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ORIGINAL ARTICLE

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Physiological responses to water stress in *Eucalyptus cloeziana* and *E. argophloia* seedlings

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Abstract Effects of water stress duration and intensity on gas exchange and leaf water potential were investigated in 7-month-old seedlings of a humid coastal provenance (Gympie) and a dry inland (Hungry Hills) provenance of *E. cloeziana* F. Muell. and in a dry inland (Chinchilla) provenance of *E. argophloia* Blakely supplied with 100% (T_{100}), 70% (T_{70}), 50% (T_{50}) of their water requirements, or were watered only after they were wilted at dawn (T_0). Seedlings of *E. argophloia* had the highest midday net photosynthetic rate (A), stomatal conductance (g_s), stomatal density and predawn leaf water potential (Ψ_{pd}) in all treatments. The *E. cloeziana* provenances did not differ in these attributes. The T_{70} and T_{50} treatments caused reductions in A of 30% in *E. argophloia*, and 55% in the *E. cloeziana* provenances. Under the T_0 treatment, *E. argophloia* maintained higher rates of gas exchange at all levels of water stress than *E. cloeziana* provenances. The estimates of Ψ_{pd} and midday water potential (Ψ_{md}) at which plants remained wilted overnight were respectively: −2.7 and −4.1 MPa for *E. cloeziana* (humid), −2.8 and −4.0 MPa for *E. cloeziana* (dry) and, −3.7 and −4.9 MPa for *E. argophloia*. Following stress relief, both A and g_s recovered more quickly in *E. argophloia* and in the dry provenance of *E. cloeziana* than in the humid provenance. We conclude that *E. argophloia* is more drought tolerant and has a potential for cultivation in the humid and semi humid climates, whilst *E. cloeziana* has greater potential in the humid subtropical climates.

Keywords Gas exchange · Acclimation · Stomatal density · Subhumid tropics · Drought tolerance

Introduction

Tree plantations have been identified as major contributors to sustainable supplies of wood products, for environmental amelioration and for carbon sequestration in tropical and sub tropical Australia (Keenan et al. 1998). In the past, tree planting in southeast Queensland has been limited to humid (>1,000 mm rainfall) coastal areas. However, with high land prices and low availability of suitable land within this zone resulting from the World Heritage Listing of large areas of coastal tropical Queensland, there is now an urgent need to explore the potential of establishing commercial plantations in marginal regions (i.e. 700–1,000 mm rainfall zone) (Loxton and Forster 2000). In these marginal forest regions, production is limited by low water availability and high summer vapour pressure deficit (Sands et al. 1999). The forest industries in both Queensland and sub tropical New South Wales have included *E. cloeziana* and *E. argophloia* for plantation establishment (Keenan et al. 1998). Knowledge of native eucalypt silviculture in these drought prone tropical and subtropical regions of Australia is limited to a few species and mainly to *E. grandis* (Doley 1978; Cromer and Jarvis 1990; Birk and Turner 1992) and *E. camaldulensis* (Blake 1980; Facanha et al. 1983). An understanding of the relationship between physiological responses of *E. cloeziana* and *E. argophloia* and the biophysical environment (particularly water

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stress) is necessary for the development of silvicultural regimes.

Plants possess three mechanisms that may counteract water deficit stress: (1) reduction of water loss through stomatal control and morphological changes; (2) increased extraction of water from the soil and; (3) increased ability to tolerate low tissue water potential (Osmond et al. 1980; Kramer 1983; Nilsen and Orcutt 1996). Stomatal closure is due directly to low turgor pressure in the guard cells (Collatz et al. 1991), which may result from root signals by abscisic acid (ABA) (Cowan 1982; Downton et al. 1988; Wartinger et al. 1990; Fort et al. 1997), or in response to increasing vapour pressure deficit (Turner et al. 1984). Avoidance of water stress by the development of a deep root system has been reported for *E. camaldulensis* and *E. saligna* (White et al. 2000), as well as for *E. grandis* (Dye 1996), and *E. marginata* (Doley 1967). Plants native to dry areas have also been reported to maintain photosynthesis at a lower leaf water potential than those native to humid areas (Kozlowski et al. 1991), favouring both survival and productivity (Ni and Pallardy 1991).

Eucalyptus cloeziana has a wide natural distribution in eastern Queensland, occurring in four disjunct geographical regions: southern coastal, southern inland, northern coastal and northern inland (Turnbull 1979). This distribution is characterised by large variations in rainfall, temperature, geology and soils, reflecting the high level of genetic variability reported by Turnbull (1979). Lee at el. (1997) reported satisfactory growth by both northern and southern coastal provenances tested on sites having an annual rainfall greater than 1,200 mm within Australia, Brazil and Congolese Peoples' Republic. Nevertheless, estimates have not been made of the relative importance of genetically based adaptation and phenotypic plasticity to specific site conditions as components of *E. cloeziana* provenance variation in site performance (Turnbull 1979; Lee et al. 1997). *E. argophloia* is a potential commercial timber species with a narrow natural distribution northeast of Chinchilla in the Southern inland Queensland where it occurs under warm subhumid conditions (Boland et al. 1984). There is very limited information on the silviculture of this species. *E. cloeziana* and *E. argophloia* have excellent form and produce strong durable timber with many uses, and both are favoured for inclusion in forest plantations.

This study reports an investigation of physiological responses to water deficit stress of a dry inland provenance of *E. argophloia* and a dry inland, and humid coastal provenance of *E. cloeziana*. It was hypothesised that due to the disjunct nature of *E. cloeziana* populations, an inland provenance (Hungry Hills) would show greater drought resistance than the coastal provenance (Gympie), rendering it a better choice for drought prone sites. It was also hypothesised that the drought response mechanisms of the dry inland provenance of *E. cloeziana* provenance would be more comparable to those of *E. argophloia* than to the humid *E. cloeziana* provenance. Accordingly the objectives of the investigation were to determine for each of the three provenances: (1) responses of photosynthesis, stomatal conductance and leaf water potential to soil water availability, and (2) the rate of recovery of physiological processes when water stress is relieved.

Materials and methods

Seedling and growth conditions

Seedlings of a humid provenance of *E. cloeziana* from the Gympie, a dry zone provenance *E. cloeziana* from the Hungry Hills and of *E. argophloia* from Chinchilla (Table 1) were raised in pots containing a 50:50 mixture of peat and vermiculite at the Queensland Forestry Research Institute Gympie (QFRI). In June 2000, the 6-month-old seedlings were moved to a University of Queensland glasshouse (Brisbane) and transferred into plastic pots (175 mm diameter ×175 mm deep) lined with polythene and containing nursery top soil (loam-textured) mixed with vermiculite and perlite in the ratio of 4:1:1. Slow release fertiliser [Osmocote Plus (3–4 months) containing micronutrients] was mixed with the potting medium at a rate of 2 kg m^{-3}. The pots were packed to a bulk density of approximately 1,100 kg m^{-3} and maintained in a naturally lit glasshouse with temperature control provided by evaporative coolers and electric fan heaters. The average daytime temperatures in the glasshouse over the experimental period (August and November 2000) ranged from 21 to 37°C.

Response to variable watering

After 8 weeks of growth under favourable conditions, 60 healthy seedlings of uniform height were chosen from each provenance. Plastic beads were spread on the top surface of each pot to minimise evaporation from the soil (Myers and Landsberg 1989). All plants were watered to field capacity and arranged on a glasshouse bench using a completely randomised design with three water application treatments. The benches were rotated every 2 days. Each treatment comprised five plants of each provenance. A

Table 1 Seedlot information on the three *Eucalyptus* provenances used in the experiments (seed provided by Queensland Department of Primary Industries—Forestry)

Taxon	Seedlot	No.	Origin				
	Number	parent trees	Locality	Latitude (S)	Longitude (E)	Altitude (m)	Mean annual rainfall (mm)
E. cloeziana (humid)	4,363	11	SF 949 Goomboorian (Downsfield LA)	26°03′	152°42′	110	1210
E. cloeziana (dry)	10,823	20	SF 57 Mungy (Hungry Hills)	25°18′	151°22′	310	780
E. argophloia	5,520	18	SF 302 Ballon	26°20′	150°20′	300	650

2-day cyclical droughting treatment was applied to all plants. Plants in the control treatment (T_{100}) were rewatered every 2 days with all the water lost through evapotranspiration. The loss was estimated by determining the weight change in the T_{100} pots of each provenance. Fresh weights of three plants treated in a similar manner and harvested every fortnight were used to adjust pot weight changes resulting from plant growth. Water stressed plants received 70% (T_{70}) and 50% (T_{50}) of water supplied to control plants. The experiment was continued for 14 weeks.

In the severe stress treatment (T_0), water was withheld from 20 plants from each provenance until *E. cloeziana* seedlings had wilted overnight. During this period, sequential measurements of water potential and gas exchange were made on five plants in each of T_0 and T_{100} treatments per provenance. The plants were then watered to field capacity and re-watered every 2nd day. Measurements of gas exchange and water potential were made on several occasions during the recovery periods until measured physiological variables of the stressed plants had recovered to the level of the control treatment (T_{100}). *E. argophloia* seedlings were subjected to a second severe water deficit stress three weeks after the first stress cycle because they did not show visible signs of wilting at the wilting point of *E. cloeziana* seedlings.

Gas exchange and Leaf water potential

Photosynthesis (A), stomatal conductance to water vapour (g_s), transpiration (E), and intercellular CO_2 concentration (ci) were measured at ambient CO_2 concentration (350–370 μmol mol^{-1}) in the glasshouse. Measurements were taken at about midday using a portable photosynthesis system (Model LI-6200, Li-Cor, Lincoln, Neb., USA) equipped with a 250 ml cuvette. Gas exchange was measured on a young, fully expanded leaf under bright sunlight when photosynthetic photon flux density (PPDF) was greater than 700 μmol m^{-2} s^{-1}. The mean midday vapour pressure deficit on the days when measurements were taken in the glasshouse ranged from 3.5 to 5.3 kPa, and mean midday air temperature measured in the leaf cuvette ranged from 30 to 39°C. Leaf conductance was partitioned into stomatal conductance (g_s) and nonstomatal or residual conductance to diffusion of CO_2 (g_{rc}) according to Farquhar and Sharkey (1982) where g_{rc} includes mesophyll conductance and carboxylation efficiency (Sun et al. 1995). Measurements of predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential were made using a pressure chamber (PMS Instruments, Corvalis, Ore., USA). The measurements were made on a fully expanded leaf occupying the same developmental position as that used for gas exchange.

Measurement of stomatal properties

One fresh leaf from a well-watered plant of each provenance was cut into approximately 5 mm^2 pieces with a sharp razor blade. The tissues were fixed in 4% glutaraldehyde in 0.05 M sodium phosphate buffer, pH 6.9, overnight at 4°C, post-fixed in 1% osmium tetroxide in 0.05 M sodium phosphate buffer of pH 6.9 for 1.5 h and then dehydrated through a graded ethanol series. The specimens were then critical point dried in a Polaron Critical Point Drier, mounted on stubs and gold coated in a BIO-RAD SC502 Sputter Coating System. Stomatal density (SD) on the adaxial and abaxial surfaces was assessed at ×400 and mean guard cell length (MGCL in μm) and total guard cell length per unit area (TGCL mm^{-2}) at ×1,000 in a JEOL 6300 field emission electron microscope (FESEM) at 5 kV accelerating voltage.

Data analysis

Analyses of variance within and among provenances were undertaken using the PROC GLM procedure of the SAS statistical program (SAS 1985), while differences between means were segregated using Duncan's multiple range tests. Water stress integral (S_Ψ) was used to derive an index of cumulative water stress (Myers and Landsberg 1989) for each treatment and provenance over the 14 weeks period. Regression analyses were undertaken using PROC REG and PROC NLIN of SAS software.

Results

Examination of stomata on young leaves showed that *E. argophloia* was amphistomatous, with more stomata on the lower leaf surface (483 mm^{-2}) than on the upper surface (377 mm^{-2}). *E. cloeziana* had stomata on the lower side only (hypostomatous) and there were no significant differences in stomatal density between the humid (674 mm^{-2}) and dry provenance (629 mm^{-2}). Moreover, there were no significant differences in mean guard cell length (MGCL) among provenances (Table 2).

The patterns of Ψ_{pd}, which resulted from the three watering treatments imposed over the experimental period, are presented in Fig. 1. In *E. cloeziana*, stress treatments T_{70} and T_{50} resulted in distinct and consistent intensities of water deficit which were more pronounced in the humid than in the dry provenance. There were significant differences in Ψ_{pd} and Ψ_{md} among T_{100}, T_{70} and T_{50} in the humid provenance of *E. cloeziana*. In the dry provenance of *E. cloeziana*, there were significant differences in Ψ_{pd} between $T_{100,}$ T_{70} and $T_{50,}$ but not in Ψ_{md} (Table 3). In *E. argophloia*, neither Ψ_{pd} nor Ψ_{md} were significantly different among the treatments (Table 3). There were significant differences ($P \leq 0.05$) between control and stress treatments of the three provenances in net photosynthesis (A). There were no significant differences between T_{70} and T_{50} in A and g_s for any of the three provenances (Table 3).

The cumulative water stress integrals (S_Ψ) for the three provenances showed that plants in T_{70} and T_{50} experienced greater water stress than the T_{100} plants (Table 3). However in all three treatments, the humid provenance of *E. cloeziana* experienced the greatest water deficit stress over the experimental period. Comparison among prove-

Table 2 Stomatal dimensions from leaves of *E. argophloia* and *E. cloeziana* seedlings watered to field capacity every second day. The values shown are means±standard errors. (*SD* stomatal density, *MGCL* mean guard cell length, *TGCL* total guard cell length)

Variable[a]	*E. argophloia*	*E. cloeziana* (humid)	*E. cloeziana* (dry)
SD abaxial (mm^{-2})	483±15 a	674±48 b	629±5 b
SD adaxial (mm^{-2})	377±23 c	0	0
MGCL abaxial (μm)	16±0 a	14±1 a	16±2 a
MGCL adaxial (μm)	17±1 a	0	0
TGCL abaxial (μm mm^{-2})	7,570±160 bc	9,210±810 ab	10,270±1280 a
TGCL adaxial (μm mm^{-2})	6,540±330 c	0	0

[a] For each of the three variables, means with the same letter are not significantly different ($P \leq 0.05$)

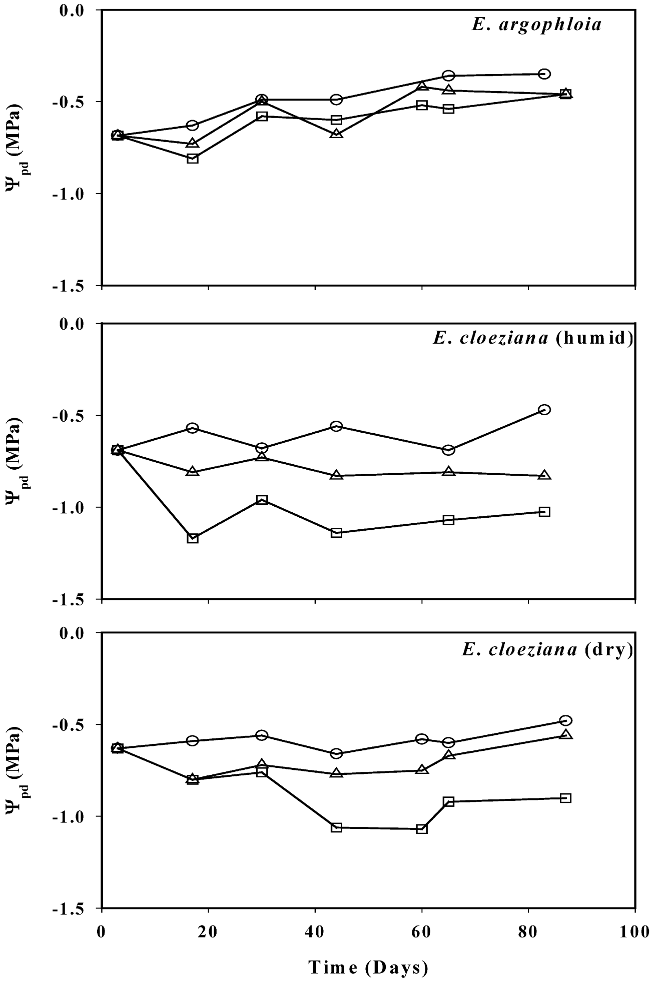

Fig. 1 Predawn water potential of *E. argophloia*, humid and dry provenances of *E. cloeziana* seedlings. Plants were watered every 2 days. Control plants (*circles*) received 100% of water lost through evapotranspiration and water stressed plants received 70% (*triangles*) and 50% (*squares*) respectively of water supplied to control plants

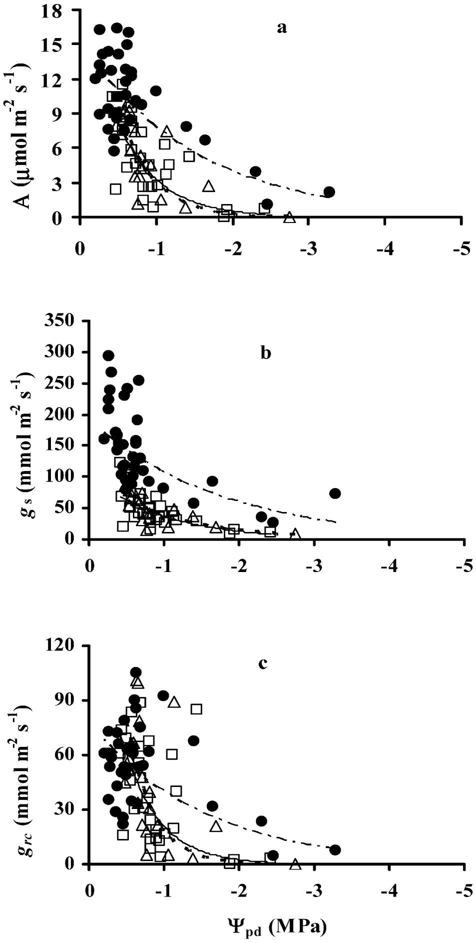

Fig. 2 Response of **a** net photosynthesis (A), **b** stomatal conductance (g_s) and **c** residual conductance (g_{rc}) to decreasing predawn leaf water potential (Ψ_{pd}) for humid (*squares, continuous line*) and dry (*triangles, dashed line*) provenance of *E. cloeziana* and *E. argophloia* (*circles, dotted line*)

nances in the three water treatments showed that *E. argophloia* had higher A, g_s, and Ψ_{pd} than *E. cloeziana* provenances which did not differ from each other (Table 3). There were no statistically significant differences between *E. argophloia* and the *E. cloeziana* provenances in Ψ_{md} (Table 3).

Regression analyses between Ψ_{pd} and gas exchange variables showed that A, g_s and residual conductance (g_{rc}) declined with decrease in Ψ_{pd} following an exponential trend of the form:

$$Y = \beta_0 e_1^{\beta\,\Psi_{pd}} \qquad (1)$$

where Y represents gas exchange variables (A, g_s and g_{rc}), β_0 is a constant, e denotes the base of the natural logarithm (2.178) and β_1 defines the rate of response. The response of *E. argophloia* to decreasing Ψ_{pd} differed from that of *E. cloeziana* (Fig. 2). It maintained higher A and g_s as the level of stress increased whereas in *E. cloeziana* provenances, rapid reduction in A occurred as Ψ_{pd} dropped from −0.3 to −1.2 MPa (Fig. 2). Generally there were limited differences between the responses of the humid and dry provenances of *Eucalyptus cloeziana* but the slope (β_1) for the relationship between Ψ_{pd} and A, g_s and g_{rc} for *E. cloeziana* provenances was greater than for *E. argophloia* (Table 4). In all three provenances, A increased with an increase in g_s and there were minor differences among the slopes of the regression lines (Fig. 3a, Table 5). However the two species differed substantially in response of A to g_{rc} (Fig. 3b) g_{rc} to g_s (Fig. 3c). The slope parameter for *E. cloeziana* provenances for this relationship was less than half that for *E. argophloia* (Table 5). In *E. cloeziana* provenances, the slope for the response of A to g_s was 2–3 times greater than that for the response of A to g_{rc}.

For all three provenances, withholding water resulted in a gradual decrease in both Ψ_{pd} and Ψ_{md} (Fig. 4). Under continuous stress, *E. argophloia* maintained significantly higher A and g_s at all levels of stress than either provenance of *E. cloeziana,* which showed no differences

Table 3 Net photosynthetic rate (A), stomatal conductance to diffusion of water vapour (g_s), predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential, and cumulative water stress (S_{Ψ}) for *E. argophloia* and *E. cloeziana* seedlings. Control plants (T_{100}) were watered to field capacity every second day, T_{70} and T_{50} were watered with 70% and 50% of water added to T_{100} respectively. The values shown are means±standard errors over all days of measurements. For each variable within each provenance, means with the same subscript for x and row are not significantly different ($P \leq 0.05$) while within each column, similar variables with the same subscript for y are not significantly different ($P \leq 0.05$)

Provenance	Variable	T_{100}	T_{70}	T_{50}
E. argophloia	A (μmol m^{-2} s^{-1})	13.4±0.6 x1 y1	9.5±0.9 x2 y1	8.7±1.0 x2 y1
	g_s (mmol m^{-2} s^{-1})	194.3±16.0 x1 y1	148.0±19.0 x1×2 y1	106.2±15.9 x2 y1
	ψ_{pd} (MPa)	−0.5±0.1 x1 y1	−0.6±0.1 x1 y1	−0.6±0.1 x1 y1
	ψ_{md} (MPa)	−1.9±0.1 x1 y2	−2.0±0.1 x1 y1	−2.3±0.1 x1 y1
	S_{Ψ} (MPa day)	68	82	86
E. cloeziana (humid)	A (μmol m^{-2} s^{-1})	7.8±0.8 x1 y2	4.1±1.2 x2 y2	2.9±1.0 x2 y2
	g_s (mmol m^{-2} s^{-1})	70.5±9.8 x1 y2	37.0±5.0 x2 y2	36.2±4.2 x2 y2
	ψ_{pd} (MPa)	−0.6±0.03 x1 y2	−0.8±0.03 x2 y2	−1.0±0.1 x3 y1y2
	ψ_{md} (MPa)	−1.6±0.1 x1 y1	−2.0±0.1 x2 y1	−2.4±0.2 x3 y1
	S_{Ψ} (MPa day)	92	114	136
E. cloeziana (dry)	A (μmol m^{-2} s^{-1})	8.1±0.4 x1 y2	4.2±1.2 x2 y2	2.3±1.7 x2 y2
	g_s (mmol m^{-2} s^{-1})	67.3±9.1 x1 y2	38.5±8.3 x2 y2	23.3±7.2 x2 y2
	ψ_{pd} (MPa)	−0.6±0.02 x1 y2	−0.7±0.04 x2 y1y2	−0.7±0.1 x2 y2
	ψ_{md} (MPa)	−1.8±0.1 x1 y1y2	−2.0±0.04 x1 y1	−2.1±0.2 x1 y1
	S_{Ψ} (MPa day)	87	97	110

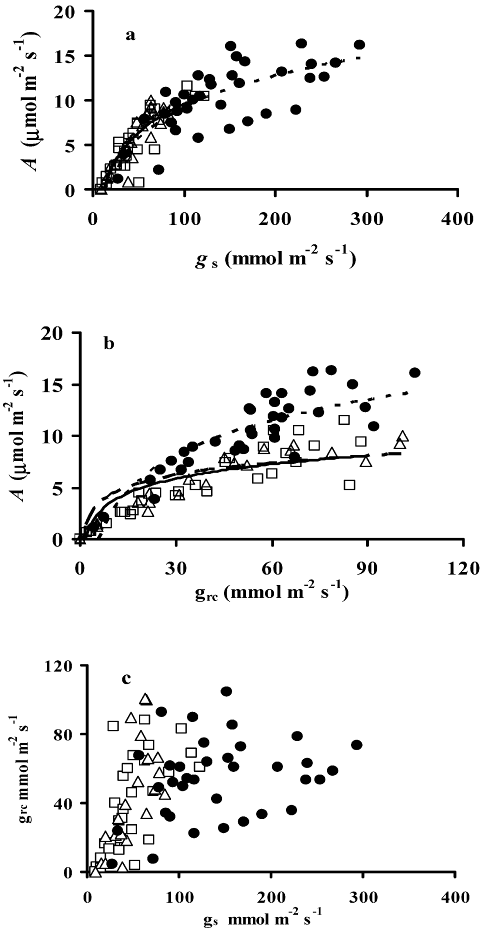

Fig. 3 Response of **a** net photosynthesis (A) to stomatal conductance (g_s) and **b** residual conductance (g_{rc}) and relationship between g_{rc} and g_s for humid (*squares, continuous line*) and dry (*triangles, dashed line*) provenance of *E. cloeziana* and *E. argophloia* (*circles, dotted line*)

in their response to stress (Fig. 4). The estimates of Ψ_{pd} and Ψ_{md} at which plants remained wilted overnight were respectively: −2.7 and −4.1 MPa for *E. cloeziana* (humid), −2.8 and −4.0 MPa for *E. cloeziana* (dry) and, −3.7 to −4.9 MPa, for *E. argophloia*. At these values of Ψ_{md}, photosynthesis could not be detected. Plants stressed beyond these limits did not recover after watering. *E. argophloia* did not portray visible signs of leaf tissue damage at the wilting Ψ_{pd} whereas *E. cloeziana* (humid) suffered extensive leaf necrosis and *E. cloeziana* (dry) showed limited necrosis.

It took approximately 10 days for water potential to recover to the level of the control treatment after rewatering in all provenances (Fig. 4a). Net photosynthesis and g_s of *E. argophloia* recovered within 10 days, *E. cloeziana* dry provenance within 2 weeks and humid provenance within 3 weeks (Fig. 4c).

Discussion

Species differ widely in their responses to reduced soil water availability (Newton et al. 1986; Kozlowski et al. 1991; Blake and Tschaplinski 1992; Kramer and Boyer 1995), so several expressions of response may be investigated. The present study concentrated on simple water relations parameters, including shoot water potential, gas phase conductances of leaves, and carbon dioxide assimilation. Each of these parameters was compared between provenances and treatments, and while not every relationship was consistent, they indicated several associations between provenance and physiological characteristics that could be useful in site-species matching. Although application of these results to field-grown saplings and adult trees is not straightforward, the differences in water stress tolerance appear to be consistent with

Table 4 The slope (β 1) and coefficient of determination (r^2) obtained from exponential decay function fitted to the relation between predawn leaf water potential (Ψ_{pd}) and net photosynthesis (A), stomatal conductance (g_s) and residual conductance to diffusion of CO_2 (g_{rc}) data from the four treatments (T_{100}, T_{70}, T_{50} and T_0) for each provenance. The values shown are parameters ± standard errors

Variable	Parameter	*E. cloeziana* (humid)	*E. cloeziana* (dry)	*E. argophloia*
A (μmol $m^{-2}s^{-1}$)	β_0	17.79±4.51	23.01±7.74	14.21±1.18
	β_1	1.46±0.37	1.76±0.51	0.46±0.13
	r^2	0.54	0.79	0.64
g_s (mmol m^{-2} s^{-1})	β_0	155.90±37.93	153.70±36.44	260.90±34.54
	β_1	1.44±0.35	1.48±0.35	0.99±0.26
	r^2	0.56	0.62	0.52
g_{rc} (mmol m^{-2} s^{-1})	β_0	17.79±4.51	23.01±7.73	14.21±1.18
	β_1	1.46±0.37	1.76±0.51	0.46±0.13
	r^2	0.57	0.71	0.46

Table 5 Parameters for regressions analyses between net photosynthesis (A, μmol $m^{-2}s^{-1}$) and stomatal conductance (g_s, mmol m^{-2} s^{-1}) and residual conductance (g_{rc}, mmol m^{-2} s^{-1}) using the function $y = \beta_1 \mathrm{Ln}(x) + \beta_0$ on combined data from the four treatments (T_{100}, T_{70}, T_{50} and T_0) in each taxon. The values shown are parameters ± standard errors

Dependent variable	Independent variable	Parameters	*E. cloeziana* (humid)	*E. cloeziana* (dry)	*E. argophloia*
A	g_s	B_0	–11.46±1.79	–12.32±2.32	–14.72±3.84
		B_1	4.56±0.47	4.86±0.61	5.19±0.79
		R^2	0.75	0.75	0.55
A	g_{rc}	B_0	–1.04±0.87	0.44±0.84	–8.78±1.84
		B_1	2.03±0.25	1.70±0.23	4.95±0.47
		R^2	0.69	0.72	0.76

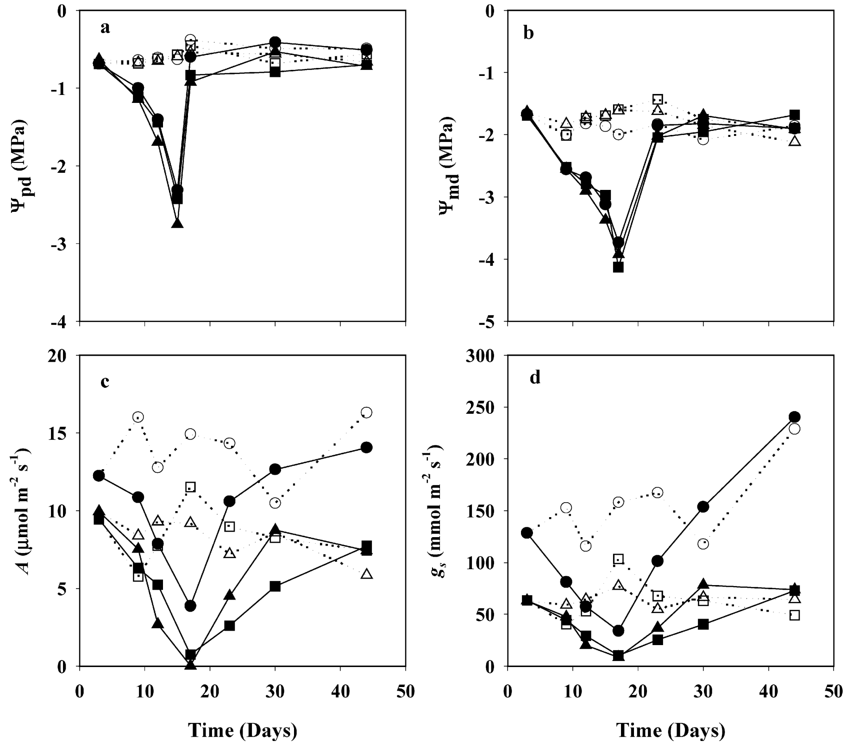

Fig. 4 Response of **a** predawn water potential (Ψ_{pd}), **b** midday water potential (Ψ_{md}), **c** net photosynthesis (A) and **d** stomatal conductance (g_s) of *E. argophloia* (*circles*) and the humid (*squares*) and dry (*triangles*) provenance of *E. cloeziana* to severe stress and subsequent stress relief at day 17. The *open symbols* and *hatched line* represents the T_{100} (control), and *closed symbols* and *continuous line* represent the (T_0) treatment

ecological observations (Turnbull 1979; Boland et al. 1984).

Distinct differences in Ψ_{pd} between treatments T_{100}, T_{70} and T_{50} in *E. cloeziana* (Fig. 1) indicate that the plants were affected by the different levels of soil water availability in the manner indicated by Schulze (1986), Myers and Landsberg (1989), Wartinger et al. (1990) and Osório et al. (1998). Whilst the patterns of predawn water potential in well-watered plants were similar in all provenances (Fig. 1), *E. argophloia* showed overnight recovery under water deficit conditions whilst increasing water deficits resulted in progressively lower values of Ψ_{pd} in *E. cloeziana*. These differences may be associated with the greater development of fine roots in *E. ar-*

gophloia than either provenance of *E. cloeziana* (Ngugi et al. 2003b). Similarly, White et al. (2000) reported that field grown trees of *E. camaldulensis* were more effective in exploitation of soil water than were *E. leucoxylon* or *E. platypus*. On the other hand, Colquhoun et al. (1984) found that *E. wandoo*, the most drought-tolerant but shallow-rooted of six species examined in the field, showed consistently lower Ψ_{pd} than the other species. It is potentially useful for rapid species evaluations that physiological differences that may be crucial to larger trees in the field may be reflected in pot-grown seedlings in the glasshouse.

Stomatal conductance (g_s) and mesophyll conductance (g_{rc}) for both provenances of *E. cloeziana* and for *E. argophloia* appeared to be very sensitive to small reductions in Ψ_{pd} between −0.2 and −0.8 MPa (Fig. 2). This relationship is quite distinct from those between gas exchange characteristics and instantaneous water potential, where linear regressions are more commonly observed (Ni and Pallardy 1991), or there may be a distinct threshold water potential above which there is little change in g_s (Schulze 1986). However, there is a close similarity between the patterns recorded in the present study and those obtained in *Eucalyptus globulus* (Pereira et al. 1987) and *Nothofagus solandri* and *N. menziesii* (Sun et al. 1995). Both *E. argophloia* and *N. solandri*, which are relatively drought-resistant species, showed higher conductance and photosynthesis values at maximum Ψ_{pd} than did the more drought-susceptible *E. cloeziana* or *N. menziesii*. *E. argophloia* also showed higher values of g_s at minimum Ψ_{pd} than did either provenance of *E. cloeziana* (Fig. 2). These differences indicate both the potential for high rates of gas exchange in *E. argophloia* when water is plentiful, and the maintenance of carbon gain under less favourable conditions.

The high maximum g_s in well-watered *E. argophloia* (Table 3) may be related to its higher mean stomatal density and total guard cell length per unit area (cf. Table 2). When the ratio, maximum g_s to mean total guard cell length (TGCL, mm^{-2}) for both leaf surfaces is calculated, *E. argophloia* has a value of 0.027, whilst the dry and humid provenances of *E. cloeziana* have values of 0.015 and 0.013, respectively. This difference may reflect the mutual interference between the diffusion shells surrounding stomata when they occur at high density on only one surface of the leaf (Meidner and Mansfield 1968). Other situations in which higher gas exchange rates have been observed in drought-resistant than in mesic taxa include provenances of *Azadarichta indica* (Kundu and Tigerstedt 1998), *Quercus stellata* in relation to *Juglans nigra* (Ni and Pallardy 1991), and *Eucalyptus camaldulensis* in relation to *E. leucoxylon* and *E. platypus* (White et al. 2000). However, where root systems of trees have access to sufficient soil water, drought stress may be avoided (e.g. in *E. marginata*), even though other species regarded as more drought-resistant (e.g. *E. wandoo*) exhibited much lower water vapour conductances and leaf water potentials (Colquhoun et al. 1984).

Under severe water stress treatments, Ψ_{pd} decreased in a similar pattern for the three provenances (Fig. 4), with *E. argophloia* consistently showing a greater *A* and g_s than *E. cloeziana* provenances (which did not differ much from each other) and a greater ability to sustain photosynthetic activity to a Ψ_{pd} value of −3.7 MPa compared to −2.7 MPa for the *E. cloeziana* provenances. The results for *E. argophloia* were consistent with drought responses of xeric species from eastern North America (Abrams 1994) whilst the responses of *E. cloeziana* provenances are consistent with those of a glasshouse experiment in which *E. cloeziana* stomata closed at water potential of −2.7 Mpa compared to −1.2 MPa for *E. grandis* (Fisher 1991). As in *E. cloeziana A* and g_s were close to zero at leaf water potential of −2.9 MPa in *Quercus stellata*, −2.6 MPa in *Q. alba* and *Acer saccharium*, and −2.2 MPa in *Juglans nigra* seedlings (Ni and Pallardy 1991). However *E. behriana* maintained photosynthesis at leaf water potential of −4.4 MPa (Küppers et al. 1987; Myers et al. 1987).

Almost identical non-linear relationships were found between *A* and g_s for *E. argophloia* and the two provenances of *E. cloeziana* (Fig. 3a). Sun et al. (1995) fitted linear regressions to these parameters for the drought-resistant *Nothofagus solandri* and the drought-susceptible *N. menziesii*, but a non-linear relationship might appear to be more appropriate for *N. menziesii*. The differences between taxa were more distinct when *A* was compared with g_{rc} in both the present study (Fig. 3b) and in *N. solandri* and *N. menziesii* (Sun et al. 1995). In both situations, the more drought-resistant species showed higher maxima for *A* and g_s, but a similar range of g_{rc} to the drought-susceptible taxa. For spring and summer conditions, *A* in *E. globulus* was linearly related to g_s up to about 80 mmol m^{-2} s^{-1}, after which there was no response (Pereira et al. 1987). The clear distinction between g_s -limited and non-limited conditions observed in *E. globulus* was not obtained in the present study (Fig. 3a), indicating greater involvement of non-stomatal limitations to photosynthesis.

A capacity for substantial variation in stomatal opening would make the leaves very responsive to the ambient conditions, whereas g_{rc} is likely to reflect internal structural and biochemical characteristics of leaves, and to be less variable than g_s. The denser packing of chlorenchyma cells in leaves of *E. argophloia* might be expected to result in a shorter mean diffusion path length for CO_2 and a higher g_{rc} than in *E. cloeziana*, but this is not reflected in Fig. 3b, which shows a similar range of g_{rc} in both species.

An insight into the variation in responses of *A* to g_s and g_{rc} may be gained by comparing these two conductances (Fig. 3c). For both *E. cloeziana* provenances, there was a reasonably close linear relationship between g_{rc} and g_s, but in *E. argophloia* high g_s was often associated with low g_{rc} and relatively low *A*. All the occasions on which *A* in *E. argophloia* exceeded the maximum value in *E. cloeziana* were associated with high values of g_s rather than of g_{rc} (Figs. 2, 3b). The cause of the varied g_{rc} values is not clear, but the majority of occasions when low g_{rc} was observed were days on which Ψ_{pd} was lower than −1MPa. In *E. globulus*, these occasions of low Ψ_{pd} were associated with low leaf conductance (Pereira et al. 1987), but in *E. argophloia*, some days of low Ψ_{pd} were associated with moderate to high g_s. Kirschbaum (1987) identified photoinhibition as a contributor to decreased g_{rc} in *E. pauciflora*, and it is possible that water deficits and the light regime of the present study could have resulted in patchy distribution of stomatal apertures (Downton et al. 1988; Beyschlag and Eckstein 1997) inducing this condition in *E. argophloia* and *E. cloeziana*.

Rewatering seedlings after an overnight wilting resulted in recovery of water potential of the three provenances over the next 10 days (Fig. 4). Gas exchange of *E. argophloia* also recovered within 10 days unlike that for the dry provenance of *E. cloeziana* and humid provenances of *E. cloeziana* that took more than 2 weeks. This difference in recovery was attributed to damaged photosynthetic tissues on the leaves. In humid provenance of *E. cloeziana* most of the leaves showed necrotic patches in regions away from the midrib during the recovery period, and many of the older leaves were shed. Similar observations have been reported for *E. cloeziana* seedlings under glasshouse conditions (Fisher 1991). Kirschbaum (1987) observed recovery of Ψ_{pd} in *E. pauciflora* within 7 h and in A within 1 day after rewatering. Although there were no significant differences in the response of the provenances of *E. cloeziana* to increasing water stress, the dry provenance recovered faster, indicating greater tolerance at low water potential.

Both *E. cloeziana* and *E. argophloia* developed fewer and smaller leaves in response to drought, but *E. cloeziana* also shed numerous lower leaves (Teskey and Hinckley 1986; Pereira and Pallardy 1987; Passioura et al. 1993; Ngugi et al. 2003a). However, these new leaves did not acclimate fully to the available water, as demonstrated by significant differences in gas exchange variables between the well-watered and water stressed treatments (Table 3).

This study confirms several aspects of the physiology of *Eucalyptus* species with respect to water deficits. It demonstrates that some degree of drought tolerance may occur (in *E. argophloia*), but that between provenances of another species, there may be no clear distinction between the drought tolerances of provenances from humid and sub-humid environments. In addition, *E. argophloia* demonstrates drought resistance through the possession of an extensive fine root system that exploits soil moisture more effectively than do either provenance of *E. cloeziana*. However, when water is freely available, the higher stomatal conductances and photosynthetic rates in the naturally restricted *E. argophloia* than in the much more widespread *E. cloeziana* indicate that the former species is likely to be suitable for planting on a greater range of sites than the latter. This finding indicates that some caution should be applied when matching species to sites simply on the basis of the climatic or distributional data associated with their natural occurrences.

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References

Abrams MD (1994) Genotypic and phenotypic variation as stress adaptations in temperate tree species: a review of several case studies. Tree Physiol 14:833–842

Beyschlag W, Eckstein J (1997) Stomatal patchiness. Prog Bot 59:283–298

Birk EM, Turner J (1992) Response of flooded gum (*Eucalyptus grandis*) to intensive cultural treatments—biomass and nutrient content of *Eucalyptus* plantations and native forests. For Ecol Manage 47:1-4

Blake TJ (1980) Effect of coppicing on growth rates, stomatal characteristics and water relations in *Eucalyptus camaldulensis* Dehn. Aust J Plant Physiol 7:81–87

Blake TJ, Tschaplinski TJ. (1992). Water relations. In: Mitchell CP, Ford-Robertson JB, Hinckley T, Sennerby-Forsse L (eds) Ecophysiology of short rotation forest crops. Elsevier Science, London, pp 66–94

Boland DJ, Brooker MIH, Chippendale GM, Hall N, Hyland BPM, Johnson RD, Kleinig DA, Turner JD. (1984) Forest trees of Australia. Thomas Nelson Australia and CSIRO, Melbourne

Collatz GJ, Ball JT, Grivet C, Berry JA (1991) Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration; a model that includes a laminar boundary layer. Agric For Meteorol 54:107–136

Colquhoun IJ, Ridge RW, Bell DT, Loneragan WA (1984) Comparative studies in selected species of *Eucalyptus* used in rehabilitation of the Northern Jarrah Forest, Western Australia. I. Patterns of xylem pressure potential and diffusive resistance of leaves. Aust J Bot 32:367–373

Cowan IR (1982) Regulation of water use in relation to carbon gain in higher plants. Enc Plant Physiol 12B:589–613

Cromer RN, Jarvis PG (1990) Growth and biomass partitioning in *Eucalyptus grandis* seedlings in response to nitrogen supply. Aust J Plant Physiol 17:503–515

Doley D (1967) Water relations of *Eucalyptus marginata* under natural conditions. J Ecol 55:597–614

Doley D (1978) Effects of shade on gas exchange and growth of *Eucalyptus grandis* Hill. ex Maiden. Aust J Plant Physiol 5:723–738

Downton WJS, Loveys BR, Grant WJR (1988) Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. New Phytol 108:263–266

Dye PJ (1996) Response of *Eucalyptus grandis* trees to soil water deficits. Tree Physiol 16:233–238

Facanha JGV, Oliva MA, Lopes NF, Barros NF (1983) Germination/growth relation in eucalypt species under water stress. Revista-Arvore 7:177–187

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annu Rev Plant Physiol 33:317–345

Fisher JR. (1991). Aspects of the water relations of *Eucalyptus grandis* and *Eucalyptus cloeziana*. MAgrSc. thesis, University of Queensland, St. Lucia, Australia

Fort C, Fauveau ML, Muller F, Label P, Granier A, Dreyer E (1997) Stomatal conductance, growth and root signalling in young oak seedlings subjected to partial soil drying. Tree Physiol 17:281–289

Keenan RJ, Ivory M, Lawson S, Lee D, Leggate W, Lewty MJ, Nikles DG, Ryan P, Walker S (1998) Hardwood plantation research and development: a strategy to support a hardwood plantation industry in Queensland. Queensland Forestry Research Institute, Brisbane

Kirschbaum MUF (1987) Water stress in *Eucalyptus pauciflora*: comparison of effects on stomatal conductance with effects on the mesophyll capacity for photosynthesis, and investigation of a possible involvement of photoinhibition. Planta 171:466–473

Kozlowski TT, Kramer PJ, Pallardy SG (1991) The physiological ecology of woody plants. Academic Press, San Diego

Kramer PJ (1983) Water relations of plants. Academic Press, New York

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic Press, San Diego

Kundu SK, Tigerstedt PMA (1998) Variation in net photosynthesis, stomatal characteristics, leaf area and whole-plant phytomass production among ten provenances of neem (*Azadirachta indica*). Tree Physiol 19:47–52

Küppers M, Neales TF, Küppers BIL, Swan AG, Myers BA (1987) Hydraulic flow characteristics in the lignotuberous mallee *Eucalyptus behriana* F. Muell. in the field. Plant Cell Environ 10:27–37

Lee D, Ryan P, Nikles G. (1997). Provenance variation of *Eucalyptus cloeziana* exhibited at Pomona in South eastern Queensland. In: Kikkawa J, Dart P, Doley D, Ishii K, Lamb D, Suzuki K (eds) Overcoming Impediments to Reforestation: Tropical Forest Rehabilitation in the Asia-Pacific Region, Proceedings of the 6th International Workshop of Biotechnology assisted Reforestation. Brisbane, Australia, pp 187–189

Loxton I, Forster S (2000) Brigalow Research Station Technical Report 1999–2000. Queensland Beef Industry Institute, Department of Primary Industries, Theodore, Queensland

Meidner H, Mansfield TA (1968) Physiology of stomata. McGraw-Hill, New York

Myers BJ, Landsberg JJ (1989) Water stress and seedling growth of two eucalypt species from contrasting habitats. Tree Physiol 5:207–218

Myers BA, Küppers M, Neales TF (1987) Effect of stem excision under water on bulk leaf water potential, leaf conductance, CO_2 assimilation and stemwood water storage in *Eucalyptus behriana* F. Muell. Aust J Plant Physiol 14:135–145

Newton RJ, Meier CE, Van Buijtenen JP, McKinley CR (1986) Moisture-stress management: Silviculture and genetics. Stress physiology and forest productivity. Martinus Nijhoff, Dordrecht

Ngugi MR, Doley D, Hunt MA, Dart PJ, Ryan P (2003a) Leaf water relations of *Eucalyptus cloeziana* and *Eucalyptus argophloia* in response to water deficit. Tree Physiol 23:335–343

Ngugi MR, Hunt MA, Doley D, Ryan P, Dart P (2003b) Dry matter production and allocation in *Eucalyptus cloeziana* and *Eucalyptus argophloia* seedlings in response to soil water deficits. New For 26:187–200

Ni BR, Pallardy SG (1991) Response of gas exchange to water stress in seedlings of woody angiosperms. Tree Physiol 8:1-9

Nilsen ET, Orcutt DM. (1996) The physiology of plants under stress: abiotic factors. Wiley, New York

Osmond CB, Bjorkman O, Anderson DJ (1980) Physiological processes in plant ecology: toward a synthesis with *Atriplex*. Springer, Berlin Heidelberg New York

Osório J, Osório ML, Chaves MM, Pereira JS (1998) Water deficits are more important in delaying growth than in changing patterns of carbon allocation in *Eucalyptus globulus*. Tree Physiol 18:363–373

Passioura JB, Condon AG, Richards RA (1993) Water deficits, the development of leaf area and crop productivity. In: Smith JAC, Griffiths H (eds) Water deficits: plant responses from cell to community. Bios Scientific, Oxford, UK, p 345

Pereira JS, Pallardy S (1987) Water stress limitations to tree productivity. In: Pereira JS Landsberg JJ (eds) Biomass production by fast-growing trees. Kluwer Academic, Dordrecht, pp 37–56

Pereira JS, Tenhunen JD, Lange OL (1987) Stomatal control of photosynthesis of *Eucalyptus globulus* Labill. trees under field conditions in Portugal. J Exp Bot 38:1678–1688

Sands PJ, Rawlins W, Battaglia M (1999) Use of a simple plantation productivity model to study the profitability of irrigated *Eucalyptus globulus*. Ecol Model 117:125–141

SAS (1985) SAS/STAT guide for personal computers. SAS Institute, Cary, N.C.

Schulze ED (1986) Carbon dioxide and water vapour exchange in response to drought in the atmosphere and in the soil. Annu Rev Plant Physiol 37:247–274

Sun OJ, Sweet GB, Whitehead D, Graeme D (1995) Physiological responses to water stress and waterlogging in *Nothofagus* species. Tree Physiol 15:629–638

Teskey RO, Hinckley TM (1986) Moisture: Effects of water stress on trees. In: Hennessey TC, Dougherty PM, Kossuth SV, Johnson JD (eds) Stress physiology and forest productivity. Martinus Nijhoff, Dordrecht, pp 9–34

Turnbull JW. (1979) Geographic variations in *Eucalyptus cloeziana*. Ph.D thesis. Australia National University, Australia

Turner NC, Schulze ED, Gollan T (1984) The response of stomata and leaf gas exchange to vapour pressure deficits and soil water contents. I. Species comparisons at high soil water contents. Oecologia 63:338–342

Wartinger A, Heilmeier H, Hartung W, Schulze ED (1990) Daily and seasonal courses of leaf conductance and abscisic acid in the xylem sap of almond trees [*Prunus dulcis* (Miller) D. A. Webb] under desert conditions. New Phytol 116:581–587

White DA, Turner NC, Galbraith JH (2000) Leaf water relations and stomatal behavior of four allopatric *Eucalyptus* species planted in Mediterranean southwestern Australia. Tree Physiol 20:1157–1165