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Elastic and osmotic adjustments in rooted cuttings of several clones of *Eucalyptus camaldulensis* Dehnh. from southeastern Australia after a drought

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Summary

Eucalyptus camaldulensis DEHNH., considered as a drought tolerant species, was examined in relation to some mechanisms linked to drought tolerance (cell-wall elastic adjustment and osmotic adjustment) and to the intraspecific variation related to those features. Rooted cuttings of five clones obtained from three different provenances from Australia (Gilgandra: 106, 109; Lake Albacutya: 119, 125; Condamine: 105) were gradually submitted to a water limitation regime. Water stress curtailed relative leaf area growth rate, pre-dawn relative water content (RWC) and noon stomatal conductance (g_s) in all clones. Shoot water parameters were estimated at the end of the drought period by pressure-volume (P–V) analysis through a repeat pressurization method. The curves obtained were analyzed by SCHULTE's P–V Curve Analysis Program. Drought decreased very significantly the osmotic potential at full turgor ($\Psi\pi_{FT}$) and at the turgor loss point ($\Psi\pi_{TLP}$), with a significant clone effect: 105 had the lowest values (–2.12 ± 0.04 MPa and –2.39 ± 0.05 MPa). Osmotic adjustment (OA) on average was 0.34 ± 0.02 MPa. Drought increased maximum bulk modulus of elasticity (ε_{MAX}) by 6.6 ± 0.7 MPa. There were no clonal differences in either OA or elastic adjustment. Water stress increased significantly turgor potential at full turgor (Ψ_{FT}), and differences between control and stress plants show that the OA recorded did not fully account for the positive changes in turgor of stressed plants. Drought decreased shoot turgid mass/dry mass ratio (TM/DM), again with a significant clone effect: 105 had the lowest value (2.66 ± 0.11). Reduced shoot TM/DM combined with increases in ε_{MAX} during stress were indicative of cell wall adjustment, reduced turgor-loss volumes and tightening of the cell walls around the protoplasts, suggesting a cell size reduction. No effects were observed on RWC at the turgor loss point. A regression model that considered ε_{MAX} and $\Psi\pi_{FT}$ explained best the response patterns of stressed plants. The mechanisms observed in *Eucalyptus camaldulensis* that delay growth while maintaining turgor and water uptake allow us to consider it as a dehydration postponement species.

Key words: Drought tolerance, pressure-volume curves, osmotic adjustment, elastic adjustment, dehydration postponement species.

Introduction

Soil water availability is one of the most limiting environmental factors for establishment and growth of *Eucalyptus* (BACHELARD 1986; MYERS & LANDSBERG 1989) and for tree species in general (MARGOLIS & BRAND 1990). *Eucalyptus* species exhibit different mechanisms activated in response to drying soils, e.g. osmotic and cell-wall elastic adjustment, changes in root/shoot dry mass partitioning, different stomatal sensitivity, leaf shedding, etc. (STONEMAN 1994; GIBSON et al. 1995). During short stress periods changes in cell osmotic potential are among the mechanisms that allow plants to maintain turgor (CLAYTON-GREENE 1983; MYERS & NEALES 1986; WANG et al. 1988). Turgor maintenance was particularly linked to leaf expansion (TYREE & JARVIS 1982), the most sensitive structural feature under water stress (HSIAO 1973).

In a previous paper we studied osmotic adjustment capacity in four *Eucalyptus* species widely grown in Argentina (LEMCOFF et al. 1994). Ranking of osmotic adjustment capacity was similar to ranking in drought tolerance based on 12 years of local experiments (GOLFARI 1985), except for *Eucalyptus camaldulensis* Dehnh. This species is drought tolerant although it has only a moderate osmotic adjustment capacity (LEMCOFF et al. 1994). Thus, other plant features must be responsible for its drought tolerance. PEREIRA & KOZLOWSKI (1976) highlighted stomatal closure as an important drought tolerance mechanism for this species. Some authors reported that both high and low tissue elasticity could add to turgor maintenance during drought periods in woody species and, therefore, cell-wall elastic adjustment has to be considered as a mechanism for water stress tolerance (FAN et al. 1994; NIELSEN & ORCUTT 1996; MARSHALL & DUMBROFF 1999). A decrease in the relative water content at the turgor loss point (RW_{TLP}) is reported to be closely associated with drought tolerance (TURNER 1986; WAN et al. 1998), and recent findings suggest that the decrease may be a function of cell-wall adjustment and tightening of the cell walls around the protoplasts (MARSHALL & DUMBROFF 1999). The decrease of the turgid mass/dry mass ratio of leaves or shoots is an index of a decrease in cell size and/or an increase in wall thickness (CORREIA et al. 1989), and both can be associated with drought tolerance.

In *E. camaldulensis* genetic variation is conspicuous probably due to its wide natural range across semiarid to humid environments (MIDGLEY et al. 1989; FARREL et al. 1996). Also, *E. camaldulensis* may be very variable reflecting the operation of local selection pressures, differing amounts of inbreeding in adjacent stands, or introgression between co-occurring members of the subgenus. When plants of this species were exposed to water restriction, interprovenance variation was documented with respect to root system, leaf production and/or seedling architecture (GIBSON et al. 1995; GIBSON & BACHELARD 1990/1991). In other *Eucalyptus* species, variation in osmotic adjustment capacity among subspecies and among provenances was reported (WANG et al. 1988; TUOMELA 1997), but little information is available regarding intra-specific variation in elastic adjustment of their cell walls during drought stress.

In Argentina, *E. camaldulensis* is found in areas where droughts of different length and intensity occur (GOLFARI 1985). Different *E. camaldulensis* provenances introduced in the last decade by the National Institute of Agricultural Technology (INTA) are performing well in local experiments. Thus, it is interesting to know the mechanisms potentially expressed under drought in order to understand and forecast better *E. camaldulensis* behavior. The objectives of this paper are a) to study the importance of elastic adjustment and osmotic adjustment as drought tolerance strategies in *E. camaldulensis,* and b) to examine intraspecific variation of those features in *E. camaldulensis.*

Materials and methods

Five clones of *E. camaldulensis* from southeastern Australia were obtained from a collection of the National Institute of Agricultural Technology (INTA), 30 km West of Buenos Aires (Castelar, Buenos Aires Province) located 34°10′S, 58°50′W, 35 m a.s.l. Clones were: 106 and 109 from Gilgandra (31°72′S, 148°66′E, New South Wales); 119 and 125 from Lake Albacutya (35°44′S, 142°02′E, Victoria); and 105 from Condamine (26°58′S, 150°07′E, Queensland). The clones were chosen because of their superior performance in preliminary field tests conducted in Argentina (J. RODRIGUEZ TRAVERSO, personal communication). Some climatic data of the

Table 1. Monthly rainfall (decile 1) and mean daily evaporation for regions of origin of 105 (Condamine), 106 and 109 (Gilgandra) and 119 and 125 (Lake Albacutya) *Eucalyptus camaldulensis* DEHNH. clones.

Provenance	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Condamine (*)												
Decile 1 Rainfall (mm)	22.1	11.2	10.3	1.3	1.0	3.4	2.0	2.0	4.9	12.4	12.1	24.6
Mean daily evaporation (mm)	7.9	7.2	7.2	5.6	4.0	3.0	2.9	3.5	4.9	5.5	7.3	8.0
Gilgandra												
Decile 1 Rainfall (mm)	5.3	7.1	2.3	0.0	3.4	6.4	9.8	8.9	5.1	8.2	5.2	6.8
Mean daily evaporation (mm)	9.4	8.4	6.7	4.6	2.6	1.8	1.8	2.6	3.9	5.8	7.7	9.0
Lake Albacutya (**)												
Decile 1 Rainfall (mm)	0.4	0.0	0.0	1.8	5.4	4.7	8.5	8.6	9.4	4.8	3.7	0.9
Mean daily evaporation (mm)	7.3	6.7	4.7	2.8	1.6	1.1	1.3	2.0	2.9	4.0	5.7	7.1

(*) Climate information for Dalby Post Office (Lat. 27° 18′S; Long. 151° 26′ E)
(**) Climate information for Ouyen Post Office (Lat. 35° 07′ S; Long. 142° 31′ E)

origin regions are given in Table 1. However, generalized meteorological data do not take rainfall variability into account (STEPHENSON 1990). Lake Albacutya, and mainly Condamine, are provenances from the toughest water regimes (Table 1).

Cuttings from 2 months old sprouts were rooted during 40 days in a greenhouse (in Castelar) with controlled temperature (26/28 °C) and humidity (95%), following the methodology of RODRIGUEZ TRAVERSO & BUNSE (1991). Then they were transplanted into plastic pots (diameter 0.1 m, height 0.2 m) containing sieved, non-fertilized topsoil of medium texture. On the surface, a thick layer of perlite was applied in order to prevent soil evaporation. Chemical analysis of soil samples revealed that total N was 0.15%; extractable P (Kurtz & Bray Nº 1) 5.9 mg kg^{-1}; K 1.1 meq 100 g^{-1}; Ca 16.2 meq 100 g^{-1}; pH 7.5; electric conductivity 15 dS m^{-1} and cation exchange capacity 12.2 $cmol_c$ kg^{-1}. Every two days, pots were relocated in order to avoid position effects inside the greenhouse. After one month forty-six plants per clone were randomly selected and placed in a greenhouse located in the experimental field of the Facultad de Agronomía (University of Buenos Aires), following a completely randomized design, at a density of 80 plants m^{-2}. Each position on the bench of the greenhouse was assigned to a combination of clone and water stress level, which means that both factors were randomized. This pattern was maintained throughout the experiment. Plants were allowed to grow with no water restriction (April to June 1996), watering was done daily at late evening, without fertilization. Day length shortened from 11h 55′ (April) to 10h 06′ (July). Average maximum vapor pressure gradient was 1.90 ± 0.27 kPa while average photosynthetically active radiation was 11.45 ± 1.92 MJ m^{-2} day^{-1}.

To perform non destructive leaf area evaluations, specific leaf area models for each single clone were developed before the beginning of the drought treatment. Six plants per clone were used randomly chosen from the 46 initially selected. Direct measurements of leaf area (obtained with a leaf area meter LI 3000, LI-COR Inc.) were correlated with calculations from blade length and width taken at half length of the blade in clone '109' and maximum width of the blade in the others, utilizing a ruler to the nearest 1 mm. Leaf shape was very similar among clones. r^2 of the regression models varied between 0.82 and 0.93, all of them being significant.

Water restriction treatment began when plants reached five months (June 27). Forty plants belonging to each clone were divided in two equal groups. One of them, control plants (C), was watered daily to soil field capacity. Daily water loss, estimated in C plants by pot weight difference, was fully replenished. The other half, water-stressed plants (S), was gradually subjected to a water restriction regime. During the first drought period (20 days), daily water supply in S plants was equivalent to 50% of the water added to the C plants. Supply was reduced to 20% of the controls in the following 21 days (second drought period) and, finally, water was withheld during the last five days (days 41 to 46 of water limitation: third drought period). The procedure mimics a drought development under field conditions.

Pre-dawn relative water content (RWC) was estimated after 18, 30 and 46 days of water limitation on five randomly chosen plants each time (and on individuals not sampled before) following BARRS & WEATHERLEY (1962). Stomatal conductance (g_s) at noon was estimated twice after 18 and 30 days of water limitation, using a transient porometer (LI 700, Li-Cor Inc.). The same intact five plants were used on both dates. Cloudy conditions precluded measurements at day 46 of water limitation.

At the end of the first and third drought periods (after 20 and 46 days of water limitation), leaf area was estimated on the five rooted cuttings used for g_s measurements using the specific models previously developed. During the second and third drought period (after 20 to 46 days of water limitation), leaf area relative growth rate (RGR) was calculated (RADFORD 1967).

Drought was interrupted when about half of the plants had reversible wilting symptoms (46 days of water limitation). Then, the five plants per clone and water restriction treatment (C and S) used for g_s and leaf area measurements were transferred for 12 hours to a humid chamber, under dimmed light (50 μmol m^{-2} s^{-1}), at 12 °C, with pot soil water at field capacity for full hydration. These plants selected for pressure-volume (PV) curves were different from those used for RWC measurements, in order to prevent any growth inhibition that could have been caused by removal of leaf disks (MITCHELL 1996). Plant water parameters were estimated by PV curves constructed with a repeat pressurization method (HINCKLEY et al. 1980) using a nitrogen gas supplied pressure chamber (PMS Instruments, Corvallis, OR). On a single distal stem portion, carrying 3 or 4 pairs of leaves (called "shoot" from now on), 10–14 pressurizations were done. Samples were allowed to dry on the lab bench between consecutive measurements. An improved version of SCHULTE's Pressure-Volume Curve Analysis Program (SCHULTE & HINCKLEY 1985), available on Internet homepage since July 1998, was used to estimate RWC at turgor loss point (RWC_{TLP}), osmotic potential at turgor loss point ($\Psi\pi_{TLP}$), osmotic potential at full turgor ($\Psi\pi_{FT}$), water potential at full turgor, pressure potential at full turgor (Ψ_{PFT}) and maximum bulk elasticity modulus (ε_{MAX}) (RICHTER 1978). Osmotic adjustment (OA) was calculated as the difference of $\Psi\pi_{FT}$, elastic adjustment (EA) as the difference of ε_{MAX}, in both cases between C and S treatments. Turgid shoot mass (TM) and dry shoot mass (DM) of the samples were estimated following CORREIA et al. (1989).

Results were processed through analysis of variance (LITTLE & HILLS 1978). Separation of means, when performed, was done using a Tukey test ($p \leq 0.05$). Simple and multiple regression analysis were used to study the relationship between most of the variables: leaf area RGR, RWC after 46 days of water limitation (RWC_{DAY46}), RWC_{TLP}, $\Psi\pi_{FT}$, $\Psi\pi_{TLP}$, ε_{MAX} and shoot TM/DM.

Results

Leaf area

At the end of the first drought period (after 20 days of water limitation) leaf area was still similar among clones and between water levels. Average value for the treatments was 77.5 ± 1.9 cm^2. However, increased water

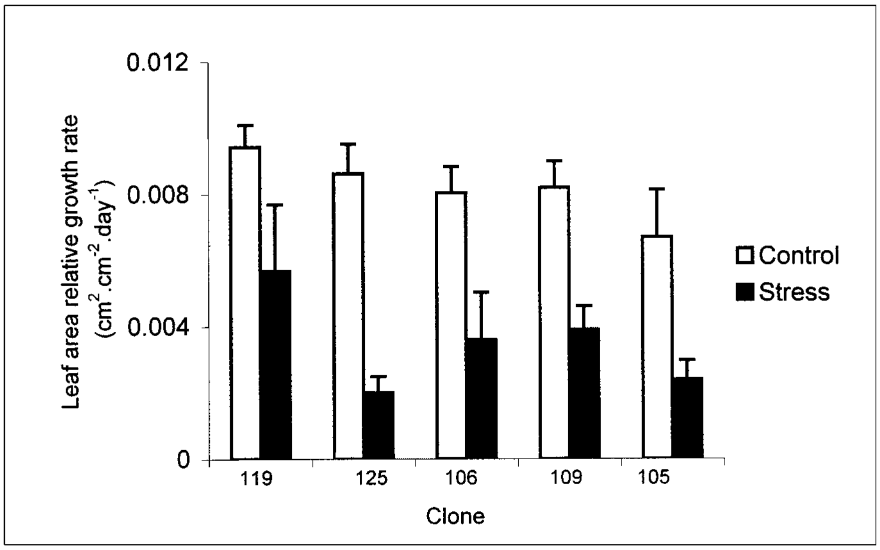

Fig. 1. Leaf area relative growth rate of *Eucalyptus camaldulensis* DEHNH. clones, with and without water stress, for the 2nd and 3rd drought periods (21–46 days after beginning of water limitation). Bars are one standard error.

Table 2. Analysis of variance for different variables of *Eucalyptus camaldulensis* DEHNH. clones, at the end of a 46 days period of water limitation, unless otherwise specified. Numbers indicate probability levels up to 0.100. NS indicates probability levels > 0.100.

Factor	Leaf area RGR	Stom. cond.	Stom. cond.	RWC	RWC	RWC	$\Psi\pi_{FT}$	$\Psi\pi_{TLP}$	ε_{MAX}	Ψ_{PFT}	RWC_{TLP}	Turgid mass / Dry mass
	21–46 d.o.w.l.	18 d.o.w.l.	30 d.o.w.l.	18 d.o.w.l.	30 d.o.w.l.	46 d.o.w.l.						
Clone (C)	NS	NS	0.048	NS	NS	NS	0.000	0.000	NS	0.070	NS	0.002
Water regime (W)	0.000	0.046	0.000	0.013	0.096	0.000	0.000	0.000	0.000	0.000	0.069	0.000
Interaction C×W	NS	0.012	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

RGR: relative growth rate.
d.o.w.l.: days of water limitation.
RWC: pre-dawn relative water content.
$\Psi\pi_{FT}$: osmotic potential at full turgor.
$\Psi\pi_{TLP}$: osmotic potential at turgor loss point.
ε_{MAX}: maximum bulk modulus of elasticity.
Ψ_{PFT}: pressure potential at full turgor.
RWC_{TLP}: relative water content at turgor loss point.

Table 3. Effect of a 46 days water limitation regime (unless otherwise specified) on pre-dawn relative content (RWC), osmotic potential at full turgor ($\Psi\pi_{FT}$), osmotic potential at turgor loss point ($\Psi\pi_{TLP}$), maximum bulk modulus of elasticity (ε_{MAX}), pressure potential at full turgor (Ψ_{PFT}), relative water content at turgor loss point (RWC_{TLP}) and shoot turgid mass/dry mass (TM/DM) in *Eucalyptus camaldulensis* DEHNH. clones (mean ± SEM, n = 25).

Water regime	RWC 18 days (%)	RWC 46 days (%)	$\Psi\pi_{FT}$ (MPa)	$\Psi\pi_{TLP}$ (MPa)	ε_{MAX} (MPa)	Ψ_{PFT} (MPa)	RWC_{TLP} (%)	TM/DM
Control	91.1 a	89.5 a	–1.77 a	–1.95 a	20.4 a	1.04 a	91.4 a	2.99 a
	(1.2)	(1.0)	(0.03)	(0.03)	(1.0)	(0.04)	(0.4)	(0.05)
Stress	86.7 b	70.8 b	–2.11 b	–2.39 b	27.0 b	1.56 b	90.5 a	2.71 b
	(1.2)	(1.2)	(0.03)	(0.04)	(0.9)	(0.04)	(0.3)	(0.04)

Note: Figures between brackets are one standard errors. Figures followed by the same letter are not different at $p \leq 0.05$.

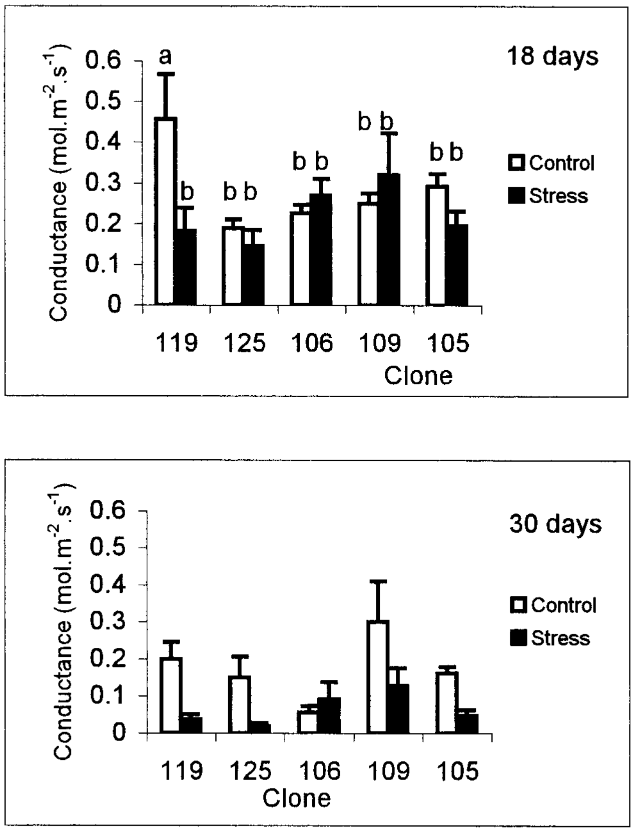

Fig. 2. Stomatal conductance at noon of *Eucalyptus camaldulensis* DEHNH. clones, with and without water stress, 18 and 30 days after beginning of water limitation. Bars are one standard error.

stress intensity, during the second and third drought periods (21–46 days of water limitation), restricted leaf area RGR significantly, confirming the sensitivity of this process to drought (Fig. 1, Table 2). No other effects were observed.

Stomatal conductance

After 18 days of water limitation, g_s of C plants of clone 119 (the only significant interaction registered for the experiment) was the largest, differing from the other treatments (Table 2, Fig. 2).

After 30 days of water limitation, drought diminished g_s significantly (Fig. 2), with clone appearing also as a main factor (Table 2): 109 had the largest value. Figures diminished when compared to the first measurement.

Plant water parameters

Water regime effect was significant for most of the parameters evaluated. Clone effect was for the osmotic potentials, the TM/DM ratio and revealed a tendency for Ψ_{PFT}.

Pre-dawn RWC after 18 and 46 days of water limitation was significantly greater in C than in S rooted cuttings (Tables 2 and 3) while at 30 days only a tendency was observed. RWC in S plants decreased with time.

Drought caused a significant decrease in $\Psi\pi_{FT}$ (Tables 2 and 3). The osmotic adjustment was 0.34 ± 0.02 MPa (Table 3, Fig. 3). A similar effect was detected in $\Psi\pi_{TLP}$ (Tables 2 and 3). A clone effect was also present: the values for clone 105 in both osmotic potentials were the lowest among the five clones (Tables 2 and 4).

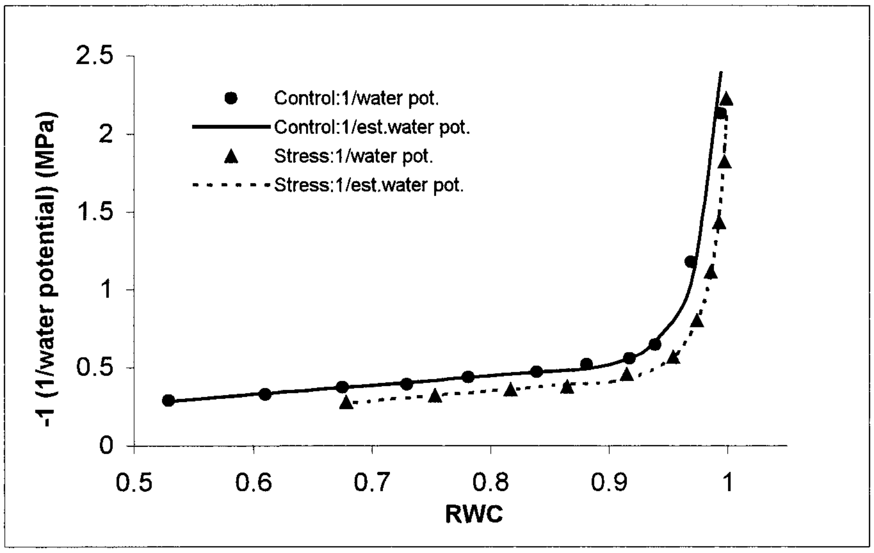

Fig. 3. Examples of pressure-volume curves for a control plant and a stressed-plant of *Eucalyptus camaldulensis* DEHNH. (clone 125), 46 days after beginning of water limitation. Solid markers are observed values; lines describe estimated values.

Table 4. Effect of a 46 days water limitation on osmotic potential at full turgor ($\Psi\pi_{FT}$), osmotic potential at turgor loss point ($\Psi\pi_{TLP}$), pressure potential at full turgor (Ψ_{PFT}), shoot turgid mass/dry mass (TM/DM), turgor changes at full turgor in stressed minus control (TC_{FT}) and TC_{FT} minus osmotic adjustment (OA) in *Eucalyptus camaldulensis* DEHNH. clones (mean ± SEM, n = 5).

Clone	$\Psi\pi_{FT}$ (MPa)	$\Psi\pi_{TLP}$ (MPa)	Ψ_{PFT} (MPa)	TM/DM	TC_{FT} (MPa)	TC_{FT}-OA (MPa)
119	–1.88 a	–2.11 a	1.21 a	2.81 ab	0.48	0.21
	(0.08)	(0.06)	(0.09)	(0.07)		
125	–1.94 a	–2.16 a	1.31 a	2.79 ab	0.46	0.14
	(0.04)	(0.05)	(0.07)	(0.05)		
106	–1.95 a	–2.16 a	1.28 a	2.99 b	0.57	0.26
	(0.05)	(0.04)	(0.13)	(0.07)		
109	–1.83 a	–2.03 a	1.22 a	3.01 b	0.50	0.12
	(0.06)	(0.07)	(0.08)	(0.08)		
105	–2.12 b	–2.39 b	1.48 a	2.66 a	0.57	0.15
	(0.04)	(0.05)	(0.06)	(0.11)		

Note: Figures between brackets are one standard errors. Figures followed by the same letter are not different at $p \leq 0.05$.

Water stress increased ε_{MAX} on average by 6.6 ± 0.7 MPa (Tables 2 and 3). The clone effect was not significant.

Ψ_{PFT} was increased by drought on average by 0.52 ± 0.03 MPa (Tables 2 and 3).

No effects on RWC_{TLP} were observed (Table 2). The average value was 90.9 ± 0.3%.

Water stress increased significantly Ψ_{PFT}, and differences between stress and control plants show that the OA recorded did not fully account for the positive changes in turgor of stressed plants.

Turgid mass/dry mass

Shoot TM/DM ratio decreased significantly in droughted plants (Tables 2 and 3). Clone had a significant effect on the relationship, 105 (Condamine) being smaller than 106 and 109 (Gilgandra) (Tables 2 and 4).

Relationship between variables

When simple regressions among stressed plants were performed, RWC_{TLP} had a significant relationship with ε_{MAX}, while RWC_{DAY46} was less strongly related (eq. 1 and 2).

[1] $RWC_{TLP} = 84.4 + 0.23\ \varepsilon_{MAX}$
($n = 25$; $r^2 = 0.43$; p (model) = 0.002)

[2] $RWC_{DAY46} = -0.50 + 0.04\ \varepsilon_{MAX}$
($n = 5$; $r^2 = 0.76$; p (model) = 0.052)

All of the regressions with $\Psi\pi_{FT}$ and those for leaf area RGR were not significant. In a regression analysis with both ε_{MAX} and $\Psi\pi_{FT}$, RWC_{TLP} was significantly related to them and r^2 increased (eq. 3).

[3] $RWC_{TLP} = 95.7 + 0.33\ \varepsilon_{MAX} - 6.69\ \Psi\pi_{FT}$
($n = 25$; $r^2 = 0.77$; p (model) = 0.000)

When both control and stressed plants were considered, $\Psi\pi_{FT}$ was inversely related to shoot TM/DM ratio (eq. 4).

[4] $\Psi\pi_{FT} = 3.97 - 0.74$ TM/DM
($n = 50$; $r^2 = 0.58$; p (model) = 0.000).

Discussion

Estimated values of $\Psi\pi_{FT}$, $\Psi\pi_{TLP}$ and OA obtained in this experiment were of the same magnitude as those previously observed in the genus (CLAYTON-GREENE 1983; TUOMELA 1997). But particularly OA was somewhat larger than previously reported for the same species and under similar growing conditions (LEMCOFF et al. 1994). Since no interaction was detected it can be concluded that there were no differences among clones in their osmotic adjustment capacity.

Estimates of ε_{MAX} were slightly larger than the previously reported bulk modulus of elasticity (ε) for the genus (CLAYTON-GREENE 1983; TUOMELA 1997). In our experiment ε_{MAX} increased significantly under drought, as it was the case for ε in *E. microtheca* (LI 1998) and for ε_{MAX} in *E. globulus* (PITA & PARDOS 2001). Neither WHITE et al. (2000) reported changes of ε for *E. camaldulensis* nor TUOMELA (1997) for *E. microtheca.* On the other hand, FAN et al. (1994) reported a small but significant decrease of ε_{MAX} in *E. grandis* as a consequence of drought. Decreases of ε or of ε_{MAX} as an effect of drought, as well as increases, have also been described in other woody species (DIAS-FILHO & DAWSON 1995; CLIFFORD et al. 1998). In our experiment there were no differences among clones in their elastic adjustment capacity. Thus, considering both paragraphs, objective b) cannot be fully confirmed at this time.

Under water stress, the more rigid the cell wall the higher the decrease in turgor pressure per unit of water loss and thus, the lower the water potential. This means that, within certain limits, cell wall stiffening as well as low $\Psi\pi_{FT}$ leads to increased soil-leaf water potential gradients and thereby promotes water uptake from drying soils (BOWMAN & ROBERTS 1985; CLIFFORD et al. 1988; MARSHALL & DUMBROFF 1999; PITA & PARDOS 2001). The relationship observed between RWC_{DAY46} and ε_{MAX} was significant, while this was not the case with $\Psi\pi_{FT}$, indicating that a stiff cell wall was a major attribute in maintaining water uptake. This behavior agrees with WHITE et al. (2000) who characterized the response of maximum stomatal conductivity to predawn leaf water potential in *E. camaldulensis* as highly sensitive. They concluded that this species avoids drought through a combination of efficient stomatal control of transpiration and access to ground water. In our experiment, g_s of the controls, mainly after 18 days of water limitation, were of the magnitude of the maximum stomatal conductance reported by WHITE et al. (2000) for the same species in winter (278 mmol m^{-2} s^{-1}). Even considering the several factors that affect stomata behavior and, as ROBICHAUX et al. (1986) described for other species, the relatively large g_s of clone 109 after 30 days of water limitation could be partially explained by the relatively high OA and the relatively low EA, while the opposite could be the explanation for the low value of clone 125.

Reduced shoot TM/DM observed in S plants indicated that they had thicker cell walls and/or smaller cell volumes than controls. These data, combined with increases in ε_{MAX}, were indicative of reduced turgor-loss volumes and tightening of the cell walls around the protoplasts and could be considered partially responsible for the maintenance of turgor. Furthermore, the high correlation between $\Psi\pi_{FT}$ and shoot TM/DM ratio (eq. 4) suggests that the osmotic adjustment can be partially attributed to a reduction in cell size, similar to results of studies by CORREIA et al. (1989) and STONEMAN et al. (1993). MARSHALL & DUMBROFF (1999) reported that maintenance of turgor during water loss in tolerant plants of *Picea glauca* was a consequence of large increases in cell-wall elastic modulus accompanied by increased wall resilience and reduced cell volumes. Turgor values at full turgor show in our case clearly that the OA recorded did not fully account for the positive changes in turgor of stressed plants in all clones (Table 4).

The significant regression between ε_{MAX} and RWC_{TLP} (eq. 1) was similar to findings by WAN et al. (1998). But a combination of both variables, ε_{MAX} and $\Psi\pi_{FT}$, explained RWC_{TLP} much better (eq. 3). A stiff cell wall and osmotic adjustment (solute accumulation and cell contraction) resulted in RWC_{TLP} estimates similar for control and stressed plants, as was also seen by CLIFFORD et al. (1998) in ber *(Ziziphus mauritiana)*.

Cell adjustments observed in this experiment, which lead to a high turgor pressure under drought, were not related to leaf area RGR. Thus, changes in tissue extension capacity (as it was seen in our work), and not necessarily reductions in cellular turgor pressure, are probably responsible for growth inhibition of expanding plant tissues under water stress (NEUMANN 1995).

$\Psi\pi_{FT}$ in clone 105 (Condamine) was the lowest one in the experiment regardless of the water availability. Under water stress this $\Psi\pi_{FT}$ could lead to a higher turgor compared to the other clones. As a matter of fact it had the highest Ψ_{PFT}, although this difference was not statistically different. It has to be considered that low values of $\Psi\pi_{FT}$ resulted from the accumulation of solute molecules, and the solutes which account for it, would have been diverted from essential processes such as protein and cell wall synthesis, thus preventing growth (MUNNS 1988). Also, this clone had the smallest TM/DM ratio, which means that it has a large cell wall and/or a small cell size, variables we did not evaluate at this time. The tendency of clone 105 to express features related to drought tolerance can be associated to its origin, since it evolved in an environment with a tendency for deluges or complete absence of rain, with this last being the dominant situation, while the other four clones evolved in less extreme environments. Gilgandra is a region transitional between summer and winter rains, with rainfall so reliable that the climate is not considered semi-arid. Lake Albacutya has winter rains in a cold climate. We suggest that plant strategies of these provenances could have been different from those at Condamine. As an example, GIBSON et al. (1995) reported that plants of *E. camaldulensis* from semi-arid environments have adopted changes in seedling architecture as a conservative strategy.

From an ecological point of view, the mechanisms observed in selected *E. camaldulensis* provenances, delaying leaf growth while maintaining RWC and probably water uptake, would be advantageous during a prolonged dry season because plants would be able to overcome this period and to grow later (DIAS-FILHO & DAWSON 1995). A decrease of ε_{MAX} as a drought effect, the classic elastic adjustment, would fit much better in a highly productive forestry scenario since it would cope with short drought periods while maintaining growth during them, as reported for *Picea mariana* by TAN & BLAKE (1997). According to the results obtained by analyzing the five clones, we conclude that drought tolerance of *Eucalyptus camaldulensis* can be partially accounted for by the concerted action of readily identifiable physiological mechanisms (OA and EA; objective a) delaying growth while increasing turgor and maintaining RWC and water uptake. Thus, *Eucalyptus camaldulensis* can be considered a dehydration postponement species following the terminology of KOZLOWSKI et al. (1991).

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References

BACHELARD, E. P. (1986): Effects of soil moisture stress on the growth of seedlings of three eucalypt species. II. Growth effects. – Aust. For. Res. **16:** 51–61.

BARRS, H. & WEATHERLEY, P. (1962): A reexamination of the relative turgidity technique for estimating water deficits in leaves. – Aust. J. Biol. Sci. **15:** 413–428.

BOWMAN, W. D. & ROBERTS, S. W. (1985): Seasonal changes in tissue elasticity in chaparral shrubs. – Physiol. Plant. **65:** 233–236.

CLAYTON-GREENE, K. A. (1983): The tissue water relationships of *Callitris columellaris, Eucalyptus melliodora* and *Eucalyptus microcarpa* investigated using the pressure-volume technique. – Oecologia: **57:** 368–373.

CLIFFORD, S.C.; ARNDT, S.K.; CORLETT, J.E.; JOSHI, S.; SANKHLA, N.; POPP, M. & JONES, H.G. (1998): The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana* (Lamk.). – J. Exp. Bot. **49:** 967–977.

CORREIA, M.J.; TORRES, F. & PEREIRA, S. (1989): Water and nutrient supply regimes and the water relations of juvenile leaves of *Eucalyptus globulus*. – Tree Physiol. **5:** 459–471.

DIAS-FILHO, M.B. & DAWSON, T.E. (1995): Physiological responses to soil moisture stress in two Amazonian gap-invader species. – Funct. Ecol. **9:** 213–221.

FAN, S.; BLAKE, T.J. & BLUMWALD, E. (1994): The relative contribution of elastic and osmotic adjustment to turgor maintenance of woody plants. – Physiol. Plant. **90:** 414–419.

FARREL, R.C.; BELL, D.T.; AKILAN, K. & MARSHALL, J.K. (1996): Morphological and physiological comparison of clone lines of *Eucalyptus camaldulensis*. I. Responses to drought and waterlogging. – Aust. J. Plant. Physiol. **23:** 497–507.

GIBSON, A. & BACHELARD, E. P. (1990/1991): Stress related changes in the architecture of seedlings of three provenances of *Eucalyptus camaldulensis* Dehnh. – Water, Air & Soil Pollut. **54:** 315–322.

GIBSON, A.; BACHELARD, E. P. & HUBICK, K. T. (1995): Relationships between climate and provenance variation in *Eucalyptus camaldulensis* Dehnh. – Aust. J. Plant. Physiol. **22:** 453–460.

GOLFARI, L. (1985): Distribución regional y condiciones ecológicas de los eucaliptos cultivados en la Argentina. Problemas inherentes. – Centro de Investigaciones y Experiencias Forestales, Buenos Aires, Argentina. Publ. Tecn. 1.

HINCKLEY, T. M.; DUHME, F.; HINCKLEY, A. R. & RICHTER, H. (1980): Water relations of drought hardy shrubs: osmotic potential and stomatal reactivity. – Plant Cell Environ. **3:** 131–140.

HSIAO, T. C. (1973): Plant responses to water stress. – Ann. Rev. Plant Physiol. **24:** 519–570.

KOZLOWSKI, T. T.; KRAMER, P. J. & PALLARDY, S. G. (1991): The Physiological Ecology of Woody Plants. New York – London (Acad. Pr.).

LEMCOFF, J. H.; GUARNASCHELLI, A. B.; GARAU, A. M.; BASCIALLI, M. E. & GHERSA, C. (1994): Osmotic adjustment and its use as selection criterion in *Eucalyptus* species. – Can. J. For. Res. **24:** 2404–2409.

LI, C. (1998): Some aspects of leaf water relations in four provenances of *Eucalyptus microtheca* rooted cuttings. – Forest Ecol. Manage. **111:** 303–308.

LITTLE, T. M. & HILLS, F. J. (1978): Agricultural Experimentation. Design and Analysis. New York (Wiley).

MARGOLIS, H. & BRAND, D. (1990): An ecophysiological basis for understanding plantation establishment. – Can. J. For. Res. **20:** 375–390.

MARSHALL, J. G. & DUMBROFF, E. B. (1999): Turgor regulation via cell wall adjustment in white spruce. – Plant Physiol. **119:** 313–319.

MEIER, C. E.; NEWTON, R. J.; PURYEAR, J. D. & SEAN, S. (1992): Physiological responses of Loblolly Pine (*Pinus taeda* L.) rooted cuttings to drought stress: osmotic adjustment and tissue elasticity. – Plant Physiol. **140:** 754–760.

MIDGLEY, S. J.; ELDRIDGE, K. G. & DORAN, J. C. (1989): Genetic resources of *Eucalyptus camaldulensis.* – Commonw. For. Rev. **68:** 295–308.

MITCHELL, C. A. (1996): Recent advances in plant response to mechanical stress: Theory and application. – HortScience **31:** 31–35.

MUNNS, R. (1988): Why measure osmotic adjustment? – Aust. J. Plant Physiol. **15:** 717–726.

MYERS, B. A. & LANDSBERG, J. J. (1989): Water stress and seedling growth of two eucalypt species from contrasting habitats. – Tree Physiol. **5:** 207–218.

MYERS, B. A. & NEALES, T. F. (1986): Osmotic adjustment, induced by drought, in seedlings of *Eucalyptus* species. – Aust. J. Plant Physiol. **13:** 597–603.

NEUMANN, P. M. (1995): The role of cell wall adjustment in plant resistance to water deficits. – Crop Sci. **35:** 1258–1266.

NIELSEN, E. & ORCUTT, D. (1996): The physiology of plants under stress. Abiotic factors. New York (Wiley).

PEREIRA, J.S. & KOZLOWSKI, T.T. (1976): Leaf anatomy and water relations of *Eucalyptus camaldulensis* and *E. globulus* seedlings. – Can. J. Bot. **54:** 2868–2880.

PITA, P. & PARDOS, J.A. (2001): Growth, leaf morphology water use and tissue water relations of *Eucalyptus globulus* clones in response to water deficit. – Tree Physiol. **21:** 599–607.

RADFORD, P. (1967): Growth analysis formulae: their use and abuse. – Crop Sci. **7:** 171–175.

RICHTER, H. (1978): A diagram for the description of water relations of plant cells and organs. – J. Exp. Bot. **29:** 1197–1203.

ROBICHAUX, R.H.; HOLSINGER, K.E. & MORSE, S.R. (1986): Turgor maintenance in Hawaiian *Dubautia* species that differ in habitat and diploid chromosome number. – Oecologia **66:** 77–80.

RODRIGUEZ TRAVERSO, J. & BUNSE, G.C. (1991): Multiplicación por estacas en especies de eucaliptos. – Proc. Jornadas Sobre Eucaliptos de Alta Productividad. CIEF, Buenos Aires, Argentina, p. 178–184.

SCHULTE, P. & HINCKLEY, T. (1985): A comparison of pressure-volume curve data analysis techniques. – J. Exp. Bot. **36:** 1590–1602.

STEPHENSON, N.L. (1990): Climatic control of vegetation distribution: the role of the water balance. – Am. Nat. **135:** 649–670.

STONEMAN, G.L.; TURNER, N.C. & DELL, B. (1993): Leaf growth, photosynthesis and tissue water relations of greenhouse-grown *Eucalyptus marginata* seedlings in response to water deficits. – Tree Physiol. **14:** 633–646.

STONEMAN, G.L. (1994): Ecology and physiology of establishment of eucalyptus seedling from seed: A review. – Aust. For. Res. **5:** 11–30.

TAN, W. & BLAKE, T.J. (1997): Gas exchange and water relations responses to drought of fast- and slow-growing black spruce families. – Can. J. Bot. **75:** 1700–1706.

TUOMELA, K. (1997): Leaf water relations in six provenances of *Eucalyptus microtheca:* a greenhouse experiment. – For. Ecol. Manage. **92:** 1–10.

TURNER, N.C. (1986): Adaptation to water stress: a changing perspective. – Aust. J. Plant Physiol. **13:** 175–190.

TYREE, M.T.; CHEUNG, Y.N.S.; MACGREGOR, M.E. & TALBOT, A.J.B. (1978): The characteristics of seasonal and ontogenetic changes in the tissue-water relations of *Acer, Populus, Tsuga* and *Picea.* – Can. J. Bot. **56:** 635–647.

TYREE, M.T. & JARVIS, P. (1982): Water in tissues and cells. In: LANGE, O.L.; NOBEL, P.S.; OSMOND, C.B. & ZIEGLER, H. (eds.): Physiological Plant Ecology. II. Water Relations and Carbon Assimilation. Encyclopedia of Plant Physiology. New Ser. **12B:** 37–77. Berlin–Heidelberg–New York (Springer).

WAN, C.; SOSEBEE, R.E. & MCMICHAEL, B.L. (1998): Water relations and root growth of two populations of *Gutierrezia sarothrae.* – Environ. Exp. Bot. **39:** 11–20.

WANG, D.; BACHELARD, E.P. & BANKS, C.G. (1988): Growth and water relations of two subspecies of *Eucalyptus globulus.* – Tree Physiol. **4:** 129–138.

WHITE, D.A.; TURNER, N.C. & GALBRAITH, J.H. (2000): Leaf water relations and stomatal behavior of four allopatric *Eucalyptus* species planted in Mediterranean southwestern Australia. – Tree Physiol. **20:** 1157–1165.