

# Optimal stomatal conductance in relation to photosynthesis in climatically contrasting *Eucalyptus* species under drought

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## ABSTRACT

Models of stomatal conductance ( $g_s$ ) are based on coupling between  $g_s$  and  $\text{CO}_2$  assimilation ( $A_{\text{net}}$ ), and it is often assumed that the slope of this relationship ( $'g_1'$ ) is constant across species. However, if different plant species have adapted to different access costs of water, then there will be differences in  $g_1$  among species. We hypothesized that  $g_1$  should vary among species adapted to different climates, and tested the theory and its linkage to plant hydraulics using four *Eucalyptus* species from different climatic origins in a common garden.

Optimal stomatal theory predicts that species from sub-humid zones have a lower marginal water cost of C gain, hence lower  $g_1$  than humid-zone species. In agreement with the theory that  $g_1$  is related to tissue carbon costs for water supply, we found a relationship between wood density and  $g_1$  across *Eucalyptus* species of contrasting climatic origins. There were significant reductions in the parameter  $g_1$  during drought in humid but not sub-humid species, with the latter group maintaining  $g_1$  in drought. There are strong differences in stomatal behaviour among related tree species in agreement with optimal stomatal theory, and these differences are consistent with the economics involved in water uptake and transport for carbon gain.

**Key-words:** drought; leaf gas exchange models; net photosynthesis; plant hydraulic conductance; stomatal optimization theory.

**Abbreviations:**  $\Gamma$ ,  $\text{CO}_2$  compensation point of  $A_{\text{net}}$ ;  $\Gamma^*$ ,  $\text{CO}_2$  compensation point of  $A_{\text{net}}$  in the absence of dark respiration;  $\lambda$ , unit marginal water cost of plant carbon assimilation;  $\Psi_l$ , leaf water potential;  $A_{\text{net}}$ , rate of net photosynthetic  $\text{CO}_2$  assimilation;  $C_a$ ,  $\text{CO}_2$  mole fraction in air at the leaf surface;  $C_{\text{st}}$ ,  $\text{CO}_2$  mole fraction in the substomatal cavity;  $E$ , transpiration rate;  $D$ , leaf-air vapour pressure difference.

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$D_0$ , parameter for base level of the  $D$  response;  $g_0$ , stomatal conductance when net photosynthesis is zero;  $g_1$ , slope of the unified stomatal optimisation model;  $g_s$ , stomatal conductance;  $K_L$ , soil-to-leaf hydraulic conductance;  $k_{\text{stem}}$ , stem specific hydraulic conductivity measured on branches;  $Q$ , quantum flux density inside the leaf cuvette.

## INTRODUCTION

Fluxes of water vapour and  $\text{CO}_2$  between plants and the atmosphere are strongly coupled because both gases must pass through the stomatal aperture. Stomata play a critical role in leaf physiology, balancing the need for photosynthetic  $\text{CO}_2$  uptake against the need to control water loss from leaves (Jones 1997; Sperry *et al.* 2002; Katul, Leuning & Oren 2003). It is widely observed empirically that there is a strong relationship between the stomatal conductance to water vapour ( $g_s$ ) and net photosynthetic assimilation ( $A_{\text{net}}$ ), in a range of environmental conditions and  $\text{C}_3$  plant species (Lloyd 1991; Jones 1997; Katul *et al.* 2003; Misson, Panek & Goldstein 2004; Buckley 2005; Galmés *et al.* 2007; Damour *et al.* 2010). This relationship between  $A_{\text{net}}$  and  $g_s$  is commonly exploited in empirical models of stomatal conductance (Leuning 1995; Damour *et al.* 2010). A range of such empirical  $g_s$  models are now employed to model vegetation-atmosphere  $\text{CO}_2$  and water vapour exchanges at scales ranging from the plot to the globe (Lai *et al.* 2000; Xu & Baldocchi 2003; Hickler *et al.* 2006; Beer *et al.* 2007; Ostle *et al.* 2009). The use of models based on the relationship between  $A_{\text{net}}$  and  $g_s$  is widespread because they only require a few empirical parameters and they are easy to incorporate into growth and ecosystem models (Van Wijk *et al.* 2000).

However, there are significant limitations to the use of these empirical models of stomatal conductance. It is unclear how parameters vary among species and therefore how parameter values should be distributed spatially. Furthermore, empirical models may not predict stomatal behaviour well in novel environmental conditions caused by climate change such as rising atmospheric  $\text{CO}_2$  concentration, warming and drought (Katul, Ellsworth & Lai 2000; Damour *et al.* 2010). Mechanistic stomatal models that

handle novel conditions arising due to climate change (Dewar *et al.* 2009) are desirable, but there is currently a lack of knowledge of the precise mechanisms responsible for stomatal responses to soil and atmospheric drought (Tardieu & Davies 1993; Tuzet, Perrier & Leuning 2003; Buckley 2005). As a consequence, many vegetation models have difficulty in reproducing water vapour and CO<sub>2</sub> fluxes when soil moisture is strongly limiting stomatal conductance (Misson *et al.* 2004; Damour *et al.* 2010). There is therefore currently much research focused on improving model formulations for stomatal conductance under drought conditions (Egea, Verhoef & Vidale 2011).

An alternative to the empirical and mechanistic approaches to modelling stomatal conductance is provided by the theory of optimal stomatal conductance, originally proposed by Cowan (1977) and Cowan & Farquhar (1977). This theory proposes that the optimal stomatal behaviour is to minimize leaf transpiration ( $E$ , mol m<sup>-2</sup> s<sup>-1</sup>) while at the same time maximizing photosynthetic carbon uptake that is, stomata act to minimise

$$E - \lambda \cdot A_{\text{net}} \quad (1)$$

The parameter  $\lambda$  (mol H<sub>2</sub>O mol<sup>-1</sup> C) is considered to be constant over the average timescale between successive rains, and describes the marginal cost of supplying water in terms of acquisition and transport to leaves to support a unit increase in carbon assimilation to the plant. Since the theory was first proposed, a number of authors have applied this theory to describe stomatal behaviour (e.g. Lloyd 1991; Thomas, Eamus & Bell 1999a; Katul, Palmroth & Oren 2009). However, while the theory is fairly general among diverse plant species, it does not apply to ancient plant phylogenies such as lycophytes and pteridophytes which regulate stomatal aperture differently than higher plants (Brodrick & McAdam 2011; McAdam & Brodrick 2012).

Recently Medlyn *et al.* (2011) showed that the optimal stomatal theory results in a model that has a very similar form to the empirical stomatal models developed by Collatz *et al.* (1991) and Leuning (1995). The resulting model, which we denote the unified stomatal optimization (USO) model, is consequently readily parameterized with field data like the empirical models, but it also provides a major advantage over the empirical models because parameter values are now meaningful. The slope parameter of the stomatal model ( $g_1$ ) is proportional to the marginal water cost of carbon gain ( $\lambda$ ) (Medlyn *et al.* 2011). Because this parameter is interpretable, we can develop hypotheses for how these slopes and  $\lambda$  should vary with species and environmental conditions.

In this paper, we propose and test two major hypotheses for the effects of water availability on the  $g_1$  parameter. Firstly, we hypothesize that plant species that are adapted to sub-humid environments will have a lower  $g_1$  parameter than species adapted to humid environments. In sub-humid regions, perennial plant species need to construct tissue that can endure unfavourable climatic periods, such as dense,

cavitation-resistant woody tissues and xerophytic leaves (Orians & Solbrig 1977; Hacke & Sperry 2001). Such tissues require substantial investments of C to construct. Following theory (Orians & Solbrig 1977; Wright, Reich & Westoby 2003), it can be hypothesized that such species will have a low marginal water cost of carbon gain ( $\lambda$ ) and consequently a low value for  $g_1$ . A subsidiary hypothesis is that wood density is highest in species from sub-humid regions (Westoby *et al.* 2002; Maharjan *et al.* 2011) and that  $g_1$  decreases with increasing wood density.

Secondly, we hypothesize that the  $g_1$  parameter will be most sensitive to variations in soil moisture availability in species adapted to humid environments. We argue that water transport pathways in sub-humid species are constructed to withstand periods of low soil moisture availability as are frequent in this sort of climate, and therefore that the carbon cost of water to the plant will not increase as soil dries, thus leading to a relatively constant value of  $g_1$ . In contrast, we argue that in humid species that rarely experience drought in their native growing environment, the cost of water in terms of acquisition and transport is likely to increase as water availability decreases, leading to a decrease in  $g_1$  as drought progresses.

We tested these hypotheses in four *Eucalyptus* species originating from different climate zones (two humid species, two sub-humid species) growing in a common garden. The use of species from the same genus avoids potential confounding factors related to phylogenetic differences. The genus *Eucalyptus* spans a large range of climates in its native growth range from sub-arid to humid and rainforest, and contains species with wide geographical ranges as well as species particular to localized conditions. Differences in stomatal responses to the environment among *Eucalyptus* species have been previously studied (Anderson *et al.* 1996; White, Turner & Galbraith 2000; Ngugi *et al.* 2004) but not within the context of optimal stomatal theory. The species selected for study occur in different sub-groups of the genus *Eucalyptus* (Table 1). They were selected to examine how stomatal behaviour and linkages to other plant traits are associated with the predominate climate of origin of these species. Diurnal patterns of stomatal conductance and assimilation were measured in these species during wet and dry periods. The USO stomatal model was fitted to these data to obtain values of  $g_1$ . The performance of the model was benchmarked against the widely used empirical stomatal model of Leuning (1995). The values of  $g_1$  obtained were used to test our two major hypotheses:

- 1 that species originating from sub-humid zones would have lower  $g_1$  than species from humid zones;
  - 1b As a corollary to the above,  $g_1$  is negatively related to wood density and positively related to hydraulic conductance or conductivity across the set of four species; and
- 2 that  $g_1$  in species from sub-humid zones would be less affected by low soil moisture availability than that of humid-zone species.

**Table 1.** Description of the seed origin and climate near the seed origin for four *Eucalyptus* tree species used in the study, grown in the common garden in Richmond, NSW, Australia

Species	Section	Species latitudinal range <sup>a</sup>	Seed source	Nearest meteorological station to seed source; Lat., Long.	Mean annual rainfall (mm)	Mean annual temperature (°C) <sup>b</sup>	Mean daily maximum summer temperature (°C) <sup>b</sup>	Species climate zone of origin
<i>Eucalyptus cladocalyx</i>	Sejunctae	32–35°S	Bundaleer Forest Reserve, SA	Yongala, SA; 33.03°S, 138.75°E	462	24.0	29.8	Sub-humid
<i>Eucalyptus dunnii</i>	Maidenaria	28–30°S	Sheepstation Creek, NSW	Green Pigeon, NSW; 28.47°S, 153.09° E	1622	25.5	28.8	Humid
<i>Eucalyptus melliodora</i>	Adnataria	24–38°S	Wagga Wagga, NSW	Wagga Wagga, NSW; 35.05°S, 147.35°E	523	24.1	30.6	Sub-humid
<i>Eucalyptus saligna</i>	Transversaria	21–36°S	Styx River State Forest, NSW	Jeogla, NSW; 30.58°S, 152.11°E	945	21.4	23.2	Humid

<sup>a</sup>From Boland *et al.* (2006).

<sup>b</sup>From Lin (2012), PhD thesis, University of Western Sydney.

The nearest meteorological station for each species' origin was within 20 km of the actual seed source location, and at a similar elevation to the seed source (unpublished data from Bureau of Meteorology, Commonwealth of Australia, Canberra, Australia). The mean annual rainfall at the common garden site is 801 mm year<sup>-1</sup>. Temperature data are not necessarily from the same meteorological station as rainfall, as sometimes the temperatures were recorded at meteorological stations located farther from the seed origin than for rainfall data.

## MATERIALS AND METHODS

### Field site

Measurements on contrasting *Eucalyptus* species were made in a common garden at the Hawkesbury campus of the University of Western Sydney in Richmond, NSW, Australia (33.621° S, 150.739° E) (see Barton *et al.* 2010). The site is located at 20 m elevation and is characterized by a temperate-subtropical transitional climate with a mean annual maximum temperature of 23.9 °C, a mean annual minimum temperature of 10.5 °C, and a mean annual precipitation of 801 mm (Bureau of Meteorology, Commonwealth of Australia 2010; Barton *et al.* 2010). The mean ratio of annual precipitation to potential evapotranspiration for this location is about 0.7 (Bureau of Meteorology, Commonwealth of Australia, unpublished data), suggesting that water limitations are common in this location. In September 2007, six *Eucalyptus* species, four of which were used in this study, were established from bare-root stock grown from seed obtained from locations within each species' native

range (Table 1). Each species was planted in two replicate monoculture blocks at ~1100 trees per ha (3.8 × 2.65 m spacing). Trees were 2½ years old at the time of measurements and varied in height from 4 m to nearly 10 m tall, and also varied in stem growth rates (Table 2). Periodic measurements of tree height to the nearest 5 cm were done with a 10 m-long telescoping height pole.

### Species

Four *Eucalyptus* species were selected within the common garden planting: *E. cladocalyx* F. Muell., *E. dunnii* Maiden, *E. melliodora* A. Cunn. ex Schauer and *E. saligna* Sm. (Table 1). Their respective growing-region precipitation ranges are 450–650 mm year<sup>-1</sup>, 1200–1500 mm year<sup>-1</sup>, 450–1300 mm year<sup>-1</sup> and 900–1800 mm year<sup>-1</sup> (Boland *et al.* 2006), with rainfall at stations near their geographical origins shown in Table 1. The four species are distinguished by different native ranges, largely separating by rainfall zones. *E. cladocalyx* and *E. melliodora* are here considered species

**Table 2.** Mean tree size and annual growth at time of measurement for plantations of contrasting *Eucalyptus* species during the course of the study

Species	Mean tree basal area (cm <sup>2</sup> at base, 10 cm above soil)	Mean tree basal area growth (cm <sup>2</sup> per year)	Mean tree height (m)	Mean height extension growth (m per year)	Mean gas exchange sampling height (m)
<i>Eucalyptus cladocalyx</i>	74.0	21.4 ± 3.3	6.1	1.49 ± 0.06	5.0
<i>Eucalyptus dunnii</i>	150.6	37.3 ± 0.1	8.8	1.95 ± 0.17	7.0
<i>Eucalyptus melliodora</i>	44.6	17.1 ± 2.3	4.1	1.29 ± 0.03	3.5
<i>Eucalyptus saligna</i>	133.2	22.8 ± 5.5	8.3	1.91 ± 0.06	5.5

Means are based on replicate plots of 35 trees each (25 trees per plot in the case of *E. saligna*), measured across 1 year beginning with winter prior to the summer gas exchange measurements through the following autumn.

from predominately low-rainfall (<800 mm year<sup>-1</sup>), sub-humid climates ('sub-humid species'). These two species grow in Mediterranean climate regions of Australia that experience a significant period of drought in summer. The other two *Eucalyptus* species, *E. dunnii* and *E. saligna*, have wet montane to coastal distributions receiving rainfall >900 mm year<sup>-1</sup>, and we therefore consider them to be 'humid species'. We avoid use of terms such as 'mesic' and 'xeric' as they presume knowledge of water source for trees, and can be misleading in terms of species drought responses if not properly characterized. While *E. cladocalyx* and *E. dunnii* have narrow geographical and climate distributions (less than 2° latitude), *E. melliodora* from sub-humid climates and *E. saligna* from humid climate zones have wide natural latitudinal distributions (more than 14° latitude; Table 1). The species differed in leaf characteristics: leaves of *E. cladocalyx* and *E. saligna* are hypostomatous while leaves of *E. melliodora* are isobilateral and amphistomatous. The fourth species, *E. dunnii*, was strongly heteroblastic and had hypostomatous leaves when juvenile, and amphistomatous leaves in a mature state. Mature leaves of this species were principally measured, but the prevailing leaf type (juvenile versus mature) of each individual measured was recorded and used in the appropriate corrections to stomatal conductance for stomatal distributions.

### Leaf gas exchange and water relations measurements

Measurements were made during summer and autumn, as prior work indicated that processes during that period have the strongest effect on intrinsic water-use efficiency and differentiate species (Anderson *et al.* 1996). Over the course of five sunny days during January–March 2010 (the austral summer period), diurnal measurements of  $A_{\text{net}}$  and  $g_s$  were made every 2 h on two recently fully expanded leaves of three replicate trees for each of the study species, using two open-flow portable photosynthesis systems (Li-6400, Li-Cor, Inc., Lincoln, NE, USA). Leaves were of similar ages in all species and had emerged since spring in all cases. Canopy access for all measurements was achieved with 4 m tall orchard ladders (in the case of trees up to 4–5 m tall), and with an articulated boom lift in the case of taller trees (Snorkel MHP13/35 Trailer Mounted Lift, Snorkel Ltd, Meadowbrook, Qld, Australia). We ensured that measurements were made within the upper third of the tree crown, for leaves in the sun over the majority of the diurnal cycle. Pre-dawn  $\Psi_l$  measurements were made on all trees prior to diurnal measurements (except 12 January) using the pressure chamber technique with a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA). Soil surface gravimetric soil moisture (upper 30 cm of the profile) was measured on all dates (see below). Meteorological data were recorded by an automatic weather station located in a clearing 200 m from the tree plantations (Barton *et al.* 2010).

Prior to leaf  $A_{\text{net}}$  and  $g_s$  measurements across diurnal courses, cuvette light levels and temperatures were adjusted

to ambient conditions, as determined for each 2 h interval on each measurement day from the weather station. Chamber humidity was maintained as close as possible to ambient during measurements by removing a small amount of water vapour commensurate with that expected to be added by leaves during measurements. The leaf cuvette was kept in the shade when not in use for measurements to minimize heating and to ensure that the temperature of the chamber remained close to ambient. For each measurement, CO<sub>2</sub> mole fraction  $C_a$  was set at ambient (nominally 385  $\mu\text{mol mol}^{-1}$  CO<sub>2</sub>) by mixing CO<sub>2</sub> from an internal source. Appropriate stomatal ratio settings were used for each species, depending on whether the species were hypostomatous or amphistomatous according to microscopic observations of stomatal peels. Once leaves were inside the leaf cuvette and reached equilibrium (coefficient of variation for  $A_{\text{net}}$  and  $g_s$  < 1%),  $A_{\text{net}}$  and  $g_s$  measurements were recorded as four measurements per leaf logged at 10 s intervals, with these later averaged to comprise an observation. No leaf gas exchange measurement was made on *E. cladocalyx* on the first measurement day (12 January), so only four sets of diurnal measurements were available for this species.

Two of the diurnal measurements on each species (or one set, for *E. cladocalyx*) were made on days with sunny, summertime conditions with low soil moisture (drought conditions), when soil moisture in the upper 50 cm was  $4.4 \pm 0.3\%$  and  $4.2 \pm 0.3\%$ . Subsequent to these drought conditions, heavy early February rainfall increased soil moisture in the stands to  $9.6 \pm 0.3\%$ , and soil moisture remained relatively high through April. Based on available data for recent rainfall, soil moisture and pre-dawn water potentials, the first two sets of diurnal measurements were considered to reflect species performance during drought and the final three sets of diurnal measurements were considered to be under well-watered conditions. These will be referred to hereafter as 'drought' and 'non-drought' periods.

To quantify leaf photosynthetic capacity at the end of the summer while plants were well watered, a set of controlled responses of  $A_{\text{net}}$  to CO<sub>2</sub> mole fraction in the substomatal cavity (i.e.  $A_{\text{net}}-C_{\text{st}}$  curves) at saturating quantum flux density ( $Q$ ) inside the leaf cuvette was conducted on mature leaves, prior to the onset of days with temperature <22 °C. The measurement procedure followed Ellsworth *et al.* (2004) using  $Q = 1800 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  as light saturation and constant leaf temperature ( $28 \pm 1$  °C).  $A_{\text{net}}-C_{\text{st}}$  curves were generated by manipulating  $C_a$  entering the leaf chamber in 9 to 10 steps, including one step measured near the CO<sub>2</sub> compensation point as well as 2–3 points above the leaf CO<sub>2</sub> saturation point. The data were fitted to the model of Farquhar, Caemmerer & Berry (1980) as described in Ellsworth *et al.* (2012).

### Plant hydraulic architecture and leaf and plant functional traits

At pre-dawn as well as immediately after the gas-exchange measurements, leaves were collected and leaf water potentials ( $\Psi_l$ ) were measured using Scholander



type pressure chamber (Plant Moisture Systems, Inc., Corvallis, OR, USA). No  $\Psi_l$  was measured on the 12th of January. Apparent soil-to-leaf hydraulic conductance ( $K_L$ ) was estimated from the slope of the relationship between leaf transpiration rate ( $E$ ) inside the leaf chamber and the difference in  $\Psi$ , and was calculated as  $-E/(\Psi_l - \Psi_{l-\text{predawn}})$  (Reich & Hinckley 1989; Sperry, Alder & Eastlack 1993), noting that  $E$  estimated within the well-stirred leaf chamber is not the same as  $E$  under ambient conditions. When average whole-tree  $E$  from sap-flow measurements was employed rather than  $E$  measured by porometry, the whole-tree results based on sap flow measurements had the same relative species ranking as for the leaf level (data not shown).

Leaves measured for the daily maximum photosynthesis peak and the  $A_{\text{net}}-C_{\text{st}}$  curves were collected to measure thickness using digital calipers (resolution 10  $\mu\text{m}$ ; Mitutoyo Handtools, South Melbourne, Vic., Australia) and for analysis of leaf area (scanner and optical planimetry software; Regent Instruments, Quebec, Canada) and dry mass (drying oven at 70 °C for >48 h). Leaf nitrogen contents were analysed by a TruSpec CHN elemental analyser with micro-digestion apparatus (LECO Corp., St. Joseph, MI, USA).

The specific hydraulic conductivity was measured on 20 cm-long stem segments of each species using a Sperry type of apparatus in the laboratory at 20 °C and pressure head of 8–10 kPa (Sperry, Donnelly & Tyree 1988). Three upper crown branch samples per species were used for the measurements, and 1 m long branches were cut when under low tension, immersed and re-cut under water, and then stem segments were collected under water and transferred to the hydraulic conductivity apparatus. Stem-specific hydraulic conductivity ( $k_{\text{stem}}$ , in  $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$ ) was expressed as the volume flow rate divided by the pressure gradient and dividing by the segment's cross-sectional area of hydroactive xylem. These measurements were made at a native state of embolism so the measurements reflect the *in situ* capacity for hydraulic conductivity by the collective xylem conduit lumens in a stem segment, assuming that conduit diameters have a strong influence on xylem flow resistance (Hacke & Sperry 2001). When branches were collected, the leaves distal to the branch segment were removed and leaf area was measured. The measurement trees were harvested by the end of April 2010 and wood density of the main stem at breast height was measured by immersion in a volumetric cylinder on an analytical balance. Total tree leaf area and Huber values were also determined, the latter based as the ratio of sapwood area of the main trunk to leaf area of the individual tree.

## Data analysis and model fitting

We evaluated the USO model (Medlyn *et al.* 2011) for estimating stomatal conductance as a function of environmental and photosynthetic parameters. The model combines the form of the Ball–Berry model of Collatz *et al.* (1991), the

photosynthetic model developed by Arneth, Lloyd & Santruckova (2002) based on Farquhar *et al.* (1980) and the optimal stomatal control model of Cowan & Farquhar (1977). This formulation was proposed in Medlyn *et al.* (2011) as:

$$g_s \equiv g_0 + 1.6 \cdot \left(1 + \frac{g_1}{\sqrt{D}}\right) \cdot \frac{A_{\text{net}}}{C_a} \quad (2)$$

where  $g_0$  is the stomatal conductance at zero photosynthesis, and  $g_1$  is the slope parameter. The term  $\frac{g_1}{\sqrt{D}}$  is large and therefore dominates the term  $\left(1 + \frac{g_1}{\sqrt{D}}\right)$ . Consequently, a close relationship between  $g_s$  and the combination of terms  $\frac{1}{\sqrt{D}} \cdot \frac{A_{\text{net}}}{C_a}$  is predicted. This approach leads to an expression for  $g_s$  that is similar in form to the Leuning (1995) and Collatz *et al.* (1991) models. The slope parameter  $g_1$  has been shown to increase linearly with the combination of terms:

$$g_1 \propto \sqrt{\Gamma^* \cdot \lambda} \quad (3)$$

where  $\lambda$  represents the marginal water cost of plant carbon gain and  $\Gamma^*$  is the  $\text{CO}_2$  compensation point of  $A_{\text{net}}$  in the absence of dark respiration used in photosynthetic models (Medlyn *et al.* 2011). Hence the USO model offers a new and simple means of quantifying  $\lambda$  by fitting Eqn 2 to  $g_s$  measurements and using the fitted parameter  $g_1$  as a proxy for  $\lambda$ , considering that  $\Gamma^*$  is known for  $\text{C}_3$  species and varies with temperature.

We used the Ball–Berry–Leuning (BBL) model (Leuning 1995) as a benchmark against which to test how well the USO model performed. The BBL and USO models have a similar form which facilitates their comparison, but there are different interpretations of their major parameters. The BBL model states that  $g_s$  can be estimated by the following expression:

$$g_s = g_0 + g_1 \cdot \frac{A_{\text{net}}}{(C_a - \Gamma)(1 + D/D_0)} \quad (4)$$

where residual conductance  $g_0$  and the slope parameter  $g_1$  and the base level of  $D$  ( $D_0$ ) are fitted parameters, and  $\Gamma$  is the  $\text{CO}_2$  compensation point of assimilation in the presence of dark respiration (Leuning 1995).

The values of the model parameters for the BBL and USO models were estimated from leaf gas exchange measurements by linear and non-linear fitting by least mean squares method using SigmaPlot (v. 11, Systat Software Inc., Chicago, IL, USA). Akaike's information criterion with a bias adjustment for small sample sizes was used to compare models (Anderson & Burnham 2002). Dummy variables were used to compare regression lines across species and environmental conditions, both in terms of slope and intercept parameters. Differences in leaf traits, leaf photosynthetic and hydraulic characteristics were evaluated by

Pearson's correlation using JMP (JMP v.8.0.2, SAS Institute, Cary, NC, USA). When the data were significantly drawn from a non-normally distributed population, square root or inverse transformations were performed to ensure normality prior to conducting statistical tests.

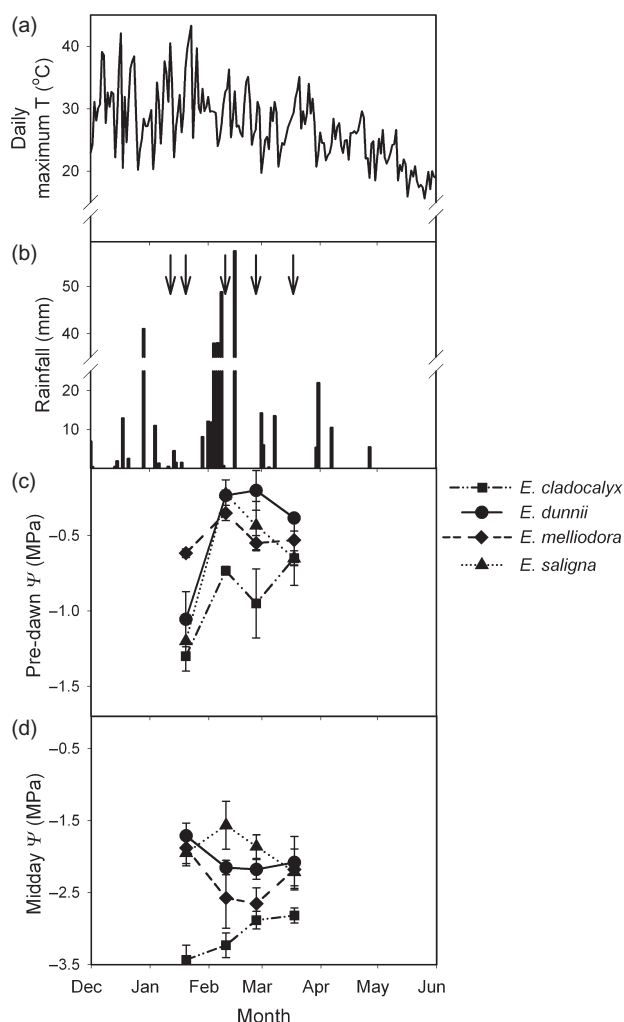
## RESULTS

To determine if there were species differences in stomatal model parameters, measurements were made under a wide range of environmental conditions on sunny days, with leaf temperatures ranging 12–43 °C and  $D$  ranging from <0.5 to 6.0 kPa (Fig. 2). In the austral summer of 2009–2010, maximum air temperature at the site exceeded 35 °C on 17 d (Fig. 1a). Temperature inside the leaf chambers generally matched outside conditions (Fig. 2). However,  $D$  inside the leaf chambers was not directly controlled, though it did parallel outside conditions measured by the nearby meteorological station (Fig. 2b). For the 5 d of measurements, the solar radiation generally followed a symmetric bell curve shape with a peak around midday between 700 and 1000 W m<sup>-2</sup> (Fig. 3a), with irregularities in these curves resulting from intermittent cloudy conditions. Monthly cumulative rainfall in December and January was 55 and 22 mm, respectively, whereas pan evaporation for each of these months was more than 150 mm, suggesting significant water deficits (Australian Bureau of Meteorology, <http://www.bom.gov.au>; unpublished data). Other than *E. melliodora*, the other three species all exhibited low pre-dawn  $\Psi$  during the drought period (e.g. pre-dawn  $\Psi \leq 1.0$  MPa) and recovery of pre-dawn  $\Psi$  during a rainy period in summer (February 2010, during which 210 mm of rain was received, compared with the long-term mean of 95 mm; Fig. 1).

### Stomatal responses and coupling to photosynthesis

Leaf  $g_s$  generally exhibited a declining pattern during each day, but was distinctly lower during drought in January 2010 compared with later in the season (Fig. 2). The humid species *E. dunnii* and *E. saligna* both exhibited  $g_s < 0.25$  mol m<sup>-2</sup> s<sup>-1</sup> at all times during January sampling dates, compared with peak  $g_s > 0.5$  mol m<sup>-2</sup> s<sup>-1</sup> under favourable conditions in February. In contrast, the sub-humid species *E. cladocalyx* and *E. melliodora* maintained higher  $g_s$  than *E. dunnii* and *E. saligna* during mid-summer in January. Later in the season when well watered, species  $g_s$  differences were less distinct (Fig. 2).

Overall, the four *Eucalyptus* species showed similarly shaped, curvilinear relationships between  $g_s$  and  $A_{\text{net}}$  (Fig. 3a,b). However, the  $g_s$ – $A_{\text{net}}$  relationship differed in magnitude between the humid and sub-humid species groups (Fig. 3a,b). The two humid species, *E. dunnii* and *E. saligna*, achieved higher maximum  $g_s$  ( $g_s > 0.5$  mol m<sup>-2</sup> s<sup>-1</sup>) than the two sub-humid species *E. cladocalyx* and *E. melliodora* (Figs 2 and 3). The  $g_s$ – $A_{\text{net}}$  relationship

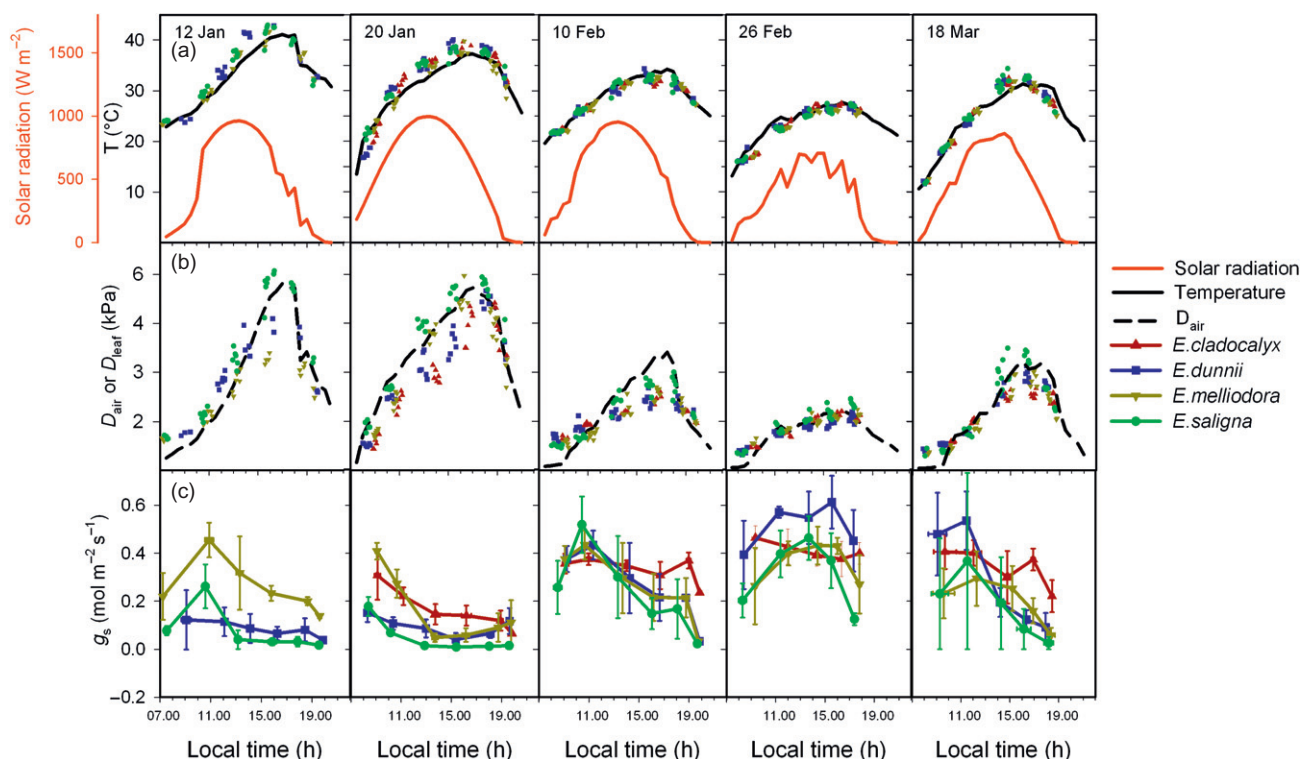


**Figure 1.** (a) Mean temperature and (b) mean precipitation  $P$  (mm) measured by two rain gauges located at the site in an open area from the first of January 2010 until the end of June 2010 for the common garden site. Black arrows represent the days of measurements. (c) Leaf pre-dawn water potential ( $\Psi$ ) for the last 4 d of measurements for the four *Eucalyptus* species. (d) Leaf mid-day water potential ( $\Psi$ ) for four *Eucalyptus* species in a common environment, measured on the same days as in panel C. Each symbol is the mean of one measurement for one or two leaves within each of three trees, with error bars indicating one standard deviation.

was apparently different among these species groups (Fig. 3a,b). All species showed strong, steep declines in  $g_s$  in response to  $D$  across the diurnal range of environmental conditions, with a shallower  $g_s$ – $D$  response for the sub-humid than humid species, most notably at  $D > 3$  kPa (Fig. 3c,d). The differences in relationships in Fig. 3 imply differences in stomatal responses to environment and their coupling to photosynthesis between these species groups.

### Comparison of model parameters across species and during drought

During the well-watered period, the species had different stomatal model parameters. The USO model had the highest



**Figure 2.** Outside climatic conditions and leaf-level gas exchange measurements for the four *Eucalyptus* species grown in a common environment. (a) Solar radiation  $E$  ( $\text{W}\cdot\text{m}^{-2}$ ) and air temperature  $T$  ( $^{\circ}\text{C}$ ) measured by the meteorological station, respectively, represented by black and red lines. Leaf temperature  $T_{\text{leaf}}$  ( $^{\circ}\text{C}$ ) as set in the leaf-level chamber is represented by symbols. (b) Vapour pressure deficit  $D$  (kPa) measured by the meteorological station is denoted by black line. Vapour pressure deficit at the leaf level  $D_{\text{leaf}}$  (kPa) as measured in the chamber is represented by symbols corresponding to the different species. (c) Stomatal conductance to water vapour,  $g_s$  ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), measured by the leaf-gas exchange measurements. Each symbol is the mean of two leaves measured on each of three trees with standard deviations. Red triangles, blue squares, yellow inverse triangles and green rounds are used to represent *E. cladocalyx*, *E. dunnii*, *E. melliodora* and *E. saligna*, respectively. Columns correspond to days of measurements and are presented in chronological order.

slope parameter for the narrowly distributed humid-zone species *E. dunnii* and the lowest slope parameter for the narrowly distributed sub-humid zone species, with intermediate slopes for the more widely distributed species, *E. saligna*, from a humid climate and sub-humid *E. melliodora* (Table 3). There was a significant difference in  $g_1$  between the humid and sub-humid species groups [ $P < 0.022$  for the dummy variable in analysis of variance (ANOVA)] for the USO model. This was the case in spite of the small difference in  $g_1$  between the sub-humid species *E. melliodora* and the humid-zone species *E. saligna* (Table 3). When data were pooled, the mean  $g_1$  for the USO model between the humid and sub-humid species groups was  $4.17 \pm 0.18$  compared with  $3.19 \pm 0.17$ , means  $\pm$  SE for these respective groups. Thus, the original growing climates for the different species were distinguished by the USO model.

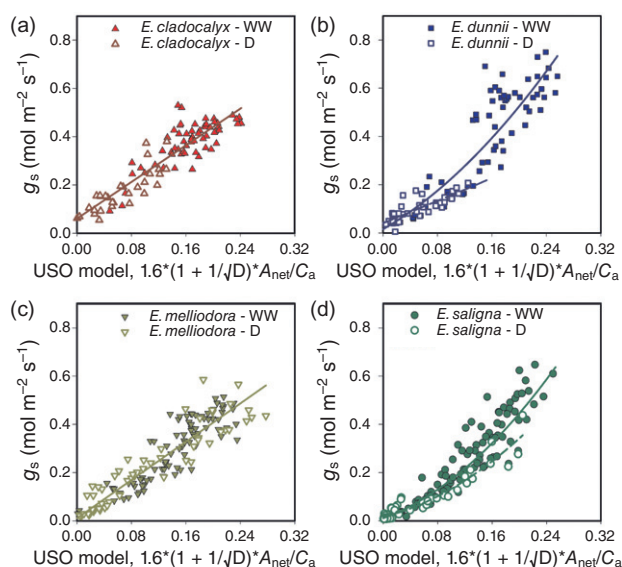
Goodness-of-fit was generally similar between the empirical BBL model and the USO model among the species, but standard errors of the BBL model parameters were larger and the fitted parameter values for the BBL model varied in a less predictable way than for the USO model. The slope parameter for the BBL model was highest for *E. dunnii*, and similar for the three other species.

Three of the four *Eucalyptus* species had model intercepts ( $g_0$  values) that were close to zero for both the BBL and USO models. The exception was *E. cladocalyx*, which had a large intercept, of the order  $100\text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Table 3). *E. cladocalyx* also had the lowest goodness-of-fit for both stomatal models ( $R^2 = 0.61$  and  $0.65$  for the BBL and USO models, respectively). The large positive  $g_0$  in both models for *E. cladocalyx* suggests a relatively large  $g_s$  when  $A_{\text{net}} \rightarrow 0$  for this species, which is as yet unexplained.

Water status affected the parameters of the stomatal models in some but not all species. On the basis of the analysis of climatic conditions and water status (Figs 1 and 2a,b) as well as whole-tree sap flow data (Bourne & Ellsworth, unpublished results), measurements performed in February and March were considered to reflect well-watered conditions, while  $g_s$  and  $A_{\text{net}}$  measured in January were considered to be limited by drought. The sub-humid species *E. cladocalyx* and *E. melliodora* both showed no effect of drought on parameters of the USO model ( $P > 0.10$  using dummy variable in ANOVA), while the humid-zone species *E. dunnii* and *E. saligna* were sensitive to drought and did show significant differences between well-watered and drought data ( $P = 0.0001$  and  $P = 0.005$







**Figure 4.** Relationships between  $g_s$  and the parameterized unified stomatal optimization model (USO) for the four *Eucalyptus* species during well-watered (WW; thick line and filled symbols) and droughted conditions (D, thin line and unfilled symbols) in a common garden. Red triangles, blue squares, yellow inverse triangles and green circles are used to represent (a) *E. cladocalyx*, (b) *E. dunnii*, (c) *E. melliodora* and (d) *E. saligna*, respectively. Each symbol represents the 1 min average gas exchange measurements made over diurnal courses, and lines show splines for the overall non-linear regression fit to the measured data. Where separate lines are shown, these fits were significantly different ( $P < 0.01$ ) using dummy variables. The slope parameter  $g_1$  (unitless) of the lines shown are (means  $\pm$  SE of parameter estimate): (a) *E. cladocalyx*,  $2.80 \pm 0.17$  across WW and D; (b) *E. dunnii* – WW,  $5.17 \pm 0.36$  (Table 3) and *E. dunnii* – D,  $1.33 \pm 0.22$ ; (c) *E. melliodora*,  $2.93 \pm 0.14$  across WW and D; (d) *E. saligna* – WW,  $3.41 \pm 0.17$  (Table 3) and *E. saligna* – D,  $2.08 \pm 0.14$ .

parameter  $g_1$  during drought (*E. dunnii* and *E. saligna*) also showed a tendency for increased  $C_{st}/C_a$  at  $D > 4$  kPa, whereas the other species showed a tendency for an approximately ‘constant’  $C_{st}/C_a$  across a range of  $D$  values from 1 to 6 kPa (Fig. 5). Thus, the constancy of  $C_{st}/C_a$  for the sub-humid species with  $D > 1$  kPa across drought and non-drought days is consistent with a similar slope  $g_1$  for these species between drought and non-drought conditions, which was not the case for humid species. Species showing decreased  $g_1$  for drought days compared with non-drought days also had higher  $C_{st}/C_a$  under high  $D$  during drought. This was also evidence of different stomatal behaviour with regard to gas exchange during drought in the humid zone species compared with the sub-humid species.

## Relationship with leaf and whole-plant traits

Following the functional coordination hypothesis (Sperry *et al.* 2002; Katul *et al.* 2003), a number of leaf and whole-plant traits were associated with the range in stomatal behaviour among the four species in this study as

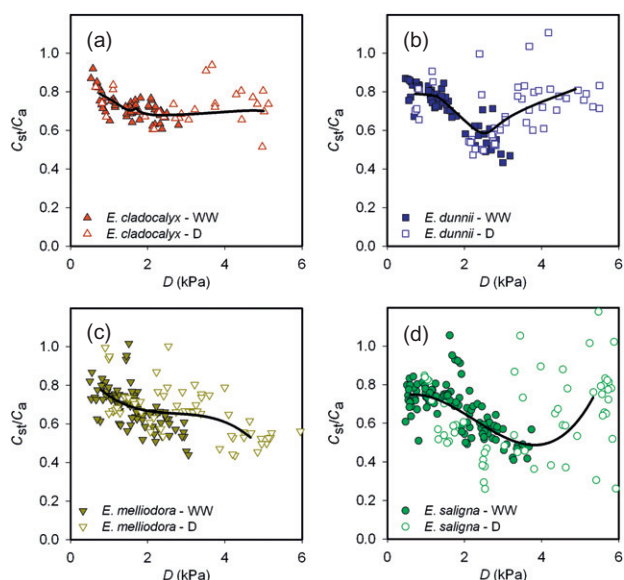
**Table 4.** Comparison of model parameters for four *Eucalyptus* species growing under drought, and the relative change in stomatal model slope parameter  $g_1$  for each species between well-watered and drought conditions

Species	Species climate origin	BBL model for drought			USO model for drought		
		$g_{0,D}$ , mol m <sup>-2</sup> s <sup>-1</sup> (SE)	$g_{1,D}$ , unitless (SE)	Drought change in slope (%)	$g_{0,D}$ , mol m <sup>-2</sup> s <sup>-1</sup> (SE)	$g_{1,D}$ , unitless (SE)	Drought change in slope (%)
<i>Eucalyptus cladocalyx</i>	Sub-humid	0.083 (0.015)	16.4 (7.99)	+46	0.056 (0.014)	2.94 (0.39)	+20
<i>Eucalyptus dunnii</i>	Humid	0.047 (0.01)	5.74 (1.76)	-74	0.042 (0.005)	1.33 (0.22) <sup>a</sup>	-74
<i>Eucalyptus melliodora</i>	Sub-humid	0.056 (0.018)	5.49 (0.926)	-36	0.043 (0.013)	2.63 (0.22)	-19
<i>Eucalyptus saligna</i>	Humid	0.009 (0.005)	5.44 (0.24)	-34	0.007 (0.004)	2.08 (0.14) <sup>a</sup>	-39
							0.897

The model intercept for drought conditions is denoted  $g_{0,D}$ , and the slope term is given as  $g_{1,D}$ , with the change in slope comparing  $g_{1,D}$  with  $g_1$  from Table 3 (see also Fig. 4). For *E. saligna*, the same  $D_0$  parameter was used as for well-watered plants. Parameter standard errors are shown in parentheses.

BBL, Ball–Berry–Leuning; USO, unified stomatal optimization.

<sup>a</sup>Indicates a significant difference between well-watered and drought for this parameter ( $P < 0.001$ ).



**Figure 5.** Variation in estimated  $C_{st}/C_a$  ratio for four *Eucalyptus* species as a function of measurement  $D$  (symbols as in Fig. 4). Filled symbols are for measurements during well-watered conditions (WW), and open symbols are from measurements during drought (D) conditions (see Fig. 4). The solid lines shown in each panel represent the overall  $C_{st}/C_a$  trends using bin-averaged data.

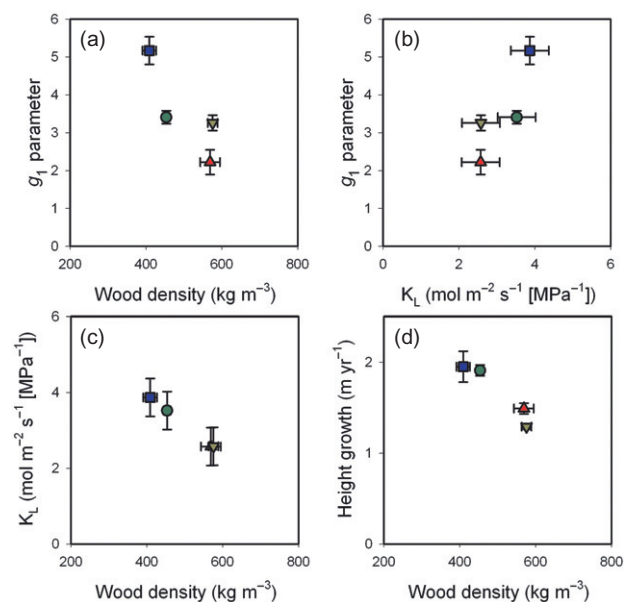
indicated by the  $g_1$  parameter for the unified stomatal model. The  $g_1$  parameter is proportional to the combination of  $\sqrt{\Gamma^* \cdot \lambda}$  (derivation in Medlyn *et al.* 2011) and hence is related to the marginal water cost of carbon gain and inversely proportional to  $\partial A_{net}/(\partial g_s \cdot D)$  as a measure of leaf-level intrinsic water-use efficiency (Barton *et al.* 2012). The  $g_1$  parameter was negatively associated with wood density (Fig. 6a) and positively associated with  $K_L$  (Fig. 6b). There was also a strong negative correlation between  $K_L$  and wood density, and between height growth and wood density (Fig. 6c,d).

## DISCUSSION

Long-standing theory of stomatal behaviour suggests that species from more arid habitats that experience frequent droughts are expected to have more conservative stomatal behaviour and more efficient use of water per unit carbon gain than species adapted to moist habitats (Cowan & Farquhar 1977; Orians & Solbrig 1977; Cowan 1982). This is predicted from the balance between the demand for  $CO_2$  regulated by the biochemistry of photosynthesis and the supply of  $CO_2$  and water to intercellular air spaces within leaves. The rate of photosynthesis increases asymptotically with regard to increasing  $g_s$ , but water loss rates from leaves increase linearly, hence species with lower  $g_s$  have lower  $\lambda$  (Cowan 1982; Anderson *et al.* 1996; Thomas *et al.* 1999a). The USO model  $g_1$  parameter can be seen as a proxy for the water cost of carbon uptake  $\lambda$  under the assumption that the temperature (and  $D$ ) range is roughly equivalent across

species within the dataset. We show that in a given environment where different species are grown together, sub-humid climate *Eucalyptus* species have different  $g_s$ – $A_{net}$  coupling and different  $g_s$ – $D$  relationships than humid-zone species, consistent with these theories and with previous empirical measurements (Anderson *et al.* 1996; Ngugi *et al.* 2004). As a result, the species differences were exemplified by lower  $g_1$  slopes for the sub-humid species in the USO model when trees were well watered than for humid-zone species (Table 3), and a lower marginal water cost of  $CO_2$  uptake,  $\lambda$ , in these species.

The  $g_1$  parameter values for the *Eucalyptus* species here are similar to those derived for *Eucalyptus delegatensis* in Medlyn *et al.* (2011) as well as the temperate species *Fagus sylvatica* and *Pinus taeda*, with correction to the USO model considered. However, all species here had lower  $g_1$  and generally better fits to the USO model than for mature *Eucalyptus paramattensis*–*Angophora bakeri* trees at a nearby site on different soils (Medlyn *et al.* 2011). Thus, while differences in the marginal water cost of carbon uptake have been identified among contrasting plant functional types or climates (Medlyn *et al.* 2011), there are also clear differences among contrasting species in the same genus at the same site that relate to their geographical distributions. That these species differences are smaller under drought (Table 4) suggest a convergence amongst species with similar biochemical capacities and high stomatal limitations to photosynthesis.



**Figure 6.** Species variation in the unified stomatal optimization model slope parameter  $g_1$ , and whole-plant variables. Each symbol denotes a species according to the colour scheme in Figs 3 and 4. Whole-plant variables wood density (a, d), root-to-leaf hydraulic conductance ( $K_L$ ; b) and height growth rate (c, d) are shown, and Pearson correlation coefficients are given in Supporting Information Table S1. See supporting information for further details.

## Relationships of $\lambda$ with plant traits and hydraulic characteristics

As an expression of whole-plant C and water economies, long-term  $\lambda$  is expected to be related to water transport parameters that integrate both the supply of water and the C cost of vascular structures over the growth of plants (Givnish 1986). Such parameters include characteristics of the vascular system such as conduit size and density, water-conducting path length, and the leaf surface area supported by conductive stem tissues. Our observation of a progressive increase in  $K_L$  across species in well-watered conditions with an increase in  $g_1$  (Fig. 5b) is consistent with this expectation. Moreover, using wood density as a proxy measure of the construction cost for hydraulic transport (Hacke *et al.* 2001; Choat *et al.* 2006) can explicitly link whole-plant C and water economies as faster-growing trees typically produce lower-density wood and this kind of wood is far less resistant to cavitation than is denser wood with thicker cell walls and greater xylem cell wall strength (Hacke & Sperry 2001; Hacke *et al.* 2001). Supporting this idea, sub-humid species with lower  $\lambda$  and lower  $g_1$  slopes also had greater wood density than the humid species in this study (Fig. 5a), and would thus be expected to endure lower  $\Psi$  without cavitation. Indeed, both sub-humid species showed lower  $\Psi_1$  during peak summer conditions than the humid species *E. dunnii* and *E. saligna*, which reached only  $-2.1$  MPa. Thus, the links among the  $g_1$  parameter,  $K_L$ , and trunk wood density quantitatively establishes long-hypothesized trade-offs associated with C costs of water transport and opportunity costs of water loss (Cowan & Farquhar 1977; Lloyd & Farquhar 1994) with a further connection to plant growth rate (Fig. 5e). However, we have only considered four species in our study, so the relationships observed here require confirmation with a larger set of related species.

## Effect of drought on optimal stomatal behaviour

Previously, different studies have found different sensitivities of the marginal water cost of carbon uptake to declining soil water availability. In tropical savanna species, the marginal water cost of carbon uptake was higher in wet versus dry seasons, indicating a decline with lower soil moisture availability (Thomas, Eamus & Bell 1999b). However, in Mediterranean oak species, it was not dependent on water availability (Xu & Baldocchi 2003). Here, we have presented evidence of both types of behaviours in different species in the same genus (e.g. humid-zone species versus sub-humid *Eucalyptus* species; Figs 4 and 5) during a 6 week summer drought with pre-dawn water potential  $<1$  MPa (Fig. 1).

We hypothesized that the sensitivity of  $g_1$  to drought should vary with the climatic origin of species. We argued that in sub-humid species, the carbon cost of water to the plant should not increase as soil dries, leading to a relatively constant value of  $g_1$ ; whereas in humid species, the cost of water would be likely to increase with decreasing water

availability, leading to a decrease in  $g_1$  with declining soil moisture. This hypothesis was largely supported by stomatal behaviour in the four species we examined in this study. We found that  $g_1$  was lower during drought periods in two humid-zone species (*E. dunnii* and *E. saligna*) but was unaffected by drought in two sub-humid zone species (*E. melliodora* and *E. cladocalyx*).

Not all the species in this study experienced drought similarly. Based on pre-dawn  $\Psi_1$  data (Fig. 1), *E. melliodora* probably had more water available in its soil/rhizosphere than the other species, as it showed little change in pre-dawn  $\Psi_1$  from the drought period in January to the wet month of February. This might explain why *E. melliodora* had a constant marginal water cost of carbon across different soil moistures, but it does not explain why so for the other sub-humid species, *E. cladocalyx*. The pattern of both pre-dawn and midday  $\Psi_1$  across the summer for *E. cladocalyx* suggests anisohydric behaviour which suggests that this species relied on other 'water-saving' strategies than conserving  $\Psi_1$ . For instance, strategies related to functional traits and hydraulic architecture are strongly suspected to be related to  $\lambda$  and therefore the USO model  $g_1$  parameter (Sperry 2000; Medlyn *et al.* 2011). Given that the set of *Eucalyptus* species studied here was small, they may not typify the broad range of environments, traits or stomatal behaviours within the genus *Eucalyptus*. Still, species did exhibit contrasting stomatal behaviour consistent with their climate of origin, and which was associated with whole-plant traits (Fig. 5). It is unclear what aspect of the climate in their original geographical range of these species is most relevant to the contrasting stomatal behaviour we observed. Adaptations to frequent drought and/or high  $D$  stress may involve decreased sensitivity of stomata to these conditions, which is consistent with maintaining  $\lambda$  during drought. The patterns observed across these four species clearly warrant further investigation across a broader set of species.

## CONCLUSIONS

Differences in stomatal conductance and its response to environment among different *Eucalyptus* species are well documented, but here we have tested the optimal stomatal behaviour hypothesis with regard to drought trade-offs in young *Eucalyptus* trees from different regions grown in a common environment. The marginal water costs of carbon uptake were lower for sub-humid climate species that are drought tolerant than humid zone species due to their structural and hydraulic attributes, with implications for species selections for plantations in these climatic regions. The differences in stomatal behaviour and its coupling to photosynthesis in humid zone species compared with sub-humid, drought-tolerant species, suggest that strong selection has occurred for leaf and whole-plant characteristics and different stomatal optimization behaviour for species from climates with contrasting rainfall and drought frequencies (see Brodribb *et al.* 2005; Franks 2006).

The  $g_1$  parameter is useful because it encompasses two universal relationships, stomatal responsiveness to  $D$



and coupling to photosynthesis, and hence it establishes the connection of stomatal behaviour with plant hydraulic functioning. As such, leaves must adjust their stomatal aperture to maximize photosynthesis while minimizing consequences of excessive water loss such as cavitation and drought-induced dieback. We advocate the use of carefully designed common garden studies of within-species variation in stomatal behaviour for this purpose.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Pearson correlation coefficients among eight leaf-level and whole-plant traits across four contrasting *Eucalyptus* species. Traits are:  $g_1$  under non-drought conditions from Table 4, dimensionless; wood density of the main trunk, kg m<sup>-3</sup>; apparent  $V_{\text{cmax}}$ ,  $\mu\text{mol m}^{-2}$  leaf s<sup>-1</sup> from fits to the Farquhar *et al.* (1980) photosynthesis model; root-to-leaf  $K_L$ , mol m<sup>-2</sup> s<sup>-1</sup> (MPa)<sup>-1</sup>; leaf thickness,  $\mu\text{m}$ ; basal area (BA) growth, cm<sup>2</sup> per year; height extension growth, m yr<sup>-1</sup>; and  $k_{\text{stem}}$ , kg s<sup>-1</sup> m<sup>-1</sup> MPa<sup>-1</sup>.

**Table S2.** Mean leaf traits for current growing season leaves measured under optimal conditions for four *Eucalyptus* species (*E. cladocalyx*, *E. dunni*, *E. melliodora* and *E. saligna*) grown in a common garden and measured at age 2.5 years. Data are means  $\pm$  SE for the three trees on which the diurnal gas exchange and  $\Psi_{\text{leaf}}$  were measured.