RWC0. The Landes provenance showed the highest tissue water content ratio (TW/DW), the water accumulation was greatest in the symplast. For the R2 treatment, there were only small differences between provenances during June and September for  $\Psi\pi_{100}$ ; however, during July  $\Psi\pi_{100}$  (figure 3) was significantly lower in the Oria and Arenas provenances (Or =  $-1.70 \pm 0.07$ ; Ar =  $-1.52 \pm 0.07$ ; SL =  $-1.13 \pm 0.06$  and Ld =  $-1.18 \pm 0.07$  MPa). Similar results were noted for  $\Psi\pi_0$ . The exposure to water stress in June led later in July to decreases in  $\Psi\pi_{100}$ ,  $\Psi\pi_0$ ,  $\epsilon_{\max}$  and TW/DW ratio, whereas  $\Delta\Psi\pi$  increased. From July to September the previously mentioned water relation parameters changed but in the opposite direction from that noted from June to July. Nevertheless, for most of the parameters the initial June values were not reached by September. For all the provenances, the decrease in TW/DW value from June to September was not due to a concomitant drop in Wap/DW (table IV); therefore, it was likely due to a decrease of symplastic water content.

### 3.2. Growth chamber experiment

Table VI shows the measured water relation parameters and their significance level based upon analysis of

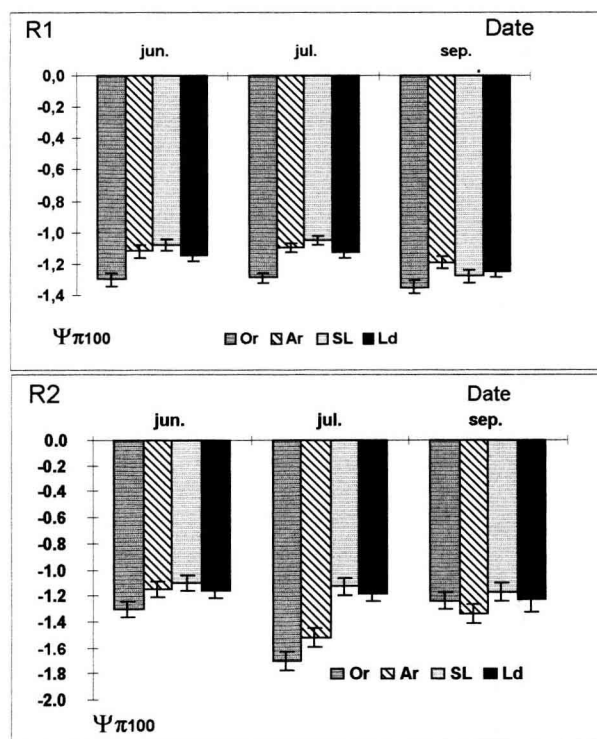


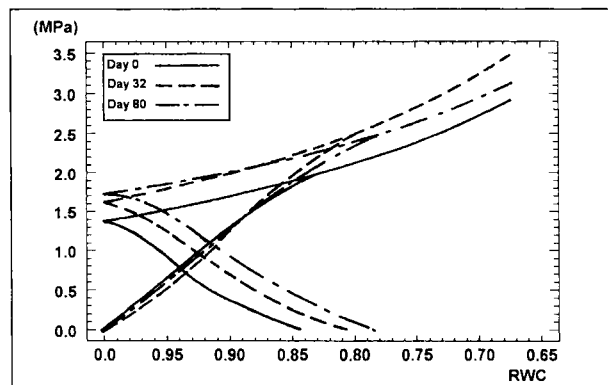
Figure 3. Osmotic potential at full turgor ( $\Psi\pi_{100}$ , MPa) for each provenance, measurement date and water supply regime.

variance (ANOVA). Differences between provenances were not significant for any parameter ( $0.122 < P < 0.888$ ), neither was the interaction of provenance  $\times$  date ( $0.124 < P < 0.917$ ). Only date was observed to have a significant effect ( $P \leq 0.040$ ).

Table VI. Mean value  $\pm$  standard error and significance level ( $P$ ) of every parameter for the plants of the growth chamber. Osmotic potential at full turgor ( $\Psi\pi_{100}$ , MPa), osmotic potential at turgor loss ( $\Psi\pi_0$ , MPa), relative water content at turgor loss (RWC0, %), maximum elasticity modulus ( $\epsilon_{\max}$ , MPa), full turgor weight to dry weight ratio (TW/DW) and apoplastic water to dry weight ratio (Wap/DW).

	Date			Provenance (P)	P value Date (D)	P $\times$ D
	Day 0	Day 32	Day 80			
$\Psi\pi_{100}$	$-1.39 \pm 0.04$	$-1.59 \pm 0.02$	$-1.72 \pm 0.05$	n.s.	***	n.s.
$\Psi\pi_0$	$-2.00 \pm 0.05$	$-2.33 \pm 0.03$	$-2.47 \pm 0.06$	n.s.	***	n.s.
RWC0	$82.2 \pm 0.9$	$81.5 \pm 0.5$	$78.2 \pm 0.9$	n.s.	**	n.s.
$\epsilon_{\max}$	$5.41 \pm 0.56$	$5.96 \pm 0.30$	$7.52 \pm 0.49$	n.s.	**	n.s.
TW/DW	$3.5 \pm 0.1$	$3.4 \pm 0.1$	$3.7 \pm 0.2$	n.s.	*	n.s.
Wap/DW	$1.0 \pm 0.1$	$0.95 \pm 0.0$	$0.72 \pm 0.1$	n.s.	*	n.s.

n.s.: no significant ( $P > 0.05$ ); \*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ .



**Figure 4.** Höfler diagram for seedlings grown in the climatic chamber (San Leonardo provenance).

The first response to water stress cycles (from day 1 to 32) was a significant decrease of  $\Psi_{\pi 100}$  and  $\Psi_{\pi 0}$  and an increase of  $\Delta\Psi_{\pi}$ . Changes in RWC0,  $\epsilon_{\max}$ , TW/DW and Wap/DW were not significant until the third measurement (day 80), then an increase of TW/DW and a decrease of Wap/DW were observed. *Figure 4* illustrates a Höfler diagram for one of the three provenances (SL). Diagrams for the other provenances were quite similar.

#### 4. DISCUSSION

In general, water potential and gas exchange values from this study were similar to those from other studies of pine species [7, 15]. For the R1 treatment, mean values were not significantly different among provenances. Predawn water potential dropped to  $-0.5$  MPa after 7 days without water. Although Tschaplinski et al. [39] observed that such predawn values can affect plants, *P. pinaster* showed no effect and continued to grow. In addition, values of noon or minimum water potential indicated no stress. Under water shortage conditions (R2), water stress was high and predawn water potential approached the survival threshold. Differences among provenances were not significant. Stomata closed, as is made evident by the values recorded for gas exchange parameters, and growth was restricted. The restriction of growth due to lack of available water is a well-known general response of plants [5]; such observations have been made in 1-year-old *P. pinaster* [16]. For comparison, threshold values of water potential that result in stomata closure and a decrease in photosynthesis of some Pinaceae species are listed:  $-1.3$  MPa for *Pinus pinaster* [9],  $-1.2$  MPa for *Cedrus atlantica*,  $-1.5$  MPa for *Pseudotsuga menziesii* (Mirb.) Franco,  $-1.9$  MPa for

*Pseudotsuga macrocarpa* [12],  $-1.5$  MPa for *Larix occidentalis* Nutt. [17],  $-1.75$  MPa for *Pinus banksiana* Lamb. and *Picea glauca* (Moench) Voss. [15].

For the R1 treatment, differences between provenances are small with regard to water relation parameters derived from pressure-volume curves. Pressure potential was always positive ( $\Psi_P > 0$ ), since water potential values close to turgor loss were never measured in spite of the high summer temperatures. Very low water potential values ( $-2.5$  MPa) on 27 July for the R2, suggested that many plants had exceeded the turgor loss point. As a consequence, an increase in mortality was observed. However, most of the plants had recovered 24 h after watering. For *Cedrus atlantica* and *Pinus nigra*, a drop of  $\Psi_{pd}$  below  $-3.0$  and  $-2.5$  MPa, respectively, reduces the possibility of surviving and if  $-4.5$  and  $-3.0$  MPa are reached, recovery is impossible [21].

Under water shortage conditions (R2), differences between provenances, water supply treatments as well as between dates were obvious. Water stress cycles led to changes in water relation parameters of plant tissues. This is in agreement with other studies of several conifer species [1, 4, 13, 35, 36, 38, 40, 41, 42, 43]. The decrease of  $\Psi_{\pi 100}$ ,  $\Psi_{\pi 0}$ ,  $\epsilon_{\max}$  and TW/DW, parallel to the increase of  $\Delta\Psi_{\pi}$ , indicate the development of strategies of acclimation to water stress conditions. However, the response of  $\epsilon_{\max}$  cannot be generalised since it is possible to find plants, resistant or hardened to dryness, with higher  $\epsilon$  values [35]; therefore,  $\epsilon$  performance depends on the species [13]. As water stress abates from July to September, water relation parameters tend to recover to values linked with periods of active growth. Such reversible changes have been described for other conifers [4, 32, 43].

When comparing the reaction of the provenances to water stress, changes in  $\Psi_{\pi 100}$  and  $\Psi_{\pi 0}$  suggest a more rapid response in the Arenas and Oria provenances. Lower  $\Psi_{\pi 100}$  and  $\Psi_{\pi 0}$  values would indicate a greater ability to absorb water to maintain turgor when plant water potential decreases.

At the beginning of the season symplastic water content of leaves is almost twofold their dry weight and this ratio decreases during July, as leaves mature and dry matter increases. It is also possible that in water-stressed plants symplastic volume diminishes as cellular integrity is lost and the permeability of the membranes is reduced [4, 32]. A modification of this pattern was shown by Joly and Zaerr [20] for several populations of *Pseudotsuga menziesii* (Mirb) Franco under water stress: in spite of the decrease in the ratio of symplastic water to dry weight, the  $\Psi_{\pi 100}$ , RWC0 and TW/DW values were not modified by water supply or stress intensity and no differences between populations were found.



In the growth chamber, a change in  $\Psi\pi_{100}$ ,  $\Psi\pi_0$  and  $\Delta\Psi\pi$  was the first response (days 1 to 32) to water stress. Observed also in *Pseudotsuga menziesii* [20], this acts as a stimulus to induce internal changes in water allocation and elasticity of tissues, which were then noted later (days 32 to 80). Water stress induced an increase of symplastic water content, the opposite response to that observed for the R2 water supply regime at the nursery. It can be assumed that in the growth chamber plants did not support such high stress and the loss in integrity of membranes was not approached. In spite of the differences previously mentioned, the three provenances showed a similar pattern for the water relation parameters, and their genetic potential for water stress acclimation may be limited by growth conditions. Because of the low level of radiation in the growth chamber, osmotic adjustment is affected [29, 41].

The results should be interpreted with some caution, since the response of the parameters under study depends on the season, cultural conditions, seed origin and species [4, 27, 31, 41, 42] and even on the nature of the tissue sampled from the plant [38]. Colombo [6], in his work with *Picea mariana*, obtained similar or opposite results to those of other authors, and he suggested some reasons to justify the lack of a uniform pattern for  $\epsilon$ . Furthermore, although water deficit induces changes in water parameters, seasonal changes have been found in well-watered plants [14]. On the other hand, differences between populations do exist but they are so small that genetic correlations are difficult to demonstrate [42].

Under moderate water stress, plants will produce as much dry matter (growth) as additional water they would be able to remove from the soil. This ability may be linked to low values of cellular elasticity [29], as occurred in plants in the growth chamber. Under severe water stress, another possibility is that maintenance of tissue water content would be more important than maintenance of water potential. Then, the increase in cell elasticity could be the mechanism for stress acclimation if other mechanisms are limited [25]. This appeared to have occurred to plants under the R2 water supply at the nursery.

In conclusion, the following can be emphasised: *i*) With adequate water supply, differences for most of the water relations parameters among provenances are not significant. *ii*) Restriction of water supply through stress cycles causes noticeable changes in water parameters. A drop in  $\Psi\pi_{100}$ ,  $\Psi\pi_0$  (osmotic adjustment),  $\epsilon$ max and TW/DW points to acclimatisation strategies by plants to water stress. Differences between Oria and Arenas de San Pedro (provenances from hotter sites) and San Leonardo and the Landes (provenances from colder sites) point to a better or a faster response by the first

two provenances to water stress. In addition, the lower specific leaf area of the Oria and Arenas provenances may be a strategy to save water. The Arenas provenance stands out because of its growth, whereas the Landes families lost the potential of biomass production they showed under high water availability. These results are in agreement with the field performance at five and eighteen years old of the same provenances at five experimental plots [2]; therefore, they indicate some validity to the use of water parameters as criteria applied for early selection to 1-year-old *P. pinaster* seedlings.

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