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Early testing for improving growth under water shortage in *Eucalyptus globulus* Labill

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Abstract

Aim of study: We aimed at identifying differences in the response to water shortage between *Eucalyptus globulus* clones.

Area of study: Field trials were established in SW Spain.

Material and methods: Potted plants from six clones were grown in a greenhouse for 35 days under two watering regimes. Two clones were F_0 genotypes and the other four were F_1 (hybrid) genotypes, including one inbred clone. Differences in stomatal conductance, hydraulic traits, growth and specific leaf area (SLA) were analyzed.

Main results: Water shortage decreased SLA, growth in height and leaf area and leaf-specific hydraulic conductivity (K_{Lmax}). We measured the highest growth in F_1 genotypes and the lowest in the clone in which SLA was lowest. The inbred clone showed the highest growth reduction under water shortage. There was substantial hysteresis between leaf water potential (Ψ) and native embolism, most probably a result of combined cavitation and refilling. High losses of hydraulic conductance were compatible with high stomatal conductances. Maximum values of stomatal conductance decreased with the soil water content estimated from predawn Ψ and were lowest in the inbred clone, showing less plasticity and a diminished ability to cope with high temperatures, which could explain its poor development under field conditions.

Research highlights: Soil water content and predawn Ψ appeared as critical factors controlling stomata closure, while stomatal conductance and SLA could be useful to predict differences in growth and survival from early trials.

Additional key words: heterosis; inbreeding depression; native embolism; specific leaf area; predawn leaf water potential; stomatal conductance; plasticity.

Abbreviations used: HW (high-watering treatment); LA (total area of all the leaves on a plant); LW (low-watering treatment); PLC (percentage loss of hydraulic conductance); PPFD (photosynthetic photon flux density); SLA (specific leaf area); SWC (soil water content); TDR (time domain reflectometry). **Parameters**: K_i (initial hydraulic conductivity); K_{Lmax} (maximum leaf-specific hydraulic conductivity); K_m (maximum hydraulic conductivity); L_A (leaf area of a single leaf); L_{Ad} (surface area of all leaves distal to the stem portion in which hydraulic conductivity was measured); L_L (leaf length); L_W (maximum leaf width); W_T (weight of each potted plant).

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Introduction

Stomatal conductance is considered a major determinant of photosynthesis and a breeding target for improving crop productivity under the expected rising temperatures and more frequent episodes of drought (Faralli et al., 2019). Evaluating the stomatal response to water shortage seems also interesting under a scenario of increasing atmospheric CO_2, Contrary to what has been frequently accepted, that plants respond with decreasing stomatal conductance to elevated CO_2, Purcell et al. (2018) found that stomatal conductance may increase in woody species adapted to warm, low-humidity conditions.

Stomata sensitivity to external and internal factors differs between and within species (Klein, 2014; Cañete-Salinas et al., 2019), affecting carbon uptake and contributing to explain why plant species differ so much in their rate of biomass production. In this regard, Brodribb & Holbrook (2004) suggested that early stomatal closure could be an explanation for the low evolutionary success of pteridophytes relative to angiosperms in seasonally and perennially dry habitats. Stomatal closure decreases the chance for evaporative cooling, a common mechanism to cope with high temperatures, which seems particularly relevant given the increased frequency of heat waves (López et al., 2022). In a previous study, we measured the lowest stomatal conductance in the *Eucalyptus globulus* Labill. clone that reached the lowest growth (Hernandez et al., 2016).

Plant growth depends on the uptake of carbon, nitrogen and other essential resources, but also on how those resources are used. Biomass allocation has widely been considered a major trait conditioning plants' performance, growth and productivity (Poorter & Sack, 2012). For fast-growing tree species, a rapid development of leaf area would be most relevant in determining growth (Mira et al., 2023). Prioritizing leaf area growth would come at the cost of decreasing biomass allocation to other organs and/or producing leaves with a higher specific leaf area (SLA). This parameter, calculated as the ratio of leaf area to leaf dry weight, is considered a key factor in determining slow growth in herbaceous species (Lambers & Oliveira, 2019) and has shown to be a reliable indicator of growth in a number of woody species (Diémé et al., 2019; Lambers & Oliveira, 2019; Rodríguez et al., 2020).

Breeding programs established in dry environments must seek a balance between improved growth and improved stress tolerance. This is a complex task, since most of the responses that help tolerate water stress involve a loss of growth for the plant. For instance, species or provenances adapted to drought-prone environments typically show a low vulnerability to water stress-induced cavitation (López et al., 2013). Cavitation resistance tends to increase with wood density (Barotto et al., 2018), which is costly in terms of biomass allocation to non-photosynthetic tissues. In addition, leaf-specific hydraulic conductance may decrease under water shortage as reported for *E. globulus* (Hernandez et al., 2016), leading to a reduction in water supply to the leaves, photosynthesis and growth (Zhang & Cao, 2009).

While numerous studies highlight the relevance of tree breeding for improved drought resistance, mainly in the scope of global change (Polle et al., 2019; Taylor et al., 2019), studies facing the challenge of improving tree growth in water-limited environments are much less abundant and show contrasting results. According to Wikberg & Ögren (2007), increased drought resistance results in decreased growth in *Salix*. Conversely, selection for both drought resistance and improved growth would be possible in *Pinus contorta* (Wang et al., 2003). Growth was unaffected by mild water stress in poplar (*Populus tremula* × *Populus alba*) selected lines showing enhanced tolerance to water deficit (Sow et al., 2021). Moreover, breeding for improved growth had opposite effects on drought tolerance in coastal and interior provenances of *Pinus radiata* in Chile (Espinoza et al., 2016).

Fast-growing species are prone to water stress in the early stages of growth because of their tendency to allocate a greater proportion of biomass to leaves than to roots (Brodribb & Hill, 2000). Eucalypt plantations established in southwest Spain often suffer from the combination of drought and high summer temperatures. Breeding programs established for *E. globulus* have taken advantage of hybrid vigor, also called heterosis, which is widely used in plant and animal production. Heterosis can be defined as the phenomenon by which hybrids (F_1 genotypes) are superior to parents (F_0) in terms of yield, growth rate, viability, and disease resistance (Wu et al., 2021). The mechanisms underlying hybrid vigor remain to be fully elucidated (Wu et al., 2021).

We carried out a greenhouse experiment with two F_0 and four F_1 *E. globulus* clones differing in survival and growth in dry Mediterranean climate conditions, as shown in two trial plantations established in SW Spain. We aimed at identifying differences in the response to water shortage between clones that could contribute to explain the observed differences in growth and survival in the field. We investigated the following hypotheses: (1) regardless of water availability, clones with lower SLA will grow less; (2) in the event of a water scarcity, the maximum leaf-specific hydraulic conductivity will drop and remain greater in clones that develop the fastest; (3) clones that grow more quickly in dry conditions should be able to achieve larger stomatal conductances.

Material and methods

Plant material, irrigation treatments and growth conditions

ENCE Energía y Celulosa S.A. supplied the plants, which belonged to two F_0 and four F_1 clones from the company's breeding program. The parental (F_0) clones, C13 and C14, have been used regularly in commercial plantations established in SW Spain. The hybrid (F_1) clones (H463, H354, H231) come from crosses of clone C14 with other

Table 1. Average survival, height and diameter (DBH_{ob}) in *E. globulus* clones, three years after plantation. Field trials were established in SW Spain: Trial 1 on less-favorable slate soil and Trial 2 on deep, sandy soil. Both trials followed a complete random block design, with 20 plants per clone and trial, and more than 147 clones at each trial.

	C13		**C14**		**H231**		**H354**		**H463**		**H491:inbred**	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Survival (%)	75	73	75	80	70	95	60	55	55	80	55	50
Height (m)	6.4	12.4	5.7	12.4	6.7	14.0	7.6	13.8	7.4	13.1	4.8	8.4
DBH_{ob} (cm)	6.4	11.0	5.4	10.9	6.8	13.7	6.9	13.5	7.1	12.9	4.5	6.0

Data kindly provided by ENCE Energía y Celulosa S.A.

F_0 clones, while clone H491 comes from the self-crossing of C14. Table 1 shows the results obtained by the company three years after planting, in two field trials. Survival and growth were higher in the trial established on better soil, as expected. The worst results in survival and growth were measured for clone H491. Excluding this clone, survival values were similar for F_0 and F_1 clones, while growth was generally higher in F_1 clones.

Twenty plants of each clone were transplanted into 3-L pots filled with the same weight of a mixture of peat and sand (3:1 v/v). At this time the plants had an average of ten whorls. The average height ranged 20-25 cm and was highest in clone C14 and lowest in H463. Plants were placed inside a greenhouse where minimum temperature remained above 16ºC and maximum temperature was kept below 32ºC. Maximum photosynthetic photon flux density (PPFD) was 1700 mmol m^{-2} s^{-1}.

The plants were maintained under optimum irrigation conditions for six weeks and then two irrigation treatments were established, as explained below. Ten plants per clone were randomly assigned to each treatment. From this day (April 29, d0) all the plants were irrigated once to three times a week, when the first symptoms of turgor loss were observed. Irrigation was carried out by placing the plants on a balance and adding water until reaching the values established as target weight in each treatment. The maximum target weight was established for the high-watering (HW) treatment based on previous tests and corresponds to the maximum amount of water that could be added guaranteeing that there would be no loss of water by percolation after irrigation. For the low-watering (LW) treatment, a target weight 10% lower than that of the HW treatment was set. These irrigation treatments were applied for 35 days. From d35 till the end (d55), all plants were watered in the same way, using the target weight of the HW treatment.

In order to better characterize the irrigation treatments, predawn leaf water potential (Ψ) was measured in the first fully expanded leaf, on days d9 and d14. Plants were moved to a growth chamber in the afternoon of days d8 and d13, kept in the dark until measured in the early morning and then returned to the greenhouse. After measuring the water potential, we recorded the weight of each plant (W_T) and measured volumetric soil water content (SWC) with a TDR probe (Trime-FM, IMKO Micromodultechnik Gmbh, Ettlingen, Germany). These measurements were made on four plants per clone and treatment on day d9 and eight plants per clone of the LW treatment on day d14. Soil water content and W_T were also measured at the time of harvest. We found a strong linear relationship between both parameters, with no differences between lines fitted to data measured on different dates, so we used the whole data set to obtain the following regression equation:

$$SWC = 0.0362 \cdot W_T - 56.195 \quad (R^2=0.84, n=199) \quad (1)$$

We used this equation to calculate SWC at the time of gas exchange measurements to minimize damages to the root system from the TDR probe.

Growth and specific leaf area

To estimate leaf area values at the beginning of the experiment, we made tracings of all leaves in a subsample of two plants per clone. We measured leaf area (L_A) (LI-3000, Li-Cor Inc), leaf blade length (L_L) and maximum leaf blade width (L_W) on the tracings. For each clone we fitted these values by linear regression (R^2>0.90):

$$L_A = a + b \cdot L_W + c \cdot L_L \quad (2)$$

Using Eqn. (2) we calculated the leaf area per plant (LA) in a total of six plants per clone, in which the length and width of the leaf blade of all leaves were measured.

At the time of harvesting (d35-d55), we measured plant leaf area directly from leaves, as well as plants' height. We separated those leaves attached to branches (auxiliary) from those in the main stem. We also measured separately all leaves distal to the stem portion used to measure the hydraulic conductance of the stem. This value was used to calculate the maximum leaf specific hydraulic conductivity. We calculated growth rates in leaf area for each plant as the difference between the leaf area measured at the time of harvesting and the initial leaf area estimated from leaf blade length and width, divided by the time elapsed between measurements.

After measuring leaf area, we oven-dried the leaves at 70ºC up to constant weight to determine leaf dry weight. We calculated SLA by dividing the surface area of the leaves by their dry weight.

Gas exchange and hydraulic conductivity

In order to answer our third hypothesis, we measured stomatal conductance in plants of the LW treatment on four occasions (d35, d43, d50, d51), on 4-9 plants per clone and day of measurement. Stomatal conductance and other gas exchange parameters were measured on a fully developed leaf of the sixth whorl with a LCA4 IRGA (Analytical Development Co, Hoddesdon, UK). Measurements were made in batches of six plants, with one plant of each clone in each batch, between 9:30 and 11:30 (local solar time), on sunny days, with a PPFD > 1100 µmol m^{-2} s^{-1}.

We carried out hydraulic measurements between d35 and d55. Measurements were made between 8:00 and 09:30 (local solar time) on sunny days, with a maximum air temperature of 32°C and minimum temperatures between 18 and 20°C. Each day, we measured between one and two plants per clone, from the same irrigation treatment. Measurements of plants from different treatments were taken on alternate days. As explained above, SWC and W_T were measured in each plant at this time, as was plant height. Then, water potential was measured on a leaf of the sixth whorl with a pressure chamber (PMS Instrument Co., Albany, OR, USA) and hydraulic conductance was measured following Sperry et al. (1988).

We used the portion of the stem between the base and the sixth whorl for hydraulic conductivity measurements. The stem was cut under water, and all leaves and branches were also removed under water. The stems were left in trays with water at room temperature for approximately 20 minutes before measuring the initial hydraulic conductance (K_i), at a pressure drop of 0.0064 MPa. A pressure of 0.08 MPa was then applied for 30 min, which was enough to eliminate any embolism in the stem. Maximum hydraulic conductance (K_m) was then measured as explained for K_i and the percentage loss of hydraulic conductance (PLC) was calculated from K_i and K_m:

$$PLC = 100 \cdot (K_m - K_i) \cdot K_m^{-1} \quad (3)$$

A filtered (0.2 µm), degassed and slightly acidified (1‰ HCl) distilled water solution was used throughout the process. We measured the length of the stems used to determine the hydraulic conductance (L). To calculate the maximum leaf-specific hydraulic conductivity (K_{Lmax}) we used Eqn. (4), where L_{Ad} is the surface area of all leaves fed by the stem.

$$K_{Lmax} = L \cdot K_m \cdot (L_{Ad})^{-1} \quad (4)$$

Statistical analyses

We carried out the analyses of variance by fitting General Linear Models, considering the effect of the watering treatment and clone, together with the possible interactions. Analysis of covariance was used to analyze the effect of clone and watering regime on leaf area and plant height, taking the day of harvest as continuous predictor. We used factorial ANOVA to analyze differences between clones and watering regimes in SLA, PLC and K_{Lmax} and one-way ANOVA to analyze the differences between clones in the maximum values of stomatal conductance. The effect of the watering treatment was further analyzed clone by clone by means of ANCOVA or one-way ANOVA. We checked the validity of the basic assumptions regarding the normality, independence and homocedasticity of residuals; and log-transformed the data when necessary. We used simple linear regression to analyze the relationship between W_T and SWC and multiple linear regression to analyze the relationship between L_A, L_W, and L_L. Percentage values were arcsin transformed prior to analysis. Mean values were compared using Fisher's LSD test. Differences were considered significant at $p<0.05$. All analyses were run using the 6.0 version of STATISTICA (StatSoft, Tulsa, OK, USA).

Results

Soil water content and predawn Ψ

Figure 1 shows the average SWC values for both treatments, calculated from W_T values using Eqn. (1). The mean minimum SWC remained above 5% in the HW treatment and decreased to 4% in plants from the LW treatment (Fig. 1). There was a tight relationship between predawn Ψ and the values of SWC estimated from Eqn. (1) (Fig. 2), which further supports the validity of the parameter W_T to estimate SWC. When SWC was higher than 5%, predawn Ψ remained above -0.8 MPa (Fig. 2). As SWC decreased below this threshold value of 5%, predawn Ψ tended to decrease steeply.

Growth and specific leaf area

We found no significant differences in leaf area between clones at d0. At harvest, leaf area differed between clones ($F_{5,92}$=3.49, p=0.006) and irrigation treatments ($F_{1,92}$=16.5, $p<0.001$). The effect of time was also significant ($F_{1,92}$=63.1, $p<0.001$). We measured the highest growth in the F_1 clones H463 and H354 grown under the most favorable HW treatment and the lowest growth in the F_0 clone C14 in both irrigation treatments. The inbred clone showed the highest decrease in growth with water shortage (Table 2).

There were significant differences in height between clones at the time of transplanting ($F_{5,113}$=14.45, $p<0.001$). Average height was largest in clone C14 (25.0±0.5 cm) and

Table 2. Mean (±SE) values of specific leaf area (SLA) measured in leaves attached to the stem (main) and attached to branches (auxiliary) and absolute growth rate in total plant leaf area (LA). Least square means ±SD of plant height at harvest. Data were measured in plants from six *E. globulus* clones grown for 35 days under two irrigation treatments. Within a row, mean values followed by the same letter are not significantly different. An asterisk denotes significant differences between treatments for each clone.

		C13	C14	H231	H354	H463	H491
SLA (main)	HW	24.60±1.00a*	19.38±0.31c	20.75±0.65bc	20.10±1.00bc	22.08±1.05b	20.85±0.74bc
(m^2 kg^{-1})	LW	21.67±0.44a*	18.78±0.55b	20.03±0.54ab	19.36±0.54b	21.52±0.79a	18.96±0.33b
SLA (aux.)	HW	27.3±1.63a	25.2±0.58ab	24.7±1.4ab	23.7±0.83b	25.5±0.83ab	25.7±0.91ab
(m^2 kg^{-1})	LW	24.9±0.88a	23.5±0.96a	23.5±0.60a	23.1±0.54a	23.7±0.83a	24.2±0.68a
Growth-LA·10^{-3}	HW	3.50±0.18abc	3.10±0.14c	3.38±0.17bc	3.65±0.16ab	3.90±0.18a	3.50±0.17abc*
(m^2 day^{-1})	LW	3.55±0.14a	2.80±0.19c	3.09±0.11bc	3.30±0.15ab	3.51±0.20ab	3.06±0.12bc*
Height (cm)	HW	98.7±3.6bc	91.7±3.1c	96.5±3.1c	108.2±3.2ab*	111.0±3.3a*	101.8±3.4bc*
	LW	93.5±3.0ab	81.2±3.3c	91.0±3.3bc	99.2±3.1a*	99.7±3.3a*	84.9±3.0c*

lowest in H463 (20.1±0.7 cm). At the time of harvest, plant height was lower in the LW treatment compared with the HW treatment ($F_{1,90}$=39.7, p<0.001). We found a significant effect of time ($F_{1,90}$=39.7, p<0.001) and clone ($F_{5,90}$=9.51, p<0.001) on plant height. The lowest values were measured in clone C14 and the highest in H463, showing no effect of the initial height on growth. The effect of the irrigation treatment was highest in the inbred clone, as found for leaf area (Table 2).

Specific leaf area (SLA) decreased under water shortage in leaves directly attached to the main stem ($F_{1,88}$=8.1, p<0.01) and also in auxiliary leaves ($F_{1,88}$=7.6, p<0.01). Auxiliary leaves had a higher SLA than main leaves (Table 2), most probably because the proportion of growing leaves was higher in auxiliary leaves than in main leaves. Differences between clones in SLA were only significant for main leaves ($F_{5,88}$=8.9, p<0.001), with no clone × treatment interaction. For main leaves, we measured the highest and lowest values of SLA in clones C13 and C14 respectively (Table 2). Interestingly, clone C14 attained the lowest growth under both watering regimes in the present study and under field conditions.

Hydraulic traits

Water shortage significantly decreased K_{Lmax} ($F_{1,92}$=7.75, p=0.0065). Neither the effect of the clone nor the interaction between clone and treatment were significant. We measured the highest values of K_{Lmax} in HW plants from clone H463 and the lowest in LW plants from the inbred clone, H491. The highest decrease in K_{Lmax} with treatment was measured

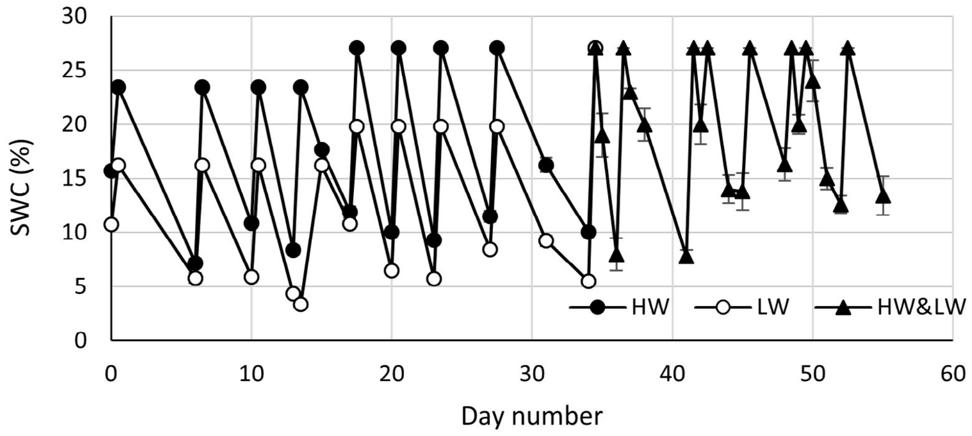

Figure 1. Average values of soil water content (SWC) calculated from the weight of the plant (W_T) before and after watering in *E. globulus* plants growing under two watering regimes: HW and LW for 35 days. From d35 onwards plants from both treatments received the same amount of water. Error bars not visible indicate SE smaller than the symbol. See text for further details.

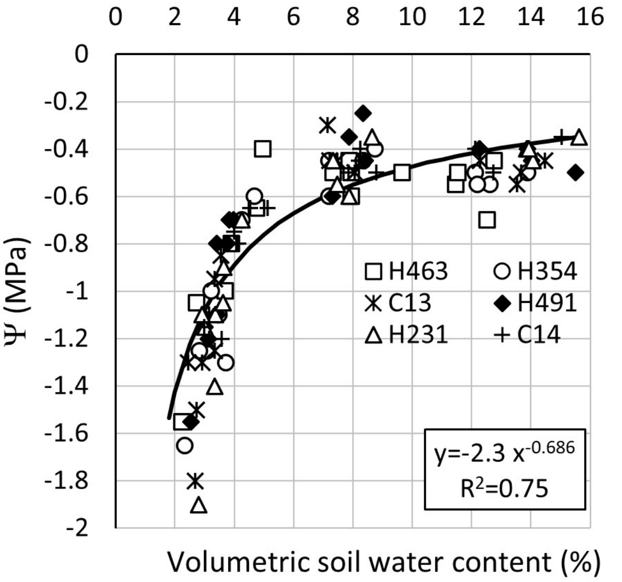

Figure 2. Relationship between soil water content and predawn leaf water potential (Ψ), measured in potted plants from six *E. globulus* clones growing under two watering regimes. All data were used to draw the regression line.

in clones H463 (40%) and H491 (37%) and the lowest in clone H354 (4.5% decrease, Fig. 3).

We found substantial hysteresis between Ψ and PLC (Fig. 4). The lower boundary of the set of points showed an almost exact coincidence with the vulnerability curve obtained by Pammenter & Vander Willigen (1998) for 7-8-year-old *E. globulus* trees (Fig. 4). There were no significant differences in PLC, either between clones or watering treatments.

Gas exchange

There was a tight relationship between stomatal conductance and the net photosynthetic rate (Fig. 5). The graphical output strongly suggested a diminished ability in the inbred clone (H491) to reach the highest values of stomatal conductance. We only measured a stomatal conductance higher than 0.5 mol m^{-2} s^{-1} in one plant from clone H491, while for the other clones 4-5 different plants were able to exceed this value (Fig. 5). We analyzed the differences between clones in the maximum stomatal conductance calculated as the average of the four highest values measured throughout the experiment. Differences between clones were close to be significant ($F_{5,18}$=2.74, p=0.052). According to the Fisher LSD test, the average value for the inbred clone was significantly lower than the average values measured in clones C13 and H463. Low-watered plants from these two clones reached the highest growth in leaf area and an average height larger than clones H491, C14 and H231 (Table 2).

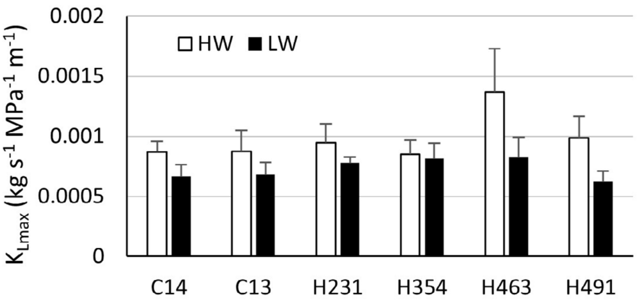

Figure 3. Mean ± SE values of maximum leaf-specific hydraulic conductivity (K_{Lmax}) measured in six *E. globulus* clones growing under two watering regimes

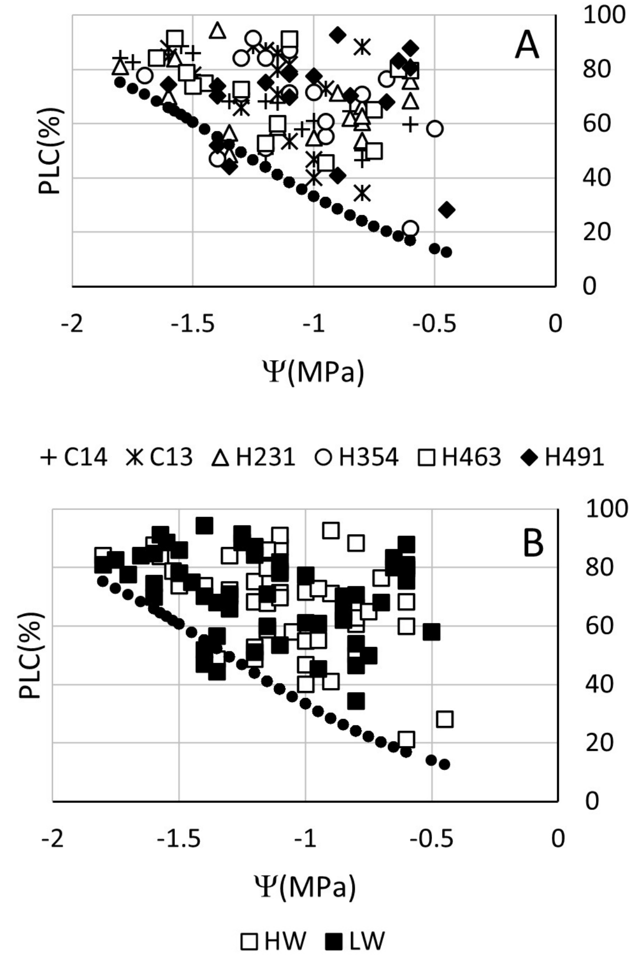

Figure 4. Relationship between native embolism, expressed as the percentage loss of hydraulic conductance (PLC) measured in plants that had undergone natural dehydration and the leaf water potential (Ψ) at the time of measuring PLC. Each data was measured in one single plant belonging to six *E. globulus* clones. Plants were grown under high (HW) or low (LW) irrigation. Panel A shows data classified by clone, panel B shows data classified by watering treatment. The dotted line corresponds to the vulnerability curve obtained by Pammenter & Vander Willigen (1998) for 7-8-year-old *E. globulus* trees.

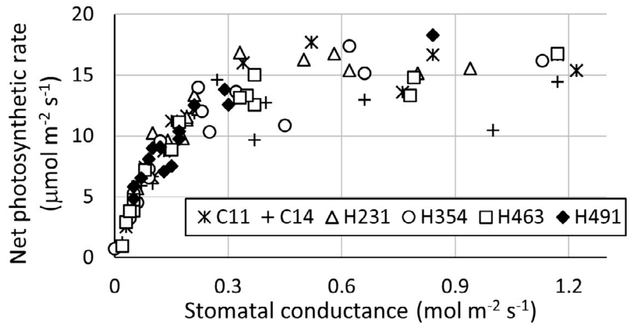

Figure 5. Relationship between stomatal conductance and net photosynthetic rate in six clones of *E. globulus*

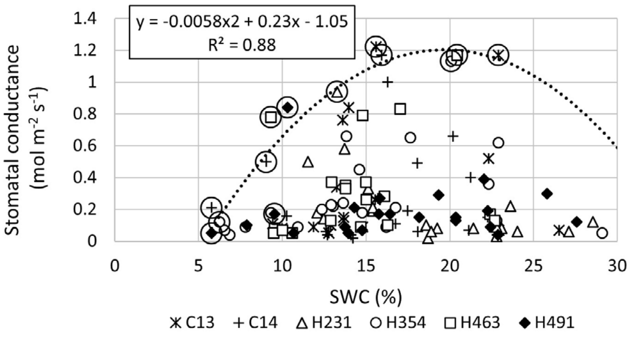

Figure 6. Relationship between stomatal conductance and volumetric soil water content (SWC), measured in six *E. globulus* clones. The second-grade polynomial was fitted to data forming the boundary, which are encircled in the figure.

The highest values of stomatal conductance were measured at a SWC higher than 15% (Fig. 6), far above the critical level of 5% for predawn Ψ drop. We fitted a second-grade polynomial to the boundary values of stomatal conductance measured in the 5.8-23% range of SWC. The regression line cut the x-axis at SWC=5.3%, showing a tendency of maximum stomatal conductance to decrease as SWC decreased and become zero before reaching the threshold for predawn leaf water potential drop.

Discussion

Breeding programs for *E. globulus* have mainly focused on improving growth. Given the economic relevance of this species, it seems crucial to gain a better understanding of the effects of climate stress on growth and survival, which could help to design management strategies to reduce climate-associated risks (Tomé et al., 2021). By analyzing the suitability of some morpho-physiological traits to characterize the response to water shortage in six selected genotypes, our study could contribute to fill this gap.

Growth and specific leaf area

Water shortage decreased growth in height and leaf area, SLA and hydraulic conductance. Our results contrast with those of Maseda & Fernández (2016), who found no significant effect of the watering regime on SLA in greenhouse-grown *E. globulus* and *Eucalyptus camaldulensis*. This discrepancy could be partly explained by a comparatively low light intensity in the study by Maseda & Fernández (2016). Decreasing SLA under water shortage would be more advantageous under high solar radiation, which increases leaf temperature and consequently increases the water vapor gradient between the mesophyll and the outside air, promoting higher rates of transpiration. A comparatively low SLA often results in an increase in net photosynthesis per unit of transpiring leaf area, which increases water use efficiency (Tortosa et al., 2022), improving plants' performance under partial stomata closure. On the other hand, a large investment in biomass to grow new leaves typically translates into diminished growth. Accordingly, we measured the lowest SLA in clone C14, which reached the lowest growth among outcrossed genotypes both in the present study and in field trials.

The highest average heights at harvest were reached by F_1 clones, independently of the watering regime. The highest growth in leaf area was also measured in F_1 clones. A similar result was observed in field trials established in SW Spain for the clones selected for the present study. Under the most favorable irrigation treatment, the inbred clone achieved higher growth in leaf area than both F_0 clones and one F_1 clone. This result contrasts with the poor development of the inbred clone in field trials established in SW Spain, under a dry Mediterranean climate. In the present study, we measured the highest reduction in growth under water shortage in clone H491. This result points at water stress as a main factor causing depressed growth in the inbred clone. Despite being a generalized trait, the magnitude of inbreeding depression may depend on the trait measured and environmental factors, often increasing in stressful environments (Sandner et al., 2021). For example, Johnsen et al. (2003) reported inbreeding depression of growth, but detected no differences in gas exchange or stable carbon isotope discrimination among inbred and out-crossed adult black spruce trees. Similarly, Isakov (2021) found both positive and negative effects of inbreeding in seedlings of *Betula* sp.

Hydraulic traits

Values of K_{Lmax} were similar to previously measured values in species from the genera *Eucalyptus* and *Populus* (Vander Willigen & Pammenter, 1998; Sparks & Black, 1999), but higher than values measured in Mediterranean species (Tognetti et al., 1998; Vilagrosa et al., 2003). The effect of losing hydraulic conductance when K_{Lmax} is high would be easier to tolerate, since it is not PLC what limits gas exchange and therefore growth, but rather the amount of hydraulic conductance that remains after embolism (Vander Willigen et al., 2000).

We measured the highest values of K_{Lmax} in HW plants from clone H463 and the lowest in LW plants from the inbred clone, H491. Interestingly, the maximum and minimum growth in leaf area were measured in these clones, under the same treatments. It is worth noting that clone H463 has been rejected as a commercial clone; because of its tendency to produce epicormic shoots under harsh conditions (F. Ruiz, ENCE, pers. comm.). Therefore, a high K_{Lmax} alone would not be a sufficiently reliable selection criterion.

Since we measured hydraulic conductance in plants that had undergone natural dehydration, PLC values quantify native embolism, which is the result of two different processes, cavitation and refilling. Results from several studies suggest that xylem conduits, in some species at least, undergo frequent cycles of cavitation and embolism repair (refilling) (Knipfer et al., 2016). Though the underlying mechanism still remains unclear, refilling may be concurrent with transpiration (Secchi et al., 2017), causing hydraulic conductance to vary diurnally as a result of both processes (Knipfer et al., 2016). Interestingly, despite the differences in the methodologies used, we found a good fit between boundary PLC values showing the highest resistance to cavitation in our study and the vulnerability curve obtained from field-grown *E. globulus* by Pammenter & Vander Willigen (1998). In their study, these authors measured embolism in branches cut from the stem and allowed to dry for two days. Not surprisingly, their set of data points showed a low degree of scattering, since there was no chance for refilling. Therefore, PLC increased progressively while water potential decreased with tissue dehydration. The high degree of scattering in our study is consistent with the phenomenon of hysteresis in the vulnerability curve (Sperry et al., 2003), which could result from partial (incomplete) refilling of the embolized vessels.

Stomatal conductance

Recent studies highlight the relevance of Ψ when modelling stomatal conductance (Anderegg et al., 2017). The dependence of stomatal conductance on Ψ has often been interpreted as an attempt to prevent hydraulic impairment from xylem embolism. In this regard, Creek et al. (2020) found that stomatal closure preceded the onset of leaf hydraulic dysfunction in three tree species. The range of PLC values suggests that this would not be the case in our study. Rather, our results point at SWC as a critical factor to stomata closure.

We measured rather small values of stomatal conductance at any SWC. The well-known lagged effect of ABA-mediated stomata closure (Klein, 2014) could contribute to explain this result. On the other hand, we found that maximum values of stomatal conductance decreased as SWC decreased, pointing at a strict stomata control to delay, as far as possible, reaching a critical threshold in SWC. Interestingly, this threshold corresponded to the SWC below which further dehydration would translate into an increasingly steeper decrease in soil water potential, as estimated from predawn leaf water potentials. In agreement with this, Carminati et al. (2020) recently reviewed the relevance of stomatal closure to prevent the formation of large gradients in water potential around the roots. Such gradients would translate into decreasing xylem water potentials, which, according to Knipfer et al. (2016) would jeopardize the refilling of embolized vessels.

The maximum values of stomatal conductance were within the range reported by Franks et al. (2009) and were lowest in the inbred clone (H491). The ability to reach high values of stomatal conductance allows plants to improve leaf cooling through transpiration and is therefore relevant to tolerate high temperatures. In Pima cotton (*Gossypium barbadense* L.), breeding for enhanced heat resistance and high yield resulted in higher stomatal conductance in the improved cultivars (Lu & Zeiger, 1994), highlighting the importance of stomatal conductance for breeding programs in heat-prone environments (Lu et al., 2000).

The diminished ability of the inbred clone to reach high values of stomatal conductance may explain its poor performance in the field, not only because of the decrease in the net photosynthetic rate, but also because of its effect in limiting the cooling capacity of the leaves. The wide range of stomatal conductance values in the other five clones shows greater plasticity, allowing the adjustment of water loss with little effect on photosynthesis. By contrast, for almost all the stomatal conductance values measured in the inbred clone, decreasing water loss by means of partial stomata closure would have a larger negative effect on photosynthesis.

Conclusions

Maximum values of stomatal conductance were the lowest in the inbred clone, showing the lowest plasticity and a diminished ability to tolerate high temperatures, which could explain its poor development under field conditions. This parameter, together with SLA could help to identify genotypes unsuitable for improving growth in drought-prone environments.

Soil water content played a relevant role in stomata control. A critical threshold for stomata closure would be the SWC at which further dehydration leads to a drop in predawn leaf water potential. This should be considered when defining watering treatments to test the response to water shortage.

We found no differences in K_{Lmax} between clones that could contribute to explain the differences observed in growth, under controlled and field conditions. Further research is needed to find the traits that allow the early selection of genotypes for improving growth in water-limited environments.

Data availability: Data will be made available on reasonable request.

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