Ann. For. Sci. 63 (2006) 941–950
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DOI: 10.1051/forest:2006077

Original article

Drought conditioning improves water status, stomatal conductance and survival of *Eucalyptus globulus* subsp. *bicostata* seedlings

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(Received 15 March 2005; accepted 29 June 2006)

Abstract – We investigated the responses of drought preconditioning in three provenances of *Eucalyptus globulus* subsp. *bicostata* (Maiden, Blakely and J.Simm) J.B. Kirkp. seedlings and assessed their effects after transplanting. After one-month moderate drought conditioning treatment, seedlings evidenced osmotic adjustment, reduction in size, leaf area, shoot/root ratio and stomatal conductance. Inter-provenance variation was found in osmotic adjustment capacity. During the first stages of transplanting period, pretreated plants showed improved water status and gas exchange capacity under drought conditions; this initial superiority was lost later on. Non-conditioned seedlings also developed morphological and physiological adjustments that allowed them to perform similarly to conditioned plants. Although preconditioning did not favour seedlings growth, it was effective in enhancing survival, an attribute correlated to shoot/root ratio and relative water content. Inter-provenances variation was found in several of the physiological and morphological responses to drought, but it was not possible to relate that variation to the dryness of the seed origin site. These results show the advantage of drought preconditioning in *Eucalyptus globulus* subsp. *bicostata* which result in better behaviour and greater survival after transplanting, factors closely associated with the establishment success.

provenances / drought acclimation / transplanting / tissue water relations / morphological characteristics

Résumé – Le conditionnement par la sécheresse améliore l'état hydrique, la conductance stomatique et la survie des semis d'*Eucalyptus globulus* subsp. *biscotata*. Nous avons étudié les réponses à un préconditionnement par la sécheresse des semis de trois provenances d'*Eucalyptus globulus* subsp. *biscotata* (Maiden, Blakely et J. Simm) J.B. Kirkp. et nous avons évalué leurs effets après transplantation. Après un mois de conditionnement par une sécheresse modérée, les semis ont montré un ajustement osmotique et une réduction de taille, de surface foliaire, de rapport partie aérienne/partie racinaire et de conductance stomatique. Nous avons trouvé des différences de capacité d'ajustement osmotique entre les provenances. Au cours des premiers jours suivant la transplantation et pendant les premières étapes de cette période, le préconditionnement par la sécheresse a permis aux semis traités d'avoir une amélioration de leur état hydrique et de leurs échanges gazeux. Ensuite leur supériorité initiale a disparu. Les semis non conditionnés ont aussi développé des changements morphologiques et physiologiques qui ont augmenté leur tolérance à la sécheresse et qui ont permis une performance similaire à celle des plants conditionnés. Cependant leurs rapports partie aérienne/partie racinaire ont été encore plus élevés. Bien que le préconditionnement n'ait pas favorisé la croissance des plants traités, il a été particulièrement efficace pour ce qui concerne la survie des plants, un attribut corrélé au rapport partie aérienne/partie racinaire et à la teneur relative en eau. Nous avons trouvé des différences dans les réponses physiologiques et morphologiques entre provenances, mais il n'a pas été possible de trouver une relation entre ces différences et la sécheresse du site d'origine des graines. Ces résultats nous permettent de confirmer l'avantage du préconditionnement par la sécheresse, pour *Eucalyptus globulus* subsp. *biscotata*, qui a pour effet un meilleur comportement pendant les premiers jours après la transplantation et une survie supérieure, facteurs étroitement associés au succès de l'installation des plants.

provenances / acclimatation à la sécheresse / transplantation / relations hydriques / caractéristiques morphologiques

1. INTRODUCTION

Seedlings establishment after transplanting is one of the most critical phases during the tree life cycle because a wide range of stressful conditions at that stage can compromise their later performance [5,13]. Water stress, which can be caused by limited contact between roots and soil, low hydraulic conductance of suberized roots and/or root confinement, represents the main constraint for plant survival and growth [3, 12, 35]. Immediately after plantation, new root growth is needed to increase water uptake and alleviate frequently occurring water stress. To assure root growth, mainly mediated by current photo-assimilates, it is necessary to maintain high plant water status and gas exchange capacity [3,48]. Therefore, factors that ameliorate plant water status after planting will be decisive for seedlings success.

The use of high quality planting stock has been identified as an effective tool to withstand field stressful conditions [3]. To harden seedlings nurseries regulate the irrigation regime by withholding irrigation or restricting the amount of water

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Table I. Location and mean climatic data of *E. globulus* subsp. *bicostata* provenances.

Provenance	Latitude	Longitude	Altitude (m)	Mean annual rainfall (mm)	Mean annual maximum temp. (°C)	Mean annual minimum temp. (°C)
Nullo Mountain	32° 43' S	150° 13' E	950	657	23	8
Wee Jasper	35° 11' S	148° 04' E	870	1077	20	6
Tumbarumba	35° 38' S	148° 09' E	720	974	19	5

supplied for short periods [23]. Seedlings of several conifer species exposed to water deficit displayed drought hardiness and were able to maintain more favorable water status, gas exchange [50,58], and greater survival after plantation compared to non-conditioned plants [51]. Similar results were observed with three representative Mediterranean species [52,53].

After submission to water restriction regimes, plants may develop different adjustments which acclimatize them to drought. It has been observed slow growth and changes in dry matter partitioning, mainly reductions in leaf area and shoot/root ratio [22,40]. Stock types with low shoot/root ratio perform better under drought conditions because a more favorable balance between water uptake and loss is reached [8,49]. Physiological changes can include osmotic adjustment, elastic adjustment and stomatal regulation [9, 10, 40, 59]. Osmotic adjustment, which allows plants to maintain turgor through the net accumulation of solutes, facilitates turgor-dependent processes such us stomatal opening and gas exchange under stressful conditions [30,46,57]. Similarly, increases in tissue elasticity allow plants to lose more water before reaching turgor loss point [46]. Thus both physiological mechanisms may contribute also to better performance after plantation [3,56].

E. globulus is one of the most appreciated and commercially important species of this genus. Besides Australia, blue gum is widely planted in the Iberian Peninsula and South America where it is considered a species of prime economical relevance. Its success as a plantation tree species has been attributed to its high productivity and superior pulping quality. During the establishment of blue gum, water deficiencies represent the main risk, causing growth reduction and affecting survival [33,41,56].

In a previous study, we analyzed the responses to three water regimes in seedlings of *E. globulus*. We observed a substantial reduction in leaf area and the development of osmotic adjustment in water-stressed seedlings of *E. globulus*, changes that were associated with drought acclimation [16]. During a 6-day drought period, imposed after preconditioning, we observed that acclimated plants showed higher stomatal conductance, predawn relative water content, water potential and greater survival than non-acclimated plants [16]. Similar responses had also been reported by Sasse and Sands [37].

Previous studies have shown the usefulness of drought preconditioning in pot-grown *E. globulus* plants [16, 37]. In contrast, little is known about seedlings performance after planting. Therefore it is necessary to evaluate the effects of preconditioning on seedlings performance after being planted [14], considering among other responses, their water status and gas exchange, processes associated with seedlings root growth and closely linked to a successful establishment [3,12].

Processes involved in drought acclimation of *E. globulus* subsp. *bicostata* have not been fully elucidated [16, 54], nor how physiological and morphological mechanisms might interact to bring about water stress tolerance in provenances coming from contrasting sites. We were particularly interested in the responses of three provenances of *E. globulus* subsp. *bicostata*, which showed promising results under field conditions in Argentina, but at the same time, exhibited some differences in survival and productivity (Pathauer, personal communication).

The objectives of the present study were: (1) to assess physiological and morphological adjustments in seedlings of three provenances of *E. globulus* subsp. *bicostata* submitted to drought preconditioning, (2) to evaluate the influence of drought preconditioning on seedlings performance after planting under water limiting conditions.

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

The experiment was carried out on three provenances of *E. globulus* subsp. *bicostata* from New South Wales, Australia. Details of their native habitats are shown in Table I. Nullo Mountain, Wee Jasper and Tumbarumba provenances were chosen due to their promising performance in southeastern Buenos Aires province, Argentina. Nullo Mountain showed the highest levels of survival, while the other two provenances had significantly the highest growth rates (Pathauer, personal communication).

An Australian tree-seed company provided the seeds. Pregerminated seeds were sown in one-liter plastic pots (diameter 10 cm, height 20 cm), filled with sieved topsoil of medium texture, and sand (3:1) (v/v) on August 1999. Seedlings were maintained during the whole experiment in a glasshouse located in the experimental field of the Faculty of Agronomy, University of Buenos Aires (34° 35' 27" S, 58° 29' 47" W, and 20 m a.s.l.). Pots on wooden benches at a density of 81 plants m^{-2}, were watered daily and periodically rotated to assure uniform growth conditions. Average temperature and relative humidity in the greenhouse were recorded during the experimental period by a meteorological station. Day-length varied from 14 h (December) to 12 h (March). Daily maximum vapor pressure deficits averaged 1.83 ± 0.08 kPa while average daily radiant energy integral was 20.28 ± 0.74 MJ m^{-2} day^{-1}.

Five months later, seedlings had an average root-collar diameter and height of 2.8 ± 0.1 mm and 18.0 ± 0.4 cm (Nullo Mountain), 2.9 ± 0.1 mm and 21.5 ± 0.4 cm (Wee Jasper), and 2.8 ± 0.1 mm and 20.3 ± 0.5 cm (Tumbarumba) respectively. Dry mass and shoot/root ratio for all provenances were 3.25 ± 0.15 g and 2.08 ± 0.1 respectively.

2.2. Drought preconditioning period

Drought preconditioning was initiated in late December 2000 (summer). Fifty seedlings per provenance were randomly selected, divided in two groups and submitted to different water regimes. During a 32-days period, 30 seedlings were watered to pot capacity daily (C plants), while the remaining 20 seedlings were submitted to a gradual water stress (S plants). Every afternoon (05.00 pm) five C plants of each provenance were weighed (W_1), watered to saturation and again weighed after 3 h (W_2). The difference ($W_2 - W_1$) yielded the amount of water lost by each provenance. S plants received a proportion of the water used by C plants of their respective provenance: at the beginning of the drought period, S plants received 50% of control and, the amount was lowered by 10% every 6 days till reached 10% of control at the end.

2.3. Plantation and post-transplanting period

When the preconditioning period ended, 30 randomly selected plants per provenance (20 C plants and 10 S plants) were planted in 10 L plastic containers, 200 µ black polyethylene (diameter 20 cm, height 35 cm), filled with sieved topsoil of medium texture, and sand (3:1) (v/v). After plantation all plants were watered to pot capacity. Five days later (on February 2000 – mid summer), for each provenance, 10 C plants were watered daily (CC); water was withheld in the other 10 C plants (CS) and the 10 S plants (SS) during a 40-days period.

2.4. Growth and dry matter allocation

To estimate seedlings attributes ten plants per treatment (combination of provenance × water regime) were randomly selected at the end of preconditioning period and five plants at the end of post-transplanting period. Seedling height (using a ruler to the nearest millimeter) and root collar diameter (using a caliper to the nearest 1/10 mm) were measured at both periods. Leaf area was measured with a leaf area meter (LI 3000, Li-Cor Inc., Lincoln, NB, USA) when preconditioning ended.

Plants were separated into stems, roots and leaves. Roots were washed thoroughly; soil was removed from roots with tap water above a 0.5-mm screen sieve. Stems, leaves and roots were oven dried at 70 °C for 72 h and weighed. Specific leaf area (*SLA*) was calculated as the ratio between leaf area and leaf dry mass. Dry mass relative growth rates (*RGR*) were calculated for both periods using the following equation: RGR (g g^{-1} d^{-1}) = $\ln M_2 - \ln M_1 / t_2 - t_1$, where M_1 and M_2 are dry mass at the beginning and the end of the sampling period, and t_1 and t_2 are the dates of sampling [19].

2.5. Stomatal conductance

Leaf stomatal conductance (g_s) was measured at ambient conditions in the glasshouse with a steady-state porometer (LI 1600, Li-Cor Inc., Lincoln, NB, USA). Measurements were done around midday during sunny days on young fully expanded leaves at the end of preconditioning, and 16, 28 and 36 days after withholding irrigation after transplanting.

2.6. Plant water potential and relative water content

Predawn relative water content (*RWC*) and leaf water potential (Ψ_w) were measured at the end of preconditioning period. After transplanting, midday *RWC* was measured 16, 28 and 36 days after initiating the differential water regime on well-expanded leaves close to those used for g_s.

Ψ_w was measured with a pressure chamber (PMS Instruments, Corvallis, OR, USA). Because seedlings of *E. globulus* subsp. *bicostata* have sessile leaves, in each selected leaf, the base of the lamina was cut with a sharp blade, and then they were placed in the chamber with the main vein protruding through the chamber opening.

RWC was measured in leaf discs that were taken to the laboratory after collection and weighed. The discs were then hydrated to full saturation, blotted gently with tissue paper and weighed. Samples were dried at 70 °C for 72 h and dry mass measured. *RWC* was calculated using the following equation: $RWC\ (\%) = (M_f - M_d)/(M_t - M_d) \times 100$, where M_f is fresh mass, M_d is dry mass and M_t is turgid mass [2].

2.7. Pressure-Volume curves

Plant water parameters were estimated through pressure-volume (P-V) curve analysis [45] at the end of both preconditioning and post-transplanting period. At dawn shoots were cut at the collar; they were re-cut under distilled water to prevent any air bubble in the conducting tissue. Shoots were maintained under distilled water and were transferred to a humid chamber with dimmed light for 12 h, at 12 °C, to allow complete re-hydration. The repeat pressurization method was used to generate the curves [18]. Samples were allowed to air dry on the lab bench between consecutive measurements and Ψ_w was determined at periodic intervals with a pressure chamber [38]. At each measurement, fresh mass was estimated by considering mean mass of the sample before and after each pressure bomb reading. When necessary, turgid mass was obtained by extrapolation of $\Psi_w = 0$ in the plot of Ψ_w versus fresh mass according to White et al. [57]. Ten to fourteen pressurizations were done in each plant, and at least five points were obtained on the linear phase of the *RWC* vs. $1/\Psi_w$ curves [47]. After these measurements, shoots were oven-dried to obtain dry mass. Schulte's PV Curve Analysis Program (version July 1998) [39], was used to estimate osmotic potential at full turgor ($\Psi\pi_{100}$), osmotic potential at turgor loss point ($\Psi\pi_0$), maximum bulk modulus of elasticity (ξ_{max}), relative water content at turgor loss point (RWC_0), apoplasmic water fraction (θ) and maximum turgor pressure (Ψ_{p100}). Turgid mass to dry mass ratio (TM/DM) was also calculated. Osmotic adjustment was evaluated as the difference in $\Psi\pi_{100}$between control and stressed plants. Elastic adjustment was calculated as the difference in ξ_{max} between control and stressed plants.

2.8. Data analysis

A multifactor analysis of variance was performed considering the effects of provenance (three) and watering regime (two or three according to the period), with five to ten replications according to the parameter. Bartlett's test was used to analyze homogeneity of variance and transformations were done when variance homogeneity was not found. When effects were significant, means were separated with Tukey's multiple range test. Simple linear regression analysis was done among variables. All statistical analysis were done using SAS statistical package, SAS Institute, Cary, NC [36].

Table II. Growth and biomass allocation of *E. globulus* subsp. *bicostata* seedlings at the end of drought preconditioning period, and dry matter relative growth rate for that period. Means ± standard error. Values followed by the same letter are not significantly different at $p < 0.05$. WR: Water regime, C: control, S: water stress. P: provenance, NM: Nullo Mountain, WJ: Wee Jasper, Tu: Tumbarumba. *LA*: Leaf area, *SLA*: specific leaf area, *RGR*: biomass relative growth rate. In the analysis of variance numbers indicate probability levels up to 0.05; ns indicates $p > 0.05$.

Factor	Level	Diameter (mm)	Height (cm)	Total DM (g)	Shoot/root biomass ratio	*LA* (cm^2)	*SLA* ($mm^2\ mg^{-1}$)	*RGR* ($g\ g^{-1}\ d^{-1}$)
WR	C	3.8 ± 0.1 a	26.6 ± 1.0 a	3.4 ± 0.1 a	2.93 ± 0.09 a	222.1 ± 9.8 a	12.1 ± 0.5 a	0.019 ± 0.001 a
	S	3.3 ± 0.1 b	22.3 ± 0.7 b	2.7 ± 0.1 b	2.48 ± 0.09 b	179.5 ± 7.1 b	12.7 ± 0.4 a	0.013 ± 0.001 b
P	NM	3.6 ± 0.1 a	21.3 ± 0.4 b	3.2 ± 0.1 a	2.61 ± 0.10 a	195.8 ± 11.6 a	11.0 ± 0.5 a	0.012 ± 0.001 c
	WJ	3.6 ± 0.1 a	24.8 ± 0.5 a	3.0 ± 0.2 a	2.73 ± 0.13 a	205.5 ± 9.1 a	12.6 ± 0.4 ab	0.021 ± 0.002 a
	Tu	3.7 ± 0.1 a	25.1 ± 0.6 a	2.9 ± 0.2 a	2.74 ± 0.14 a	204.9 ± 13.4 a	13.5 ± 0.4 b	0.015 ± 0.002 b
Two way ANOVA (*p* values)								
	WR	0.004	< 0.001	< 0.001	< 0.001	< 0.001	ns	< 0.001
	P	ns	< 0.001	ns	ns	ns	0.002	< 0.001
	WR × P	ns	ns	ns	ns	ns	ns	ns

3. RESULTS

3.1. Drought preconditioning period

3.1.1. Growth and biomass allocation

Drought preconditioning reduced seedlings growth and modified most seedlings attributes. No interactions were detected, thus the three provenances responded similarly to drought (Tab. II). Water-stressed seedlings had significantly lower diameter, height and leaf area compared to control plants. Total biomass exhibited an average reduction of 20% in water stress seedlings, and because of a lower proportion of biomass allocated to aboveground components, shoot/root biomass ratio decreased by an average of 15%. Among all the attributes, biomass *RGR* evidenced the most severe reduction, 32% lower in drought-conditioned plants. Specific leaf area was not affected by drought.

Irrespective of the nursery water regime, provenances showed significant variations. Nullo Mountain seedlings showed the lowest height, SLA and biomass *RGR* (Tab. II). In addition, there was a significant difference in the biomass *RGR* between Wee Jasper and Tumbarumba.

3.1.2. Stomatal conductance

Leaf stomatal conductance decreased progressively as water deficit intensified (data not shown). At the end of the drought preconditioning treatment, the reduction followed the same trend in all three provenances. Stressed seedlings showed significantly ($p < 0.001$) lower g_s (14.3 ± 1.8 mmol $m^{-2}\ s^{-1}$) than well-watered plants (610.7 ± 51.9 mmol $m^{-2}\ s^{-1}$). Considering stressed plants separately, Tumbarumba exhibited higher g_s (22.5 ± 6.8 mmol $m^{-2}\ s^{-1}$) than Nullo Mountain (10.2 ± 0.8 mmol $m^{-2}\ s^{-1}$) and Wee Jasper plants (13.5 ± 1.1 mmol $m^{-2}\ s^{-1}$) ($p = 0.045$).

3.1.3. Plant water potential and relative water content

Drought decreased Ψ_w of the three provenances by 2.86 MPa ($p < 0.001$). *RWC* was also significantly decreased by preconditioning but inter-provenance differences were found ($p < 0.001$) (Fig. 1). No differences were observed in the *RWC* among well-watered plants. Among stressed plants, Tumbarumba had significantly higher *RWC* than Wee Jasper.

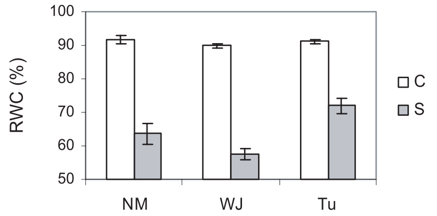

Figure 1. Relative water content (*RWC*, %) in seedlings of *E. globulus* subsp. *bicostata* after drought preconditioning. Vertical bars represent standard error. C: Control, S: water stress. NM: Nullo Mountain, WJ: Wee Jasper, Tu: Tumbarumba.

3.1.4. Pressure-Volume curves

There were significant changes in parameters derived from P-V curves at the end of preconditioning period (Tab. III). Osmotic potential at full turgor decreased significantly in water-stressed plants of Tumbarumba ($p = 0.035$), which showed an average osmotic adjustment of 0.25 MPa (Fig. 2). No significant changes were observed in the $\Psi\pi_{100}$ of Nullo Mountain and Wee Jasper. By contrast, all provenances exhibited a similar decrease in $\Psi\pi_0$ and RWC_0, and a similar increase in the θ fraction. Drought did not significantly change ξ_{max} or TM/DM. Despite differences in $\Psi\pi_{100}$ between control and stressed plants, no significant changes were observed in Ψ_{p100}, probably because this parameter depends not only on the effects of solute accumulation, but also on the cell wall elasticity, which tended to decrease ($y = -0.74\ \Psi\pi_{100} + 0.0174\ \xi_{max}$, $r^2 = 0.69$, $p < 0.001$).

Irrespective of the water regime, Tumbarumba showed the largest values of Ψ_{p100}. The lowest ξ_{max} and Ψ_{p100} were observed in Wee Jasper plants, while the largest θ corresponded to Nullo Mountain (Tab. III).

Table III. Tissue water parameters of *E. globulus* subsp. *bicostata* at the end of drought preconditioning period. Means ± standard error. Values followed by the same letter are not significantly different at $p < 0.05$. WR: Water regime, C: control, S: water stress. P: provenance, NM: Nullo Mountain, WJ: Wee Jasper, Tu: Tumbarumba. $\Psi\pi_0$: Osmotic potential at turgor loss point, $\xi_{\max}$: maximum bulk modulus of elasticity, RWC_0: relative water content at turgor loss point, θ: apoplasmic water fraction, Ψ_{p100}: maximum turgor pressure, TM/DM: turgid mass/dry mass. In the analysis of variance numbers indicate probability levels up to 0.05; ns indicates $p > 0.05$.

Factor	Level	$\Psi\pi_0$ (MPa)	$\xi_{\max}$ (MPa)	RWC_0 (%)	θ	Ψ_{p100} (MPa)	TM/DM
WR	C	–1.64 ± 0.03 a	16.34 ± 0.73 a	87.7 ± 0.45 a	0.17 ± 0.02 b	1.39 ± 0.02 a	3.12 ± 0.06 a
	S	–1.94 ± 0.04 b	15.09 ± 0.60 a	85.2 ± 0.52 b	0.33 ± 0.02 a	1.44 ± 0.03 a	3.12 ± 0.06 a
	NM	–1.74 ± 0.04 a	17.42 ± 0.72 a	87.5 ± 0.50 a	0.28 ± 0.03 a	1.42 ± 0.03 a	3.11 ± 0.07 a
P	WJ	–1.81 ± 0.04 a	13.00 ± 0.51 b	85.6 ± 0.75 a	0.26 ± 0.03 ab	1.35 ± 0.03 b	3.10 ± 0.05 a
	Tu	–1.82 ± 0.07 a	16.71 ± 0.81 a	86.2 ± 0.66 a	0.20 ± 0.02 b	1.48 ± 0.04 a	3.16 ± 0.09 a
Two way ANOVA (p values)							
WR		< 0.001	ns	< 0.001	< 0.001	ns	ns
P		ns	< 0.001	ns	0.042	0.031	ns
WR × P		ns	ns	ns	ns	ns	ns

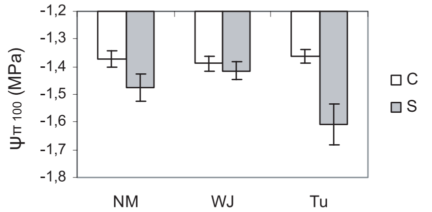

Figure 2. Osmotic potential at full turgor ($\Psi\pi_{100}$) in seedlings of *E. globulus* subsp. *bicostata* after drought preconditioning. Vertical bars represent standard error. C: Control, S: water stress. NM: Nullo Mountain, WJ: Wee Jasper, Tu: Tumbarumba.

3.2. Post-transplanting period

3.2.1. Growth and biomass allocation

Under well-watered conditions the three provenances had similar total biomass, but significant differences were observed among water regimes within each provenance ($p = 0.049$) (Fig. 3A). Biomass of CS and SS plants of Nullo Mountain were similarly reduced by an average of 72% and 75% respectively. By contrast, CS plants of Wee Jasper and Tumbarumba were less affected (68% and 53%) than their respective SS plants (77% and 75%).

Drought caused a large effect on aboveground biomass which resulted in a significant reduction in shoot/root biomass ratio ($p < 0.001$), and the three provenances responded similarly. Under water stress, seedlings had lower shoot/root biomass ratio than controls, with SS plants showing lower values than CS plants (Fig. 3B). In addition, provenances differed in biomass allocation ($p = 0.014$). Wee Jasper (3.84 ± 0.27) showed higher shoot/root biomass ratio than Nullo Mountain (3.32 ± 0.27) and Tumbarumba (3.27 ± 0.22).

Drought-induced decreases of biomass *RGR*, diameter and height varied according to the parameter (Tab. IV). Biomass *RGR* followed the same pattern as that observed for total biomass. Stressed plants of the three provenances had similar diameter, with differences among controls. In contrast we found significant differences in height among control plants and stressed plants. Both CS and SS seedlings of Nullo Mountain had similar height, while CS plants of Wee Jasper and Tumbarumba were larger than their respective SS plants.

3.2.2. Stomatal conductance

Plants exposed to soil water deficit exhibited a rapid decline in g_s, showing a different pattern according to the provenances 16-days and 28-days after initiating the water restriction (Fig. 4). In both cases, the provenance × water regime interaction was significant. Well-irrigated plants (CC) showed similar g_s, while preconditioned seedlings (SS) showed a slower decline than those not previously stressed (CS). Higher g_s were detected in SS seedlings of Tumbarumba. In the last evaluation under relative severe drought conditions, no significant differences were found between CS and SS plants ($p = 0.045$).

3.2.3. Relative water content

Withholding irrigation caused a significant decline in midday *RWC* of stressed seedlings (Fig. 4). However, 16 and 28-days after water withholding, SS plants had significantly higher levels of *RWC* ($p < 0.001$) than CS plants. Among the SS plants, Tumbarumba exhibited a significantly lower decline than those observed in the other two provenances. The decrease in *RWC* was more pronounced after 36-days of drought ($p < 0.001$), but differences between CS and SS seedlings were not significant.

3.2.4. Pressure-Volume curves

By the end of the post-transplanting period, drought had significant effects on most of tissue water parameters (Tab. V). Plants under water stress exhibited a decrease in $\Psi\pi_{100}$ and an increase in $\xi_{\max}$, with no significant differences in magnitude

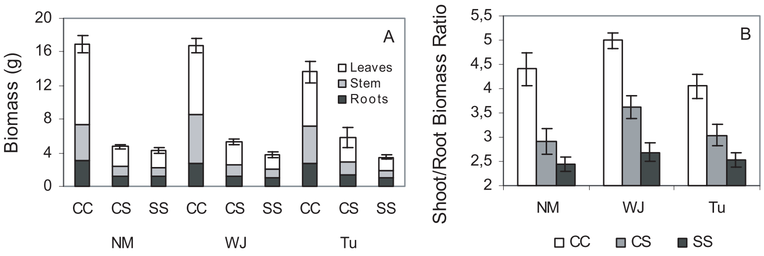

Figure 3. Total biomass (A) and shoot/root biomass ratio (B) in seedlings of E. *globulus* subsp. *bicostata* after transplanting period. Vertical bars represent standard error. CC: Control, CS: water stress in transplanting period, SS: water stress in both periods. NM: Nullo Mountain, WJ: Wee Jasper, Tu: Tumbarumba.

Table IV. Growth of *E. globulus* subsp. *bicostata* seedlings at the end of post-transplanting period, and biomass relative growth rate for that period. Means ± standard error. Values followed by different letters are significantly different at $p < 0.05$. WR: Water regime, CC: control, CS: water stress in transplanting period, SS: water stress in both periods. P: provenance, NM: Nullo Mountain, WJ: Wee Jasper, Tu: Tumbarumba. *RGR*: Biomass relative growth rate. In the analysis of variance numbers indicate probability levels up to 0.05; ns indicates $p > 0.05$.

Treatment	Diameter (mm)	Height (cm)	*RGR* ($g\ g^{-1}\ d^{-1}$)
NM CC	5.4 ± 0.1 b	38.6 ± 1.2 c	0.040 ± 0.002 a
NM CS	4.1 ± 0.1 c	26.9 ± 1.1 f	0.013 ± 0.001 cd
NM SS	3.8 ± 0.2 c	23.4 ± 1.0 f	0.010 ± 0.002 cd
WJ CC	6.2 ± 0.2 a	52.5 ± 1.3 a	0.042 ± 0.001 a
WJ CS	4.1 ± 0.2 c	35.6 ± 0.9 cd	0.015 ± 0.002 bc
WJ SS	3.6 ± 0.2 c	28.0 ± 0.7 f	0.007 ± 0.002 d
Tu CC	5.8 ± 0.1 ab	46.4 ± 1.4 b	0.035 ± 0.002 a
Tu CS	3.8 ± 0.2 c	32.3 ± 1.3 de	0.020 ± 0.004 b
Tu SS	3.4 ± 0.2 c	27.4 ± 0.4 f	0.008 ± 0.002 cd
Two way ANOVA (*p* values)			
WR	< 0.001	< 0.001	< 0.000
P	ns	< 0.001	ns
WR × P	0.013	0.048	0.008

of osmotic and elastic adjustment between CS and SS plants. Stressed plants exhibited a similar increase in Ψ_{p100}, but no change was detected in their RWC_0. Seedlings of CS treatment showed higher θ and lower TM/DM than SS seedlings, while Nullo Mountain had higher θ than Wee Jasper and Tumbarumba.

3.2.5. *Survival*

At the end of this period seedlings survival was 100% for all provenances under well watered conditions (data not shown), but water stress reduced survival rates. Mortality was more pronounced among CS seedlings. Thus, CS and SS plants of Nullo Mountain had 80% and 86% of survival, Wee Jasper, 60% and 69%, and Tumbarumba 62% and 73% respectively. Regression analysis showed relationships between survival with shoot/root biomass ratio (Fig. 5) and midday *RWC* measured 36-days after withholding irrigation (Fig. 6).

4. DISCUSSION

4.1. Preconditioning effects on physiology, growth and carbon allocation

Seedlings of *E. globulus* subsp. *bicostata* exposed to drought preconditioning experienced several changes in their physiological parameters. Plants of Tumbarumba provenance developed osmotic adjustment, a mechanism of drought adaptation in *E. globulus* [6, 15, 16, 34, 54]. This adaptive response to drought allows water to move into cells, thereby maintaining the pressure potential. But, besides the effects of osmotic and elastic properties, pressure potential depends on the interaction between these adjustments and apoplasmic water fraction [28,55]. In fact, osmotic adjustment was achieved through active solute accumulation, but the simultaneous increase in θ fraction, which causes cell reduction, might have also promoted the lowering of the $\Psi\pi$ in stressed-plants. This process, defined as passive osmotic adjustment, seems to have predominated during growing periods [32], and was observed previously in this species [6, 15]. The provenance with no significant osmotic adjustment (Wee Jasper) had the lowest value for $\xi_{\max}$, which in some way can be considered a mechanism to overcome water stress by keeping cell turgidity at low relative water contents [15, 32].

At the same time seedling exposed to water stress preconditioning experienced morphological adjustments, which were consistent with those previously reported [16]. The decrease in total biomass, leaf area and shoot/root biomass ratio, as well as their physiological changes, can be associated with their drought hardening [7, 16, 34]. Small seedlings sometimes performed better than large plants under soil water deficit [21]. The structural adjustment in leaf area would imply an effective way to limit water loss, and the greater allocation to roots would inevitably improve water uptake, allowing a more favorable plant water balance and gas exchange capacity under drought [20, 49].

4.2. Transplanting and drought effects on physiology, growth and survival

Our results showed that drought preconditioning was quite effective in improving *E. globulus* subsp. *bicostata* performance transplanted under non-irrigated conditions. The effectiveness was evidenced through the higher levels of *RWC* and

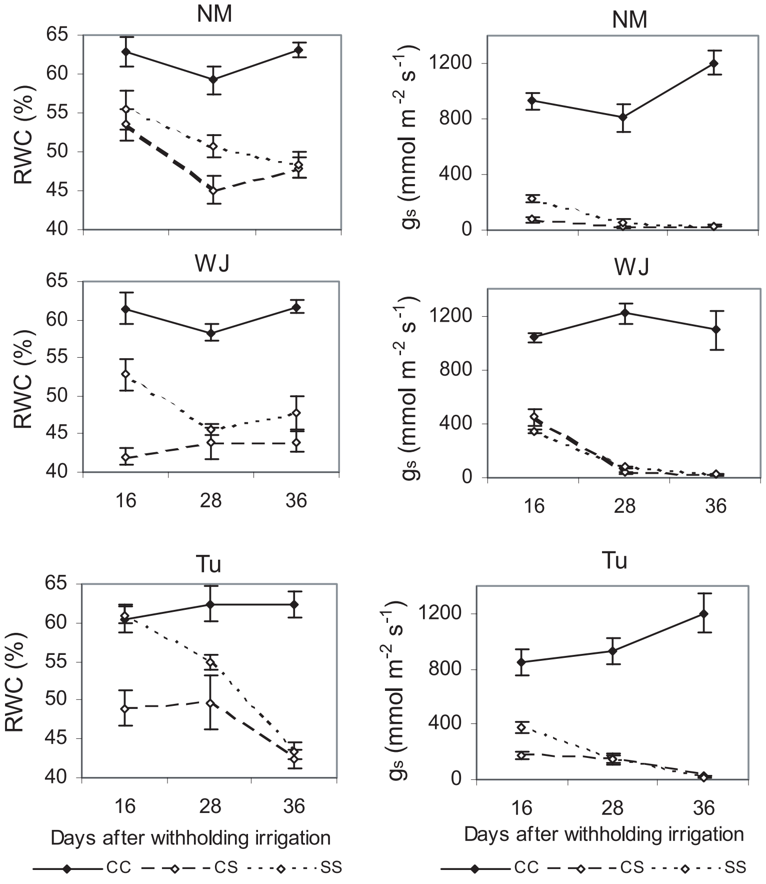

Figure 4. Midday relative water content (*RWC*, %) and stomatal conductance (g_s) in seedlings of *E. globulus* subsp. *bicostata* over the transplanting period. Vertical bars represent standard error. CC: Control, CS: water stress in transplanting period, SS: water stress in both periods. NM: Nullo Mountain, WJ: Wee Jasper, Tu: Tumbarumba.

Table V. Tissue water parameters of *E. globulus* subsp. *bicostata* at the end of post-transplanting period. Means ± standard error. Values followed by different letters are significantly different at $p < 0.05$. WR: Water regime, CC: control, CS: water stress in transplanting period, SS: water stress in both periods. P: Provenance, NM: Nullo Mountain, WJ: Wee Jasper, Tu: Tumbarumba. $\Psi\pi_{100}$: Osmotic potential at full turgor, $\Psi\pi_0$: osmotic potential at turgor loss point, $\xi_{\max}$: maximum bulk modulus of elasticity, RWC_0: relative water content at turgor loss point, θ: apoplasmic water, Ψ_{p100}: maximum turgor pressure, TM/DM: turgid mass/dry mass. In the analysis of variance numbers indicate probability levels up to 0.05; ns indicates $p > 0.05$.

Factor	Level	$\Psi\pi_{100}$ (MPa)	$\Psi\pi_0$ (MPa)	$\xi_{\max}$ (MPa)	RWC_0 (%)	θ	Ψ_{p100} (MPa)	TM/DM
	CC	–1.07 ± 0.04 a	–1.30 ± 0.05 a	8.7 ± 0.58 b	85.2 ± 0.94 a	0.14 ± 0.01 b	0.91 ± 0.04 b	4.25 ± 0.15 a
WR	CS	–1.37 ± 0.03 b	–1.68 ± 0.04 b	11.7 ± 0.70 a	85.6 ± 0.43 a	0.21 ± 0.02 a	1.16 ± 0.06 a	3.66 ± 0.17 b
	SS	–1.44 ± 0.02 b	–1.78 ± 0.03 b	11.3 ± 0.55 a	83.6 ± 1.03 a	0.14 ± 0.01 b	1.24 ± 0.05 a	3.76 ± 0.18 ab
	NM	–1.31 ± 0.05 a	–1.61 ± 0.07 a	10.9 ± 0.74 a	85.3 ± 0.69 a	0.22 ± 0.02 a	1.10 ± 0.05 a	3.85 ± 0.17 a
P	WJ	–1.31 ± 0.06 a	–1.60 ± 0.07 a	10.6 ± 0.74 a	84.7 ± 0.61 a	0.14 ± 0.01 b	1.11 ± 0.07 a	3.79 ± 0.23 a
	Tu	–1.26 ± 0.06 a	–1.55 ± 0.08 a	10.2 ± 0.71 a	84.3 ± 1.20 a	0.14 ± 0.01 b	1.10 ± 0.07 a	4.04 ± 0.14 a
Two way ANOVA (p values)								
WR		< 0.001	< 0.001	0.002	ns	< 0.001	< 0.001	0.046
P	ns	ns	ns	ns	< 0.001	ns	ns	ns
WR × P	ns	ns	ns	ns	ns	ns	ns	ns

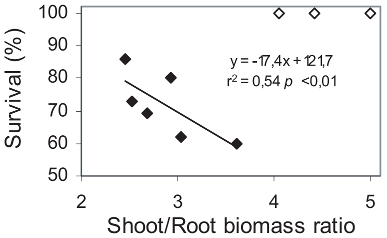

Figure 5. Relationship between survival and shoot/root biomass ratio 36-days after transplanting in seedlings of *E. globulus.* subsp. *bicostata* Each point represents the mean value of five observations of each water stressed treatment. Open symbols: CC plants values; not included in the fitted line.

g_s detected in SS plants during almost one month after withholding irrigation, and observed particularly in Tumbarumba. Indeed, the maintenance of water status and g_s are relevant factors for successful establishment of tree seedlings [3]. It is likely that the better performance of SS seedlings was mediated by their previous adjustments in morphology, carbon allocation, and physiology. All factors might have improved water absorption and restricted transpiration improving seedlings behavior during the first stages of this period.

But, the advantage of preconditioning did not last for the whole period after planting. In the last 10-days, under severe drought conditions, CS and SS plants performed similarly, having very low water status. When the second drought period ended, CS and SS seedlings displayed similar tissue water parameters, with few differences among them. The simultaneous osmotic and elastic adjustments lead to a significant increase in Ψ_{p100} of stressed plants. Both mechanisms contribute to increase the Ψ_w gradient between plant and soil, promoting water uptake at low soil water potential [32, 46], in agreement with a previous report in potted-plants of *E. globulus* [34]. The lower tissue elasticity and the higher level of osmotic adjustment observed at the end of this stage compared to those detected at the preconditioning period, could be associated to the greater severity of the drought imposed after transplanting [16]. High bulk modulus of elasticity has been related to processes of cell maturation [34]. Decrease in tissue elasticity has been identified in several species of *Eucalyptus* as a mechanism contributing to turgor maintenance under drought conditions [15, 56, 57], and after drought periods during wintertime [15]. Recently Clifford et al. [4] proposed that in species with high osmotic adjustment capacity, it is more advantageous to have rigid cell walls as these may facilitate the maintenance of cell integrity during the rehydration occurring after the drought ends. In contrast to SS plants, CS plants experienced an increase in the θ and a decrease in TM/DM, facts that probably facilitated their osmotic adjustment capacity [6, 32]. It has been suggested that this change is a strategy for plant turgor maintenance under short-term water stress [52, 55].

The results of the present experiment revealed that preconditioning did not have a clear effect on seedlings growth after transplanting in spite of the fact that drought hardening triggered several traits associated with drought tolerance, related to plant survival and growth [13, 44]. Thus, drought conditions affected CS as well as SS plants. A large change occurred in CS plants, which exposed for the first time to soil water deficit, experienced a significant decrease in growth and in their shoot/root biomass ratio almost reaching SS values. Probably stress after transplanting was too severe, adaptations triggered by preconditioning were overrun and growth was strongly reduced.

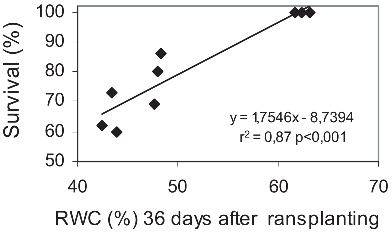

Figure 6. Relationship between survival and *RWC* 36-days after transplanting in seedlings of *E. globulus.* subsp. *bicostata* Each point represents the mean value of five observations of each treatment.

A successful establishment is characterized by high rates of survival and growth [3, 13]. Preconditioning had a positive effect on survival level. Present results are similar to those informed by Villar Salvador et al. [53] in oak seedlings. It is clear under severe drought conditions, plants generally adopt conservative strategies to avoid serious damage, which sometimes hinder growth, and survival is generally mediated by maintenance of hydraulic conductance [7]. Survival was inversely correlated with shoot/root biomass ratio. As observed previously a greater allocation to root growth improves seedling survival [8, 20]. Midday *RWC* was also closely correlated with survival, similarly to Mena-Petite et al. conclusions [29]. These results confirm that both traits can be considered reliable indicators of initial survival [13].

4.3. Provenance differences

Several studies have revealed variability in physiological and morphological responses to water stress among provenances of *Eucalyptus* from different locations [11, 26, 43]. But sometimes, species with a broad geographical distribution does not show physiological variability [17]. Differences in the osmotic adjustment capacity were observed among provenances of several *Eucalyptus* [25, 42], between and within subspecies of *E. globulus* [15, 54], as well as among clones of *E. globulus* subsp. *globulus* [34]. After preconditioning we detected osmotic adjustment in seedlings of Tumbarumba, which is indicative of inter-provenance variation in *E. globulus* subsp. *bicostata*.

Many authors observed positive effects of osmotic adjustment on gas exchange [16, 34, 57]. These effects, previously observed in pot-grown plants of blue gum [16], were also valid after transplanting: Tumbarumba maintained the highest levels of g_s and *RWC* both at the end of preconditioning and during the first weeks after plantation, probably as a consequence of its osmotic adjustment capacity.

Nullo Mountain and Wee Jasper irrespective of their previous adjustments in morphology displayed a sharper decline in g_s and *RWC*, showing a more conservative strategy than Tumbarumba.

The extended and severe water stress imposed during the post-transplanting period triggered consistent differences in the physiology and morphology of the three provenances, detected mainly among CS plants. Wee Jasper and Tumbarumba showed the highest values in several growth characteristics. Nullo Mountain plants, irrespective of the water regime, had the smallest *SLA*, which generally leads to a lower water loss per unit of leaf dry mass [1], and the lowest shoot/root biomass ratio, which should enhance survival capability during drought periods [26]. It also showed the lowest values of diameter and height under well watered conditions. Therefore this provenance favored survival over growth showing lower levels and dry mass *RGR*.

Drought resistance has been associated with low annual rainfall at seed origin, and the distribution of the species of *Eucalyptus* is influenced by drought resistance [24, 26, 27, 54]. In this study, neither the magnitude of osmotic adjustment capacity nor absolute values of $\Psi\pi_{100}$ and levels of stomatal activity were related to the dryness of the sites of origin [57].

Despite its growth responses, Nullo Mountain was quite well adapted to drought survival, which can be taken as indicative of a high drought tolerance, consistent with its dry natural habitat (Tab. I). The other two provenances, adapted to mesic conditions (Tab. I), exhibited mechanisms of drought adaptation that seemed to favor seedling growth rather than survival [27, 31]. But, to assess more accurately the relationships among seedlings responses to drought and the dryness of seed origin it would be necessary to study a larger number of provenances of *E. globulus* subsp. *bicostata*.

5. CONCLUSIONS

This study showed that drought preconditioned plants of *E. globulus* subsp. *bicostata* exhibited a better performance than non-conditioned seedlings in response to drought after transplanting. But, preconditioning had a positive effect on seedlings physiology as far as drought was not to severe; and it also improved their ability to survive water stress even though the drought severity imposed after plantation strongly reduced growth. Better performance of preconditioned seedlings during the initial phases after transplanting was facilitated by their lower shoot/root biomass ratio and lower leaf area, as well as, by their osmotic adjustment capacity. Morphological and physiological changes observed in non-conditioned plants helped them to withstand water stress conditions at later stages. Inter-provenance differences were found in several morphological and physiological traits in response to drought. However it was not possible to relate these differences to the dryness of the seed origin. These results support the expectation suggested in a previous work that preconditioned seedlings would tolerate water stress better than non-conditioned plants and would have greater chances of survival during the establishment in sites where water is a limiting factor.

Acknowledgements: This research was supported by grants from FONCyT (PIP 1495) and CONICET (BID 802 OC-AR). We are grateful to Efraín Snirman and Germán Raute for their technical assistance. Thanks are also extended to Gerald Stanhill and to two anonymous reviewers for their critical and constructive comments on the previous version of the manuscript.

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