

Leaf water relations of *Eucalyptus cloeziana* and *Eucalyptus argophloia* in response to water deficit

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Summary Leaf water relations responses to limited water supply were determined in 7-month-old plants of a dry inland provenance of *Eucalyptus argophloia* Blakely and in a humid coastal provenance (Gympie) and a dry inland provenance (Hungry Hills) of *Eucalyptus cloeziana* F. Muell. Each provenance of *E. cloeziana* exhibited a lower relative water content at the turgor loss point, a lower apoplastic water content, a smaller ratio of dry mass to turgid mass and a lower bulk modulus of elasticity than the single provenance of *E. argophloia*. Osmotic potential at full turgor and water potential at the turgor loss point were significantly lower in *E. argophloia* and the inland provenance of *E. cloeziana* than in the coastal provenance of *E. cloeziana*. There was limited osmotic adjustment in response to soil drying in *E. cloeziana*, but not in *E. argophloia*. Between-species differences in water relations parameters were larger than those between the *E. cloeziana* provenances. Both *E. cloeziana* provenances maintained turgor under moderate water stress through a combination of osmotic and elastic adjustments. *Eucalyptus argophloia* had more rigid cell walls and reached lower water potentials with less reduction in relative water content than either of the *E. cloeziana* provenances, thereby enabling it to extract water from dryer soils.

Keywords: *apoplastic water, bulk modulus of elasticity, drought tolerance, leaf anatomy, osmotic adjustment, pressure–volume curves, subtropics.*

Introduction

Currently, the planting of forests in Queensland, Australia is concentrated in the humid coastal region where annual rainfall is greater than 1000 mm. However, with increasing scarcity and cost of land suitable for commercial forestry, further expansion of plantation forestry will increasingly occur on lands subject to occasional drought (annual rainfall between 600 and 1000 mm). The bulk of forest plantations in southeast Queensland comprise species of *Pinus*, whereas *Eucalyptus*

argophloia Blakely and *Eucalyptus cloeziana* F. Muell. are favored commercial species in subtropical eastern Australia (Sewell 1997, Keenan et al. 1998). Because a physiological understanding of the responses of these native hardwood species to soil water availability is lacking, it is difficult to predict which species is most suitable for a particular site.

Maintenance of positive leaf turgor as relative water content and leaf water potential decrease is an important adaptation to water stress (Ladiges 1974, 1975, Clayton-Greene 1983, Turner 1988, Prior and Eamus 1999). A decline in leaf water potential in response to soil drying is accompanied by a decline in osmotic potential resulting from either passive concentration of solutes in response to dehydration or active accumulation of solutes (Girma and Krieg 1992, Mitchell 1992). Lowering of osmotic potential in response to dehydration can result from a decrease in water content per unit of dry mass, a change in leaf tissue elasticity, or the relative partitioning of water between the symplast and apoplast (Girma and Krieg 1992). Although osmotic adjustment, defined as the lowering of osmotic potential due to net solute accumulation (Munns 1988, Girma and Krieg 1992), may not prevent decreases in growth and gas exchange in response to drought (Jones and Rawson 1979), it allows normal physiological activity to continue at reduced rates (Turner 1988), and helps maintain tissue integrity and the potential for the rapid resumption of growth when water stress is relieved (Mitchell 1992).

In many eucalypt species, the capacity to maintain turgor through osmotic adjustment (Clayton-Greene 1983, Myers and Neales 1986, Lemcoff et al. 1994, Tuomela 1997, White et al. 2000, Pita and Pardos 2001) and tissue elasticity (White et al. 1996, White et al. 2000) accounts for their tolerance of environmental water deficits. However, little is known of drought responses of *E. cloeziana* and *E. argophloia*, which are among native hardwoods viewed as promising candidates for reforestation in subtropical Queensland (Keenan et al. 1998). *Eucalyptus cloeziana* has a wide natural distribution in

coastal (annual rainfall >1400 mm) and inland (annual rainfall < 700 mm) Queensland (Boland et al. 1984). The large differences in rainfall, temperature, geology and soil between these habitats suggests great genetic variability within this species. However, little is known about the physiological basis of intraspecific variation in growth and survival during drought in this species. *Eucalyptus argophloia* has a narrow natural distribution near Chinchilla in southern inland Queensland, where it occurs under warm subhumid (annual rainfall < 700 mm) conditions (Boland et al. 1984).

Here, we report on leaf water relations of *E. argophloia* and *E. cloeziana* in response to soil drying. We hypothesized that provenances originating from inland areas with low rainfall are more drought resistant and better able to respond to water stress by adjusting their leaf water relations than provenances originating from high-rainfall coastal areas. Specifically, we determined: (1) overall differences in drought resistance among *E. argophloia* and inland and coastal provenances of *E. cloeziana*; and (2) whether the ability to adjust to water stress relates to the place of origin of each population. Plants were exposed to variable water regimes for a prolonged period, or to a severe drought.

Materials and methods

Plants and growth conditions

Seedlings of *E. argophloia* from Ballon, a coastal provenance of *E. cloeziana* from Gympie and a dry inland provenance of *E. cloeziana* from the Hungry Hills (Table 1) were grown in pots containing a 50:50 (v/v) mix of peat and vermiculite at the Queensland Forestry Research Institute nursery, Gympie. In June 2000, the 6-month-old seedlings were moved to the University of Queensland greenhouse facility (Brisbane) and transferred to plastic pots (175 mm diameter × 175 mm deep) lined with polyethylene and containing a 4:1:1 (v/v) mix of loam-textured nursery top soil, vermiculite and perlite. Slow-release fertilizer containing micronutrients was mixed with the potting medium at a concentration of 2 kg m⁻³ and the pots were packed to a bulk density of about 1100 kg m⁻³. White plastic beads were spread on the soil surface of each pot to minimize evaporation (Myers and Landsberg 1989). The seedlings were grown in a semi-controlled environment in a natu-

rally illuminated greenhouse that provided a maximum irradiance of 2000 μmol m⁻² s⁻¹ and daytime temperatures ranging from 21 to 37 °C during the experimental period (August to November 2000).

Response to variable watering

Eight weeks after plants were transferred to the greenhouse, 60 seedlings of uniform height were chosen from each provenance. Initial heights (± SE) for the coastal and inland provenances of *E. cloeziana* and the single provenance of *E. argophloia* were 21.4 ± 1.2, 19.9 ± 0.8 and 33.8 ± 1.8 cm, respectively. Twenty seedlings per provenance were assigned to each of three treatments (100% (T₁₀₀), 70% (T₇₀) and 50% (T₅₀) of field capacity) and arranged on the greenhouse benches in a completely randomized design, with benches being rotated every 2 days. The treatments were imposed for 96 days. Every 2 days, T₁₀₀ plants received an amount of water equivalent to that lost through evapotranspiration, estimated as the change in mean weight of five pots per provenance. The T₇₀ and T₅₀ plants received 70 and 50%, respectively, of water supplied to the corresponding T₁₀₀ plants following the methods of Myers and Landsberg (1989), Tuomela (1997), Osório et al. (1998) and Nativ et al. (1999). Sequential measurements of leaf size and number were made on the same five plants per treatment and provenance. Every 2 weeks, mean fresh mass of an additional three plants per treatment and provenance was measured to correct for pot weight changes resulting from plant growth.

Severe water regime

Responses to severe water stress (T₀) relative to the T₁₀₀ treatment were examined in 20 seedlings per provenance. Water was withheld until *E. cloeziana* plants had wilted overnight. During this period, sequential measurements of leaf water variables were made on five plants in each of the T₀ and T₁₀₀ treatments per provenance. During the T₀ treatment, *E. cloeziana* leaves developed necrotic spots leading to mortality of some plants, whereas *E. argophloia* leaves showed no visible signs of wilting. All plants were watered to field capacity and their recovery was compared. Thereafter, the plants were re-watered to field capacity every second day. Because *E. argophloia* plants recovered more quickly than *E. cloeziana* plants,

Table 1. Some characteristics of the three *Eucalyptus* provenances studied (seeds were provided by the Queensland Forestry Research Institute).

Provenance	Seedlot no.	No. parent trees	Origin				
			Locality	Latitude S	Longitude E	Altitude (m)	Mean annual rainfall (mm)
<i>E. cloeziana</i> (Gympie, humid coastal)	4363	11	SF 949 Goomboorian	26°03'	152°42'	110	1210
<i>E. cloeziana</i> (Hungry Hills, dry inland)	10823	20	SF 57 Mungy	25°18'	151°22'	310	780
<i>E. argophloia</i> (Ballon, dry inland)	5520	18	SF 302 Ballon	26°20'	150°20'	300	650

they were subjected to another drying cycle after a 3-week recovery period to determine their wilting point.

Leaf water potential, relative water content and osmotic potential

Predawn and midday leaf water potentials were measured with a Scholander-type pressure chamber every 2 weeks in T₁₀₀, T₇₀ and T₅₀ plants and on four occasions in T₀ plants (Ritchie and Hinckley 1975), taking the precautions outlined by Turner (1988). Two leaf portions (2 × 3 cm) were cut from a young fully expanded leaf of each plant at midday and immediately stored in an airtight 1.5-ml tube. After the fresh mass (W_F) of the leaf portions had been recorded, the portions were floated on deionized water and illuminated at the light compensation point (50 μmol m⁻² s⁻¹) for 4 h to rehydrate. The portions were then weighed to obtain turgid mass (W_T). Dry mass (W_D) was determined after drying the leaf sample at 80 °C to constant mass. Relative leaf water content (R^*) was determined as (Barrs and Weatherley 1962):

$$R^* = \frac{(W_F - W_D)}{(W_T - W_D)} \times 100 \quad (1)$$

One leaf similar to that used for relative water content and water potential measurements was excised from each plant at midday, wrapped in a polyethylene film and placed in a numbered aluminum foil envelope. The samples were immediately frozen in liquid nitrogen and stored at -10 °C. The samples were thawed for 30 min before measurement. Leaf sap was expressed with a 1-ml syringe and 10 μl was placed on a filter paper disc in a Wescor 5500 vapor pressure osmometer chamber (Wescor, Logan, UT) and osmotic potential (π) determined. Values of π and R^* were used to calculate leaf osmotic potential at 100% R^* (π_s) (Wilson et al. 1979):

$$\pi_s = \pi \left(\frac{R^* - A_{wc}}{100 - A_{wc}} \right) \quad (2)$$

where A_{wc} is relative apoplastic water content derived from pressure–volume curves. Osmotic adjustment was estimated as the difference between π_s of T₁₀₀ plants and π_s of T₇₀ or T₅₀ plants (Li 1998) averaged over the 96-day treatment period.

Pressure–volume curves

Pressure–volume analysis (Tyree and Hammel 1972) was used to derive leaf water relations parameters at the end of the experiment. Pressure–volume curves were generated by the free transpiration method (Parker and Pallardy 1987, Lemcoff et al. 1994). A young, fully expanded leaf was excised from each plant under fresh deionized water. The cut petiole was maintained in deionized water to allow the leaf to rehydrate overnight in the dark in an airtight container (Clayton-Greene 1983, Parker and Pallardy 1987). The following morning, each leaf was surface-dried and initial mass and water potential were determined. Leaves were allowed to dry on a bench while W_F and water potential (Ψ_w) were measured periodically.

The procedure was repeated until Ψ_w values were within -3 to -5 MPa. The leaves were then oven-dried at 80 °C to obtain W_D . Five curves were constructed per provenance and treatment. The value of W_T at zero Ψ_w was calculated from the linear relationship between Ψ_w and W_F (Ladiges 1975, Clayton-Greene 1983), and R^* at each measured Ψ_w was determined by Equation 1.

Osmotic potential at full turgor (π_s), water potential at the turgor loss point (Ψ_0), relative water content at zero turgor (R^{*0}) and A_{wc} were determined from a plot of $1/\Psi$ against R^* (Ladiges 1975, Wilson et al. 1979, Turner 1988). Osmotic potentials at each Ψ_w were calculated as (Wilson et al. 1979):

$$\pi = \frac{\pi_s(100 - A_{wc})}{R^* - A_{wc}} \quad (3)$$

Turgor pressure (Ψ_p) was calculated as the difference between Ψ_w and osmotic potential (π). The extent of change in tissue volume with decreasing water availability was measured as the bulk modulus of elasticity (ϵ) (Mitchell 1992) by the method of Turner (1988):

$$\epsilon = \left(\frac{\Delta\Psi_p}{\Delta R^*} \right) R^* \quad (4)$$

where $\Delta\Psi_p/\Delta R^*$ is the slope of the linear relationship between Ψ_p and R^* in the positive turgor region.

Leaf anatomy

Fresh leaf pieces were taken from T₁₀₀ plants of each provenance on Day 96 and fixed in 70% formalin in acetic acid (Luna and Armed Forces Institute of Pathology (USA) 1968) for 12 h, dehydrated in a tertiary butyl alcohol series and embedded in paraffin wax (56–58 °C melting point). Thin sections (6 μm) of tissue were obtained in a retraction rotary microtome, mounted on gelatin-coated slides and stained with Safranin O and Fast green. The sections were examined with a light microscope, and the thicknesses of the total leaf (adaxial to abaxial thickness), palisade parenchyma, spongy mesophyll, cuticle and epidermal layer were measured.

Data analysis

Effects of water availability, provenance and species on water relations variables were evaluated by two-way and one-way analyses of variance. Relationships between variables were analyzed by linear regression methods. Means were compared by Duncan's multiple range test (1985; SAS Institute, Cary, NC). Differences were considered significant at $P \leq 0.05$.

Results

Plant growth, relative water content and prolonged water stress

Responses to water deficit differed between *E. cloeziana* and *E. argophloia*. Stem diameter and leaf area of T₁₀₀ plants of

both *E. cloeziana* provenances were significantly greater than those of the T₇₀ and T₅₀ plants, which did not differ significantly from each other (Table 2). Stem diameter and leaf area both decreased with increasing water stress in *E. argophloia* (Table 2). Stem diameter and leaf area were strongly correlated (Figure 1a) and regression coefficients for the coastal (0.0012) and inland (0.0013) provenances of *E. cloeziana* were smaller than for the single provenance of *E. argophloia* (0.0029). Stem diameter was strongly correlated to leaf volume (Figure 1b), but the difference between species was small. In the coastal provenance of *E. cloeziana*, T₅₀ plants maintained a significantly ($P \leq 0.05$) lower R^* than T₁₀₀ and T₇₀ plants, which did not differ significantly from each other (Figure 2a). For the inland provenance of *E. cloeziana*, R^* did not differ significantly between T₇₀ and T₅₀ plants. There were no significant differences in R^* among T₁₀₀, T₇₀ and T₅₀ plants of *E. argophloia*, which maintained a mean R^* of 86.7% (Figure 2a). There were no significant differences in mean R^* between species in the T₁₀₀ and T₇₀ treatments, but T₅₀ plants differed significantly between species, with *E. argophloia* having the highest R^* and the coastal provenance of *E. cloeziana* having the lowest R^* (Figure 2a).

Osmotic adjustment

In both *E. cloeziana* provenances, π_s was significantly greater in T₁₀₀ plants than in T₇₀ and T₅₀ plants, which did not differ from each other (Figure 2b). There were no significant treatment effects on π_s of *E. argophloia* (Figure 2b). Although there were no significant differences in π_s between species in the T₁₀₀ and T₅₀ treatments, T₇₀ plants of *E. argophloia* had significantly higher π_s than T₇₀ plants of the *E. cloeziana* provenances (Figure 2b). Mean osmotic adjustments of 0.29 and 0.22 MPa occurred in the coastal and inland provenances of *E. cloeziana*, respectively, but no osmotic adjustment was observed in *E. argophloia*.

Table 2. Effects of three 96-day watering regimes (100, 70 and 50% of field capacity) on the growth of the coastal and inland provenances of *E. cloeziana* and an inland provenance of *E. argophloia*. Each value is the mean of five plants \pm standard error of the mean. Within each provenance, means followed by different letters are significantly different at $P < 0.05$.

Provenance	Water regime (%)	Shoot height (cm)	Stem diameter (mm)	Leaf area (cm ²)
<i>E. cloeziana</i> (coastal, humid)	100	10.9 \pm 2.1 a	3.3 \pm 0.2 a	2100.5 \pm 98.6 a
	70	9.7 \pm 1.6 a	1.5 \pm 0.2 b	623.5 \pm 188.2 b
	50	10.2 \pm 2.1 a	1.0 \pm 0.1 b	279.3 \pm 43.3 b
<i>E. cloeziana</i> (inland, dry)	100	14.9 \pm 2.6 a	3.1 \pm 0.7 a	1650.3 \pm 147.5 a
	70	11.0 \pm 1.3 a	2.4 \pm 0.4 ab	678.1 \pm 76.2 b
	50	6.7 \pm 1.6 a	1.0 \pm 0.2 b	147.9 \pm 25.3 b
<i>E. argophloia</i> (inland, dry)	100	34.6 \pm 3.1 b	6.4 \pm 0.2 a	1792.4 \pm 124.0 a
	70	52.1 \pm 4.3 a	4.6 \pm 0.4 b	1166.8 \pm 132.0 b
	50	21.0 \pm 2.0 c	3.4 \pm 0.1 c	762.0 \pm 64.7 c

Severe water stress

Withholding water resulted in declines in R^* and predawn and midday Ψ_w of each provenance (Figures 3a, 3c and 3d), with minor differences among provenances. Variability in osmotic potential obscured evidence of possible osmotic adjustment (Figure 3b). However, π_s of water-stressed plants of the *E. cloeziana* provenances was consistently lower than π_s of water-stressed plants of *E. argophloia*. There were no significant differences in π_s between T₁₀₀ and T₀ plants of the coastal provenance of *E. cloeziana*. Osmotic potential of T₀ plants of *E. argophloia* increased in response to severe water stress (predawn Ψ_w less than -1.5 MPa) (Figure 2b), suggesting some limitations in the Wilson et al. (1979) osmotic potential technique in this species. Ratios of W_D to W_T were similar among provenances and ranged between 0.87 and 0.98. The R^* values after overnight wilting for the coastal (64.2%) and inland (64.8%) provenances of *E. cloeziana* and for the single provenance of *E. argophloia* (61.5%) were not significantly different, but there were significant differences between the corresponding midday Ψ_w values of -4.13 , -3.92 and -4.9 MPa, respectively.

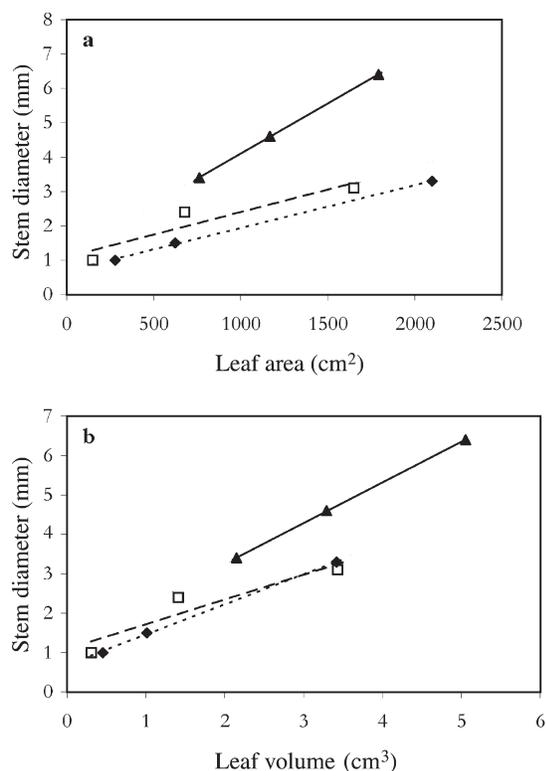


Figure 1. Relationships between stem diameter and leaf area (a) and leaf volume (leaf thickness \times total leaf area) (b) of an inland provenance of *E. argophloia* (\blacktriangle , —), and coastal (\blacklozenge , \cdots) and inland (\square , ---) provenances of *E. cloeziana* in response to three watering treatments (100, 70 and 50% of field capacity) over a 96-day period. Regression equations for *E. argophloia*: $y = 0.0029x + 1.19$, $r^2 = 0.99$; the inland provenance of *E. cloeziana*: $y = 0.0013x + 1.08$, $r^2 = 0.88$; and the coastal provenance of *E. cloeziana*: $y = 0.0012x + 0.68$, $r^2 = 0.99$.

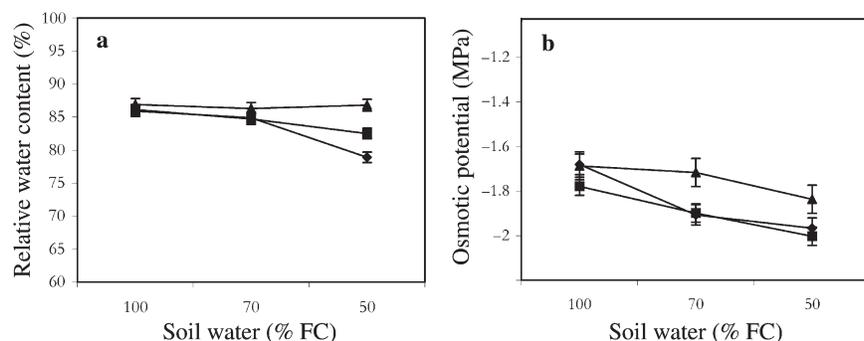


Figure 2. Mean relative water content (a) and osmotic potential at full turgor (b) of an inland provenance of *E. argophloia* (▲), and coastal (◆) and inland (■) provenances of *E. cloeziana* in response to three watering treatments (100, 70 and 50% of field capacity (FC)) over a 96-day period.

Pressure–volume curves

A comparison among T_{100} , T_{70} and T_{50} treatments within provenances showed that water stress did not influence the shape of the Ψ_w versus R^* curves (Figures 4a–4c). However, because leaves of both provenances of *E. cloeziana* were generally less rehydrated than leaves of *E. argophloia*, their curves were slightly shifted to the right (Parker and Pallardy 1987). Comparison among provenances showed that plants of *E. argophloia* had a smaller decrease in R^* with decreasing Ψ_w than plants of the *E. cloeziana* provenances (Figure 4).

There were no significant differences ($P \leq 0.05$) in π_s , Ψ_0 , R^{*0} , or A_{wc} between the coastal and inland provenances of *E. cloeziana* in any treatment. In *E. argophloia*, the treatments did not significantly affect π_s or ϵ . Means of water relations parameters for the T_{100} , T_{70} and T_{50} plants were compared among provenances, because there were only small differences between treatments. The W_D/W_T ratio was lowest and intermediate in the coastal (0.293) and inland (0.313) provenances of *E. cloeziana*, respectively, and highest in *E. argophloia*

(0.338) (Table 3). Values of π_s and Ψ_0 of the coastal provenance of *E. cloeziana* were significantly higher than those of *E. argophloia* and the inland provenance of *E. cloeziana* (Table 3). Relative water content at the turgor loss point, A_{wc} and ϵ were significantly higher in *E. argophloia* than in *E. cloeziana*, but were not significantly different between the *E. cloeziana* provenances (Table 3).

Leaf anatomy

Both the *E. argophloia* provenance and the inland provenance of *E. cloeziana* had greater total leaf, palisade, epidermal layer and cuticle layer thicknesses than the coastal provenance of *E. cloeziana* (Table 4). Palisade parenchyma in *E. cloeziana* was present on the adaxial side and spongy parenchyma was present on the abaxial side (dorsiventral). In both provenances of *E. cloeziana*, the spongy mesophyll contained large intercellular spaces as well as vascular bundles and few oil cavities, whereas mesophyll parenchyma in *E. argophloia* was composed of elongated palisade parenchyma (isobilateral),

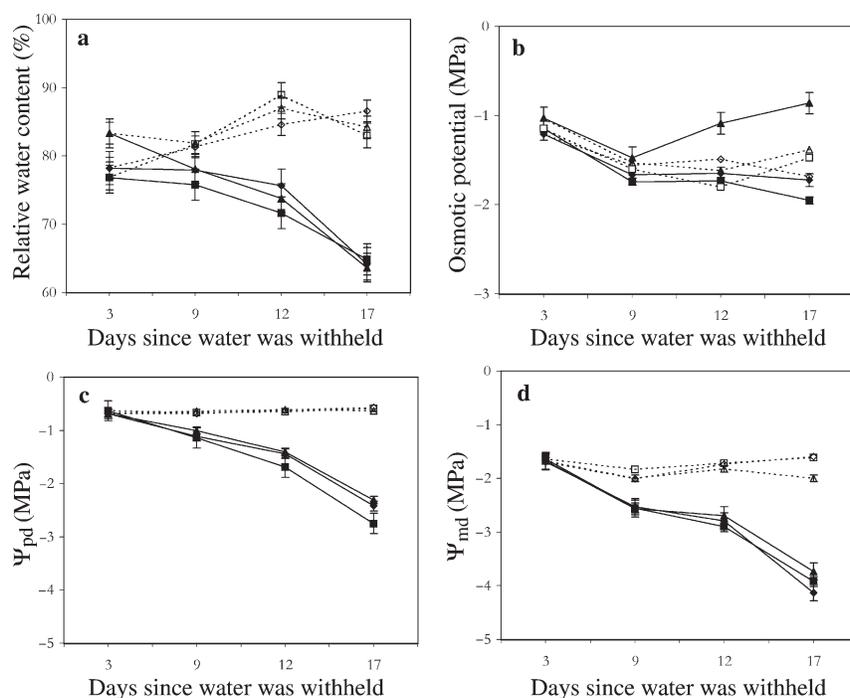


Figure 3. Effects of withholding water (T_0) on relative leaf water content (a), osmotic potential at full turgor (b), predawn leaf water potential (Ψ_{pd}) (c) and midday leaf water potential (Ψ_{md}) (d) of an inland provenance of *E. argophloia* (▲, △), and coastal (◆, ◇) and inland (■, □) provenances of *E. cloeziana*. The open symbols and hatched lines represent the T_{100} (control) treatment, and closed symbols and solid lines represent the (T_0) treatment.

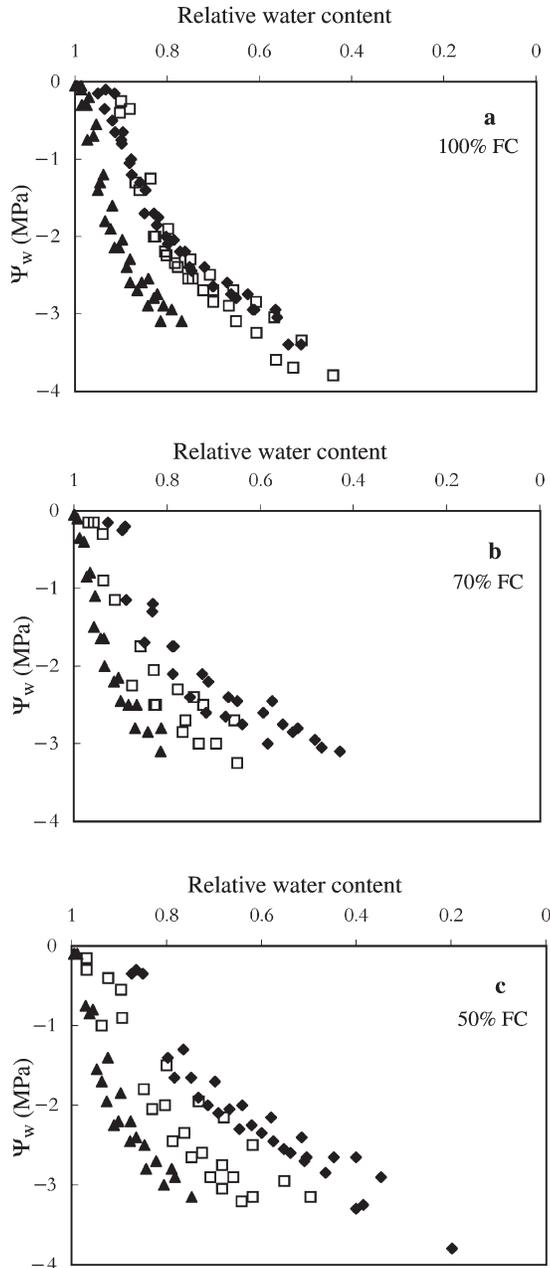


Figure 4. Relationship between leaf water potential (Ψ_w) and relative leaf water content in an inland provenance of *E. argophloia* (\blacktriangle), and coastal (\blacklozenge) and inland (\square) provenances of *E. cloeziana* in response to three watering treatments: (a) 100%, (b) 70% and (c) 50% of field capacity (FC).

vascular bundles, few oil cavities and reduced air space between the palisade cells.

Discussion

The response of plants to drought can be expressed in several ways, and the capacity of a plant to tolerate water stress is usually determined by the combination of attributes expressed

Table 3. Water relations attributes of an inland provenance of *E. argophloia* and two provenances of *E. cloeziana* maintained for 96 days. For each attribute, means followed by different letters are significantly different ($P < 0.05$). Abbreviations: W_D/W_T = ratio of dry mass to turgid mass; π_s = osmotic potential at full turgor (MPa); Ψ_0 = water potential at zero turgor (MPa); R^* = relative leaf water content at zero turgor (%); A_{wc} = apoplastic water content (%); and ϵ = bulk modulus of elasticity.

Attribute	Provenance		
	<i>E. cloeziana</i> (humid, coastal)	<i>E. cloeziana</i> (dry, inland)	<i>E. argophloia</i> (dry, inland)
W_D/W_T	0.293 ± 0.002 c	0.313 ± 0.002 b	0.338 ± 0.001 a
π_s	-1.50 ± 0.07 b	-1.72 ± 0.07 a	-1.77 ± 0.04 a
Ψ_0	-1.97 ± 0.08 b	-2.25 ± 0.11 a	-2.23 ± 0.05 a
R^*	76.44 ± 0.84 b	71.24 ± 7.10 b	89.93 ± 0.63 a
A_{wc}	8.30 ± 1.10 b	10.75 ± 1.21 b	51.57 ± 3.09 a
ϵ	9.36 ± 0.43 b	11.00 ± 1.53 b	18.71 ± 0.67 a

during development of drought. In the present study, constant water availability was not maintained, and the plants responded to the water supply regimes with changes in some water relations attributes and in growth allocation.

The treatments had little effect on shoot length (Table 2) in the *E. cloeziana* provenances, whereas T_{70} plants of the single *E. argophloia* provenance had increased shoot length and T_{50} plants had a significantly decreased shoot length compared with T_{100} plants. Leaf area and stem diameter were closely correlated and differed significantly among treatments. The regression coefficient for *E. argophloia* was smaller than for the provenances of *E. cloeziana*, which had similar regression coefficients (Figure 1a). These differences suggest that stem growth potential per unit leaf area was greater in *E. argophloia* than in *E. cloeziana*, but the differences were largely eliminated when comparisons were made on the basis of leaf volume (Figure 1b). Therefore, each provenance appears to have maintained leaf and stem diameter growth commensurate with the quantity of water available.

At the end of the 96-day treatment period, R^* in *E. argophloia* was not significantly affected ($P < 0.05$) by any treatment, whereas R^* was reduced significantly in T_{50} plants of the coastal provenance of *E. cloeziana* (Figure 2a). The generally small differences in most water relations parameters within provenances (Figure 2) suggest that the T_{70} and T_{50} plants adjusted to the reduced volume of soil, reaching a high Ψ_w at each rewatering. This adjustment presumably resulted in some root death and leaf shedding, and led to the associations between treatment and growth illustrated in Figure 1. Similar watering methods, sometimes extending to 25% of field capacity, have been used successfully by Lemcoff et al. (1994), Osório et al. (1998), Tuomela (1997) and Nativ et al. (1999).

Differences in estimates of water relations attributes between vapor pressure osmometry and pressure–volume curves may arise either because of cell sap dilution when apoplastic water is expressed (Tyree 1976, Tyree and Jarvis 1982, Myers and Neales 1986) or a right shift in the pressure–volume

Table 4. Tissue dimensions (means \pm standard error) of leaves of well-watered *E. argophloia* and *E. cloeziana* plants. For each variable, values followed by different letters are significantly different ($P < 0.05$).

Provenance	Total leaf thickness (μm)	Palisade thickness (μm)	Spongy mesophyll thickness (μm)	Epidermis		Cuticle thickness (μm)
				Upper width (μm)	Lower width (μm)	
<i>E. argophloia</i> (dry, inland)	282.2 \pm 2.6 a	241.3 \pm 3.0 a	nil	18.1 \pm 0.9 a	17.2 \pm 0.9 a	9.5 \pm 0.5 a
<i>E. cloeziana</i> (dry, inland)	208.0 \pm 3.1 b	69.3 \pm 1.3 b	98.8 \pm 1.5 a	18.6 \pm 1.1 a	16.4 \pm 0.7 a	8.9 \pm 0.2 a
<i>E. cloeziana</i> (humid, coastal)	162.7 \pm 3.1 c	58.9 \pm 1.3 c	58.9 \pm 2.8 b	14.5 \pm 0.5 b	11.4 \pm 0.6 b	5.9 \pm 0.2 b

curves in *E. cloeziana* as a result of incomplete rehydration of excised leaves (Parker and Pallardy 1987). Cell sap dilution could explain the relatively high π_s observed in severely drought-stressed *E. argophloia*, and is consistent with the conclusions of Gaff and Carr (1961) and Teoh et al. (1967) that apoplastic water becomes available to the symplast during extreme drought despite low water potentials.

Although estimates of apoplastic water content based on pressure–volume curves can be inaccurate (Tyree and Ritcher 1982), deviations from linearity in our study were minimal ($r > 0.95$, Figure 4). Estimates derived from pressure–volume curves (Table 3) indicated significant differences among provenances in most leaf water relations attributes. The *E. cloeziana* provenances had similar values for R^{*0} , A_{wc} and ϵ , but differed significantly in π_s and Ψ_0 . Large differences in R^{*0} , A_{wc} and ϵ were evident between the inland provenances of *E. cloeziana* and *E. argophloia* (Table 3), because *E. argophloia* retained more water in the whole leaf and in cell walls in response to low water availability than either of the *E. cloeziana* provenances. High A_{wc} values have also been reported for water-stressed *E. globulus* Labill. (Gaff and Carr 1961) and *E. viminalis* Labill. (Ladiges 1975). A comparison between the inland provenances of *E. cloeziana* and *E. argophloia* indicated that the former reached a similar Ψ_0 as *E. argophloia*, but it was associated with a much lower R^{*0} . The ϵ of *E. cloeziana* leaves ranged from 6.4 to 13.8 MPa, which is comparable to values of 4.2–11.2 MPa reported for juvenile *E. globulus* (Correia et al. 1989, White et al. 1996, Pita and Pardos 2001), *E. nitens* (Deane and Maiden) Maiden (White et al. 1996) and *E. grandis* W. Hill ex Maiden (Blake and Tschaplinski 1992). Values of ϵ for *E. argophloia* ranged from 18.1 to 20.2 MPa, which is within the range of 15.3 to 25.8 MPa reported for *E. camaldulensis* Dehnh., *E. saligna* Sm., *E. leucoxylon* F. Muell. and *E. platypus* Hook. in both winter and summer seasons in Western Australia (White et al. 2000).

Tissue survival has been related to the maintenance of water content and the avoidance of physical distortion of membranes (Turner 1979, Clayton-Greene 1983, Bowman and Roberts 1985, Dale and Sutcliffe 1986, Fan et al. 1994). These studies indicate that increased mechanical rigidity of the leaf and a high A_{wc} associated with less alteration in cell and tissue dimensions is beneficial, even though it is associated with a lower Ψ_w . Low tissue Ψ_0 would enable *E. argophloia* and the inland provenance of *E. cloeziana* to maintain water uptake from dry soils, as has been demonstrated in *Callitris*

columellaris F. Muell. (Clayton-Greene 1983), *E. pulchella* Desf. (Davidson and Reid 1989), *E. viminalis* (Ladiges 1975), *E. nitens* (White et al. 1996) and chaparral shrubs (Bowman and Roberts 1985). The observation that *E. argophloia* possessed a more extensive fine root system than either provenance of *E. cloeziana* explains the superior ability of *E. argophloia* to extract water from dry soils.

Limited osmotic adjustment occurred in the coastal (0.29 MPa) and inland (0.22 MPa) provenances of *E. cloeziana* during the 96-day T_{70} and T_{50} treatments (Figure 1b), and in the inland provenance, but not in the coastal provenance, during severe water deficit stress (T_0) (Figure 3b). These results suggest that osmotic adjustment is of limited importance (Munns 1988) in determining drought tolerance in the coastal provenance of *E. cloeziana*, as has been reported for *E. grandis* (Lemcoff et al. 1994) and several other woody species (Fan et al. 1994). However, osmotic adjustment appeared to be more important in the inland provenance of *E. cloeziana*, as reported for *E. viminalis* and *E. tereticornis* Sm. (Lemcoff et al. 1994). No osmotic adjustment occurred in *E. argophloia*, indicating that drought tolerance in this species may be attributed solely to mechanical characteristics associated with the maintenance of relative leaf water content. The thick leaves and lack of spongy mesophyll in *E. argophloia* are consistent with the xeromorphic characteristics reported for *E. camaldulensis* (Fahn 1990), whereas the extensive spongy mesophyll in *E. cloeziana* is similar to that found in *E. saligna* (Jones et al. 1993) and other mesomorphic species (Fahn 1990).

Trees of the coastal provenance of *E. cloeziana* typically occur on deep loam soils in warm, subtropical coastal Queensland (Table 1), where rainfall is high, summers are humid and severe droughts are uncommon. As reported by Blake and Suiter-Filho (1988), this provenance exhibits some delay in total plant dehydration through partial leaf shedding. The higher W_D/W_T ratio, lower π_s and Ψ_0 and smaller decrease in R^* per unit decrease in Ψ_w for the inland provenance of *E. cloeziana* relative to the coastal provenance (Figure 4) indicate a higher degree of drought tolerance in the inland provenance.

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