Contrasting physiological responses of two co-occurring eucalypts to seasonal drought at restored bauxite mine sites

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This study describes the physiological response of two co-occurring tree species (Eucalyptus marginata and Corymbia calophylla) to seasonal drought at low- and high-quality restored bauxite mine sites in south-western Australia. Seasonal changes in photosynthesis ($A$), stomatal conductance ($g_s$), leaf water potential ($\Psi$), leaf osmotic potential ($\Pi$), leaf relative water content (RWC) and pressure–volume analysis were captured over an 18-month field study to (i) determine the nature and severity of physiological stress in relation to site quality and (ii) identify any physiological differences between the two species. Root system restriction at the low-quality site reduced maximum rates of gas exchange ($g_s$ and $A$) and increased water stress (midday $\Psi$ and daily RWC) in both species during drought. Both species showed high stomatal sensitivity during drought; however, $E$. marginata demonstrated a higher dehydration tolerance where $\Psi$ and RWC fell to $-3.2$ MPa and 73% compared with $-2.4$ MPa and 80% for $C$. calophylla. Corymbia calophylla showed lower $g_s$ and higher $\Psi$ and RWC during drought, indicating higher drought tolerance. Pressure–volume curves showed that cell-wall elasticity of $E$. marginata leaves increased in response to drought, while $C$. calophylla leaves showed lower osmotic potential at zero turgor in summer than in winter, indicating osmotic adjustment. Both species are clearly able to tolerate seasonal drought at hostile sites; however, by $C$. calophylla closing stomata earlier in the drought cycle, maintaining a higher water status during drought and having the additional mechanism of osmotic adjustment, it may have a greater capacity to survive extended periods of drought.

Keywords: Corymbia calophylla, drought resistance, ecological restoration, Eucalyptus marginata, osmotic adjustment, photosynthesis, stomatal conductance, water relations.

Introduction

In a recent review, Cooke and Suski (2008) highlighted the fact that very few studies have applied physiological tools to determine the nature and magnitude of the stresses encountered by restored vegetation following major disturbance. Quantifying the physiological response of vegetation to stress in altered environments is critical to determining its capacity for survival and growth (Schmidt et al. 1999, Cooke and Suski 2008, Grigg et al. 2010), particularly in light of predicted future trends in climate (Shi et al. 2008, Hartmann 2011). In highly altered systems, such as restored mine sites, existing knowledge of physiological functioning of vegetation in unaltered systems may not apply (Aber 1987, Ehleringer and Sandquist 2006), as the physical, chemical and biological properties of the soil have often changed (Izquierdoa et al. 2005, Schroeder et al. 2010). Furthermore, the vegetation structure and species mix in restored systems may differ from those of the original vegetation (Dobson et al. 1997, Herath et al. 2009), especially where co-occurring species possess different physiological strategies for coping with stress (Chen et al. 2005). Many studies have shown that stress responses of co-occurring species of the same functional group/life form in resource-limited environments frequently differ in
mechanism or magnitude (Kolb and Stone 2000, Gebrekirstos et al. 2006, David et al. 2007, Grigg et al. 2008, West et al. 2008, Austin et al. 2009); therefore, it is possible that the physiological mechanisms of sub- or co-dominant species in undisturbed systems may facilitate their dominance in restored landscapes.

In seasonally dry environments, physiological mechanisms that enhance survival during extended periods of drought are common. Morphological adaptations, such as deep root systems, enhance the survival and productivity of mature vegetation (Jipp et al. 1998, Schenk and Jackson 2002, Goldstein et al. 2008); however, physiological mechanisms for resisting drought are more critical to the survival of developing juvenile vegetation (Donovan and Ehleringer 1991, Crombie 1997) at restored sites. Physiological mechanisms that enhance drought resistance include, but are not limited to, high stomatal sensitivity to leaf-air moisture gradients and/or leaf water status (Mott and Parkhurst 1991, Monteith 1995, Oren et al. 1999), the capacity to maintain turgor during drought by lowering osmotic potential (Clifford et al. 1998, Carter et al. 2006, Merchant et al. 2007) and a high tolerance to leaf tissue dehydration (Pook et al. 1966, Davidson and Reid 1989, Gulias et al. 2002).

In the Mediterranean climate of south-western Australia, <20% of annual rainfall occurs over the warmest six months of the year, which is why the vegetation is highly adapted to surviving periods of extended drought (Specht and Specht 1999). The jarrah (Eucalyptus marginata Sm.) forest is a ‘dry sclerophyll’, ‘open forest’ system in south-western Australia. The two key upper-canopy evergreen tree species, E. marginata and Corymbia calophylla (Lindl.) K.D. Hill & L.A.S. Johnson, are well adapted to seasonal drought and heterogeneous soils (Abbott and Loneragan 1986, Abbott et al. 1989). As a mature tree in the forest, E. marginata accesses water deep in the soil profile (Farrington et al. 1996) through ancient root channels (Dell et al. 1983), which allows it to transpire over summer while maintaining a stable water status (Doley 1967, Carbon et al. 1981, Colquhoun et al. 1984). Studies of C. calophylla physiology have largely been restricted to mature forest stands as a comparison with E. marginata; however, C. calophylla is typically discussed in less detail than E. marginata, presumably because it represents less of the stand (typically 20–40%) and has less economic value. Corymbia calophylla tends to colonize areas where root development is limited and access to soil moisture is highly variable, such as shallow soils and riparian zones susceptible to waterlogging (Harris 1956). Eucalyptus marginata maintains higher daily transpiration rates (Grieve 1956, Carbon et al. 1981) and midday stomatal conductance (Crombie 1992), and lower predawn (Crombie et al. 1988) and midday (Carbon et al. 1981, Colquhoun et al. 1984, Crombie 1992) leaf water potentials during drought than C. calophylla. The two species clearly demonstrate physiological differences in the undisturbed forest; however, they have not been explored in detail.

The jarrah forest predominantly occurs on deep lateritic soil profiles rich in aluminium hydroxide minerals (Sadlier and Gilkes 1976, Churchward and Dimmock 1989), which have been mined as bauxite since the 1960s (Gardner and Bell 2007). Bauxite mining in the jarrah forest is a shallow, open-cut style of mining, where sand and gravel layers above the bauxite deposit are stripped, and the bauxite is blasted and removed to a depth of 5–8 m. After mining, pits are contoured into the surrounding landscape, the ex-mine floor is deep-ripped, topsoil layers are returned, native species are broadcast in the surrounding landscape, the ex-mine floor is deep-ripped, topsoil layers are returned, native species are broadcast in the surrounding landscape, and the jarrah forest is re-established (Enright and Lamont 1992). The first aim of this study was to identify the nature and severity of stress encountered by E. marginata and C. calophylla trees at a low-quality restored site where root system development is known to be severely limited (Szota et al. 2007).

At low-quality restored sites, C. calophylla shows lower mortality than E. marginata (J. Koch, Alcoa World Alumina Australia, AU, personal communication), raising the possibility that C. calophylla has mechanisms that enhance its survival at low-quality restored sites. The second aim of this study was to investigate whether there are any inherent differences in the physiological response to drought between E. marginata and C. calophylla which would have implications for survival and productivity of tree species at restored or regenerating forest stands. Direct comparisons of the physiological responses to water deficits in the field can be complicated by the fact that co-occurring species often access different water sources (Eberbach and Burrows 2006, Schwinning 2008). To avoid this complication, many comparative physiological studies are performed in the glasshouse (e.g., Merchant et al. 2006, Austin et al. 2009); however, the behaviour of young seedlings in the glasshouse may not necessarily reflect that of juvenile trees in the field. The present study addresses these issues by comparing the physiological mechanisms of developing (11–13 years old) E. marginata and C. calophylla trees,
at two adjacent field sites: a ‘low-quality’ site with a known limitation to growth (restriction of coarse root systems to the top 0.5 m of the soil profile), and a ‘high-quality’ site with subsoil access to at least 1.5 m and no apparent growth limitation (Szota et al. 2007).

Materials and methods

Study sites

This study was carried out in a 13-year-old restored bauxite mine pit, located ~10 km north-west of Dwellingup (32.71°S, 116.06°E), Western Australia, Australia. The study area was restored according to the following general procedure used at the time (1992). Mine pit walls were smoothed down to create more natural contours and blend the pit into the surrounding landscape. Sandy gravel was spread over the mine floor to a depth of ~0.5 m followed by topsoil to a depth of 0.1 m. The site was then deep-ripped using a Caterpillar D11 bulldozer with a single winged tine capable of ripping to a depth of 1.5 m, with 2 m spacing between riplines. Seeds were broadcast in autumn to achieve a tree stand density of 2500 stems ha\(^{-1}\) with 80% *E. marginata* and 20% *C. calophylla* and nitrogen and phosphorus fertilizers were applied in spring. Two 1250 m\(^2\) plots (each measuring 25 m × 50 m) were established, one in an area of small trees (classed as ‘low-quality’) and another in an adjacent area of taller trees (‘high-quality’) within the same restored pit.

Szota et al. (2007) carried out excavations and descriptions of root-system morphologies at these sites and found that an impenetrable quartz layer (Kew et al. 2007) restricted coarse roots (>5 mm in diameter) to sandy/gravel material in the top 0.5 m of the soil profile at the low-quality site, while riplines at the high-quality site facilitated access of coarse roots to the kaolinitic clay subsoil to a depth >1.5 m.

Weather data

The weather data presented here were recorded at the Dwellingup weather station (009538) by the Australian Bureau of Meteorology. Dwellingup has a Mediterranean-type climate with long-term (76-year average) and 30-year average annual rainfall of 1243 and 1177 mm. Only 196 mm (16% of long-term average annual total) occurs in the 6 months from November to May. The bulk of the study took place during 2004, which recorded an annual rainfall of 1162 mm and an annual pan evaporation of 1402 mm (Figure 1). Rainfall received in the two drought cycles in the present study was 93 and 140 mm for 2003/2004 and 2004/2005. Vapour pressure deficit (VPD) increased over the day in each month, with the highest value recorded at 15:00 h (Australian Western Standard Time), with the exception of the winter months where there was no increase from 12:00 to 15:00 h. Average daily VPD increased from 1.2 to 3.0 kPa in summer and from 0.1 to 0.5 kPa in winter from 09:00 to 15:00 h over the study period.

Stand characteristics

Initial stand characteristics were measured at both sites in May 2003. Stand density was determined by counting all *E. marginata* and *C. calophylla* trees taller than 2 m at the two 1250 m\(^2\) plots. Tree height (tallest living section of crown) and diameter over bark at breast height (1.3 m; DBH) were measured for all *E. marginata* and *C. calophylla* trees >2 m tall at both sites. Thirty pre-selected trees representative of the size-class distribution range for each species in both stands were re-measured in May 2005 using the same methods to determine growth over the study period. Specific leaf area (SLA) was measured prior to emergence of new leaves in August 2004, using 36 leaves per site, per species. Leaf area was measured on fresh leaves with a LAI-310°C (LI-COR Inc., Lincoln, NE, USA). Leaves were then oven-dried for 48 h at 70 °C to determine leaf dry weight. Specific leaf area was calculated as leaf area divided by leaf dry weight.

Study tree selection for physiological measurements

Physiological measurements were taken on trees in the median size class for that species at each site. At the low-quality site, *E. marginata* trees were 4–6 m tall and *C. calophylla* trees were 3–6 m tall. At the high-quality site, *E. marginata* trees were 7–9 m tall and *C. calophylla* trees were 5–8 m tall. Adjacent
E. marginata and C. calophylla trees were selected where possible in order to make direct comparisons between the two species. Physiological measurements described below were carried out over two to three consecutive days each month between November 2003 and April 2005. Three trees per species were sampled at each site between 07:00 and 17:00 h in three blocks: morning (07:00–10:30 h), midday (11:30–13:30 h) and afternoon (14:00–17:00 h). Twelve new trees were selected for measurement each month and measurements were blocked over the day to make valid comparisons between sites and species. Data from measurement days within the month were pooled where weather conditions were similar; otherwise the most representative measurement day was used.

**Leaf stomatal conductance and photosynthesis**

Gas exchange was measured on sun-exposed, freshly cut branches (30–50 cm long) from the upper third of the crown. Previous studies on E. marginata have successfully used cut sections for gas exchange measurements (Crombie 1992, 1997), and tests run periodically over the present study confirmed no effect of excision during the time required for measurement (data not shown). Diurnal patterns of stomatal conductance \( (g_s) \) and photosynthesis \( (A) \) were captured each month for three trees per species, per site, with a LI-6400 gas exchange system (LI-COR Inc.). Three mature sun-exposed leaves were measured on each study tree at each measurement time (4–6 measurement times per day). Photosynthetic photon flux density (red–blue light source) was set at 1500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and \( \text{CO}_2 \) concentration in the chamber ranged from 374 to 394 \( \text{mmol mol}^{-1} \) over the course of the study. Temperature in the chamber was set at 25 °C; however, when ambient temperature was high in summer (>35 °C) the chamber temperature increased to 30–33 °C.

**Leaf water potential, osmotic potential and relative water content**

Diurnal patterns of leaf water potential \( (\Psi) \), osmotic potential \( (\Pi) \) and relative water content \( (\text{RWC}) \) were measured 4–6 times per day between 07:00 and 17:00 h at monthly intervals over the study period. Leaf water potential was measured using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA). Three twigs bearing 3–4 mature sun-exposed leaves were excised from the top of the canopy on the northern side of each of the 12 study trees (3 trees per species per site) measured each month. The sections were measured immediately following excision. Sections were placed inside a zip-lock bag with only the cut end protruding while inside the chamber, which was lined with wet cloth in order to minimize evaporative losses during pressurization (Turner 1988).

Three leaves from each of the 12 study trees (3 trees per species per site) selected each month were placed in an airtight 5-ml cryovial (Simport, Canada) and immediately stored on dry ice to determine leaf osmotic potential. Samples were transferred to a −20 °C freezer in the laboratory until analysis. Samples were thawed and then crushed using a leaf press. The sap was analysed with a Fiske 101 freezing-point depression osmometer (Fiske Associates, Model 110, MA, USA). The osmometer was regularly calibrated with 50, 850 and 1200 mmol kg\(^{-1}\) standards during analysis of the samples. In order to compare osmotic adjustment between sites and species over the course of the year, osmotic potential values were corrected for seasonal changes in leaf RWC.

Three sections were excised from the top of the canopy on the northern side of the 12 study trees (3 trees per species per site) selected each month to determine leaf RWC. From these three sections per tree, three mature leaves were sealed in small zip-lock bags and placed in an insulated box with ice packs. Depending on the time of day sampled, leaves remained in the box for 3–10 h prior to being weighed (fresh weight). Samples where condensation was obvious inside the zip-lock bag were discarded. Leaves were wrapped in wet tissue paper and stored in plastic zip-lock bags at 4 °C in the dark for 12 h to facilitate hydration. Leaves were then blotted dry and their saturated, or turgid, weights recorded. The leaves were then oven-dried at 70 °C for 48 h, and re-weighed to determine their dry weights. Relative water content was calculated as RWC = \( [(\text{FW}–\text{DW})]/(\text{SW}–\text{DW})] \times 100 \), where FW = fresh weight, DW = dry weight and SW = saturated weight.

**Pressure–volume curves**

Pressure–volume curves were derived in March and August 2004. Sun-exposed branches were taken from the exterior of the canopy from the northern side of five E. marginata and five C. calophylla trees at each site. Stems were immediately recut under water in 50-ml plastic vials and leaves were wrapped in plastic film. The samples were left to hydrate overnight in the laboratory for ~12 h. The following morning the material was used to produce pressure–volume curves. Some leaves developed blotchy, dark staining which may be due to over-saturation (Bleby 2003, Warren et al. 2007), and these leaves were not selected for producing curves. Youngest fully expanded leaves were excised with a razor blade and immediately weighed and transferred into a pressure bomb to determine leaf water potential (as above). Leaves were placed on the laboratory bench between measurements to facilitate dehydration. Pressure-bombing finished when water potentials of −4 to −6 MPa were reached, and when the relationship between 1/balancing pressure (BP) and 1 – RWC became linear. Osmotic potential at full turgor \( (\Pi_{\text{max}}) \), osmotic potential at zero turgor \( (\Pi_z) \), relative water content at zero turgor \( (\text{RWC}_z) \) and the turgid weight to dry weight ratio were calculated from the pressure–volume curves (Tyree and Hammel 1972, Turner 1988). Bulk modulus of elasticity \( (\epsilon_{\text{max}}) \) was calculated from the slope of the
relationship between pressure potential and RWC in the positive turgor range (Turner 1988).

Data analyses

Two-way analysis of variance (ANOVA) was used to determine differences between sites and species and the interaction site × species for the stand and tree characteristics. Two-way ANOVA was also used to determine differences between sites and species between and within seasons for pressure–volume curve parameters. Two-way ANOVA was used to test for significant differences in \( g_s \), \( A \), \( \Psi \), \( s_{max} \) and RWC between sites and species within a given month and within a given time of day (morning, midday or afternoon). One-way ANOVA was used to test for significant differences within site, species and time of day between months. One-way ANOVA was also used to test for significant differences between time of day within month, for each species at each site. Linear regression analysis was used to determine the correlation between \( g_s \) and VPD, maximum \( g_s \) (\( s_{max} \)) and \( \Psi \); \( s_{max} \) and RWC; and \( \Psi \) and RWC. Results are only referred to as significantly different where \( P < 0.05 \). All data were tested for normality using a Shapiro–Wilk test, and transformations were performed where appropriate.

Results

Stand characteristics

The low-quality site had 61% fewer trees and 74% less standing basal area than the neighbouring high-quality site (Table 1). *Eucalyptus marginata* trees represented 80% of the stand at the low-quality site and 76% of the stand at the high-quality site. *Eucalyptus marginata* trees constituted 88% of the stand basal area at the low-quality site and 86% at the high-quality site. *Eucalyptus marginata* and *C. calophylla* trees at the low-quality site were 42 and 40% shorter and had 28 and 26% smaller diameters than those at the high-quality site. The mean annual increase (2003–2005) in height of *E. marginata* and *C. calophylla* at the low-quality site was 41 and 38% less than that at the high-quality site. There was no significant difference in the annual increase in DBH between sites. *Eucalyptus marginata* trees did, however, have annual increases in DBH 43 and 33% higher than *C. calophylla* trees at the low-quality and high-quality sites. There was no significant difference in SLA between sites or species.

### Table 1. Stand and tree characteristics at low-quality and high-quality restored bauxite mine sites containing jarrah (*E. marginata*) and marri (*C. calophylla*) (mean standard error in parentheses with \( n = 168, 407, 42 \) and 126 for low-quality jarrah, high-quality jarrah, low-quality marri and high-quality marri). Mean annual growth rates (2003–2005) for height and DBH are also presented with mean standard error in parentheses (\( n = 36 \) leaves per site, per species). All \( P \) values are derived from two-way ANOVA. \( \rho_{site} \) and \( \rho_{species} \) represent the \( P \) values for differences between sites and species, and \( \rho_{site × species} \) represents the interaction site × species. Within rows, different letters indicate significant differences between sites and species (using the maximum least significant difference from the interaction site × species). n.s. refers to no significant difference (\( P > 0.05 \)).

<table>
<thead>
<tr>
<th></th>
<th>Low-quality</th>
<th>High-quality</th>
<th>Low-quality</th>
<th>High-quality</th>
<th>( \rho_{site} )</th>
<th>( \rho_{species} )</th>
<th>( \rho_{site × species} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stand density (trees ha(^{-1}))</td>
<td>1344</td>
<td>3256</td>
<td>336</td>
<td>1008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stand basal area (m(^2) ha(^{-1}))</td>
<td>11.4</td>
<td>43.4</td>
<td>1.5</td>
<td>6.8</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Tree height (m)</td>
<td>4.6 (±0.1)(^b)</td>
<td>8.0 (±0.1)(^c)</td>
<td>3.9 (±0.3)(^a)</td>
<td>6.5 (±0.2)(^c)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Tree DBH (cm)</td>
<td>8.4 (±0.3)(^b)</td>
<td>11.6 (±0.2)(^c)</td>
<td>5.9 (±0.5)(^a)</td>
<td>8.0 (±0.3)(^b)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Height growth rate (m year(^{-1}))</td>
<td>0.43 (±0.03)(^b)</td>
<td>0.73 (±0.04)(^a)</td>
<td>0.26 (±0.02)(^a)</td>
<td>0.42 (±0.02)(^b)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.04</td>
</tr>
<tr>
<td>DBH growth rate (cm year(^{-1}))</td>
<td>0.60 (±0.04)(^b)</td>
<td>0.57 (±0.01)(^a)</td>
<td>0.42 (±0.03)(^a)</td>
<td>0.43 (±0.04)(^a)</td>
<td>n.s.</td>
<td>&lt;0.001</td>
<td>0.05</td>
</tr>
<tr>
<td>SLA (cm(^2) g(^{-1}))</td>
<td>57.0 (±0.9)</td>
<td>55.3 (±0.8)</td>
<td>57.1 (±1.2)</td>
<td>59.2 (±0.9)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Morning \( g_s \) and \( A \) were highest in late spring and declined over the dry season (December–March) for both species at both sites, whereas midday and afternoon values peaked earlier, at least in 2004 (Figures 2 and 3; refer to least significant difference for each time of day for significant differences between months). Both species at both sites maintained high stomatal conductance and rates of photosynthesis in the morning (07:00–10:30 h) for the major part of the year, with the exception of the winter months when the highest \( g_s \) and \( A \) were recorded at midday (11:30–13:30 h) (Figures 2 and 3; refer to ‘†’ symbols below the \( x \)-axis for significant differences between time of day for each species at each site). Both species at both sites showed decreasing \( g_s \) and \( A \) over the course of the day during the dry season (Figures 2 and 3). *Eucalyptus marginata* and *C. calophylla* at the low-quality site showed lower morning \( g_s \) and \( A \) over the dry season than at the high-quality site (Figures 2 and 3; refer to ‘*’ symbols for each time of day for significant differences for the interaction site × species within each month). *Eucalyptus marginata* at the low-quality site showed higher midday and afternoon \( g_s \) over spring and early summer than at the high-quality site (Figure 2). *Eucalyptus marginata* maintained higher \( g_s \) and \( A \) over the course of the year than *C. calophylla* did at both
Seasonal patterns of leaf water potential, osmotic potential and RWC

_Eucalyptus marginata_ and _C. calophylla_ at both sites showed their highest midday Ψ in winter and their lowest in summer (Figure 4). _Eucalyptus marginata_ at the low-quality site maintained higher Ψ over spring and early summer, and lower Ψ over mid to late summer than it did at the high-quality site (Figure 4). There was no significant difference in Ψ between sites over the dry season for _C. calophylla_ (Figure 4). _Eucalyptus marginata_ maintained significantly lower Ψ over the dry season than _C. calophylla_ did at both sites (Figure 4).

Seasonal variation in Π was similar to that for Ψ, with highest values in winter and lower values in summer (Figure 4). _Eucalyptus marginata_ showed no consistent seasonal difference between sites during the year; however, _C. calophylla_ had lower Π at the low-quality site compared with that at the high-quality site over the dry season.

There was no significant diurnal trend in leaf RWC on any of the measurement days over the course of the study (data not shown). Consequently, all RWC results are expressed as averages for the measurement day(s) within a given month. _Eucalyptus marginata_ and _C. calophylla_ at both sites had their highest RWCs in winter and their lowest late in the dry season (Figure 4). _Eucalyptus marginata_ leaves at the low-quality site dried out to a greater extent (73% RWC) than _C. calophylla_ leaves did (80% RWC) in late summer (Figure 4).

Stomatal sensitivity in relation to VPD, leaf water potential and RWC

During winter and spring, _E. marginata_ and _C. calophylla_ at both sites maintained high _gs_ over the course of the day as...
VPD increased (Figure 5). In mid-summer, however, leaves of both species showed a significant decrease in $g_s$ over the day as VPD increased, with *E. marginata* and *C. calophylla* at the low-quality site maintaining lower $g_s$ for any given VPD (Figure 5). Conductance was low for both species at both the low- and high-quality sites in late summer/autumn at the start of the day and declined further over the day in response to increasing VPD, even though the maximum VPD on the measurement days was lower than that recorded during winter/spring and early summer (Figure 5).

For *E. marginata*, the highest daily value for $g_s$ (typically recorded in the morning) decreased linearly with midday water potential, whereas there was no correlation between morning $g_s$ and $Ψ$ for *C. calophylla* (Figure 6). Leaves of both species, however, showed a similar linear decrease of morning $g_s$ with RWC (Figure 6).

**Pressure–volume analysis**

There was no significant difference in $Π_{100}$, RWC$_0$ or TW:DW between sites, between species or between seasons. Values for $Π_0$ of *E. marginata* did not differ between winter and summer at either site; however, $Π_0$ was significantly lower ($P < 0.02$) for *C. calophylla* leaves analysed in summer compared with those in winter at both sites (Table 2). There was no significant difference in $Π_0$ between *E. marginata* and *C. calophylla* in winter at either site; however, *E. marginata* had significantly higher $Π_0$ ($P < 0.02$) than *C. calophylla* in summer at both sites. *Eucalyptus marginata* leaves analysed in winter had significantly higher bulk modulus of elasticity, $ε_{max}$ ($P < 0.001$), than those analysed in summer at both sites. Elasticity did not differ between winter and summer for *C. calophylla* at either site. *Eucalyptus marginata* at both sites had significantly higher $ε_{max}$ ($P < 0.001$) than *C. calophylla* in winter; however, there was no

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**Figure 3.** Morning (a and b), midday (c and d) and afternoon (e and f) photosynthesis ($A$) of *E. marginata* (jarrah) and *C. calophylla* (marri) at low-quality (black) and high-quality (white) restored bauxite mine sites. Bars on mean values represent mean standard error ($n$ = 3–9). Bold bars represent least significant difference ($P < 0.05$) between months within site and species for the low-quality (LQ) and high-quality (HQ) sites. Significant differences ($P < 0.05$) for the interaction site × species within any given month are represented by an asterisk. Significant differences ($P < 0.05$) between time of day within site and species are represented by † below the x-axis of the figure.
the long-term survival during severe drought events (White et al. 2009) compared with E. marginata at sites with adequate root access. In the first substantial study of the physiological response of E. marginata to drought at restored bauxite mine sites, Bleby (2003) showed that 6–9-year-old saplings at a low-rainfall (~600 mm year⁻¹) site maintained similar minimum water potentials (~2.5 MPa) to those at a high-rainfall (~1200 mm year⁻¹) site (~2.7 MPa) during drought. In unmined forest, E. marginata saplings at low-rainfall (630–750 mm year⁻¹) sites recorded similar minimum Ψ to those at high-rainfall (1250–1350 mm year⁻¹) sites (~2.35 and ~2.46 MPa), while mature trees often recorded much higher Ψ (~1.40 to ~2.03 MPa) at high-rainfall sites compared with low-rainfall sites (~2.29 to ~2.95 MPa) (Colquhoun et al. 1994, Cribb 1992, 1997).

Although water content of soils at depth was not measured in this study, previous studies in the E. marginata forest have demonstrated that soil water stores at depth are at their maximum from August to November (Farrington et al. 1996). Over this period in the present study, E. marginata at the high-quality site showed stomatal closure over the day and a lower water status (midday Ψ) compared with the low-quality site. These results suggest that the higher stand density and growth rates at the high-quality site increased competition for water over the period of leaf expansion (Abbott et al. 1989), indicating that stand density (4264 stems ha⁻¹) was too high. A reduction in stocking has been recommended by Grant et al. (2007) for overstocked sites as a means of decreasing competition between trees, a strategy also recommended for E. globulus plantations in south-western Australia (White et al. 2009).

**Discussion**

**Key physiological differences between low- and high-quality restored sites**

The present results show clear physiological differences between trees at low- and high-quality restored bauxite mine sites. Plant water status and physiological functioning were substantially reduced at the low-quality site for both species during summer, most likely due to root systems being restricted to the top 0.5 m of the soil profile (Szota et al. 2007). This result suggests that a lack of access to moisture was responsible for poor growth at the low-quality site, and supports the suggestion by Cooke and Suski (2008) that physiological tools can be usefully applied to diagnose the nature of stressors in restored environments. In the unmined forest, mature trees survive the summer drought via access to moisture-holding kaolinitic clay subsoil (Carbon et al. 1980, Farrington et al. 1996). Previous studies have shown that plants with restricted root systems caused by mining-related earthworks are more susceptible to water stress and less productive (Enright and Lamont 1992, Varelides and Kritikos 1995, Ashby 1997, Rokich et al. 2001).

The severity of water stress at the low-quality site was substantially greater than previously reported for E. marginata (~3.2 MPa) in a high-rainfall zone (1243 mm year⁻¹), indicating an elevated level of risk to its long-term survival during severe drought events (White et al. 2009) compared with E. marginata at sites with adequate root access. In the first substantial study of the physiological response of E. marginata to drought at restored bauxite mine sites, Bleby (2003) showed that 6–9-year-old saplings at a low-rainfall (~600 mm year⁻¹) site maintained similar minimum water potentials (~2.5 MPa) to those at a high-rainfall (~1200 mm year⁻¹) site (~2.7 MPa) during drought. In unmined forest, E. marginata saplings at low-rainfall (630–750 mm year⁻¹) sites recorded similar minimum Ψ to those at high-rainfall (1250–1350 mm year⁻¹) sites (~2.35 and ~2.46 MPa), while mature trees often recorded much higher Ψ (~1.40 to ~2.03 MPa) at high-rainfall sites compared with low-rainfall sites (~2.29 to ~2.95 MPa) (Colquhoun et al. 1994, Cribb 1992, 1997).

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Key physiological differences between species in response to site conditions

Eucalyptus marginata and C. calophylla differed in their water status and physiological functioning during drought. Eucalyptus marginata maintained higher gs and A than C. calophylla for the majority of the year, especially over the dry season, a result that has also been recorded in unmined forest (Crombie 1992). This result is surprising as C. calophylla leaves are anatomically suited to having a higher photosynthetic capacity than E. marginata leaves, in that the leaves of C. calophylla are thicker, and have a higher proportion of mesophyll and a higher stomatal density per unit leaf area (Ridge et al. 1984). Growth rates are typically poorly correlated with photosynthetic rates and generally positively correlated with SLA (Lambers and Poorter 1992). However, in the absence of differences in SLA between the two species, higher photosynthetic rates over the year may contribute to the faster growth rates observed for E. marginata compared with C. calophylla.

Eucalyptus marginata operated at higher gs and A and at lower Ψ compared with C. calophylla. A number of studies have shown that E. marginata maintains lower Ψ than C. calophylla across a range of ages and size classes at both mined and unmined sites (Carbon et al. 1981, Colquhoun et al. 1984, Crombie et al. 1988, Crombie 1992); however, this difference has never been discussed in detail. At the height of drought in the present study, RWC fell to 73% for E. marginata and 80% for C. calophylla, which indicates that E. marginata leaf tissue has a higher tolerance to dehydration (Pook et al. 1966, Davidson and Reid 1989, Gulias et al. 2002). Maintenance of a lower Ψ may give E. marginata a greater ability to access soil moisture during drought compared with C. calophylla, which may be a contributing factor to its dominance in the forest. Superior exploitation of soil water resources can allow dominant eucalypts to out-compete subdominants during drought (Eberbach and Burrows 2006); however, this is unlikely in the present study, particularly at the low-quality site, where all
coarse roots were restricted to the top 0.5 m of the soil profile (Szota et al. 2007). The fact that *C. calophylla* leaves operated at lower $g_s$ and higher $\Psi$ and RWC under the same conditions in the field indicates that *C. calophylla* uses water more conservatively than *E. marginata* and therefore has an enhanced ability to survive extended periods of drought. The more conservative water use strategy of *C. calophylla* may also coincide with lower vulnerability of xylem vessels to cavitation (West et al. 2007, 2008). A more conservative water use strategy does not guarantee enhanced capacity to survive extended drought, as shown by piñon-juniper woodland species in the south-western USA, where the more conservative hydraulic strategy of *Pinus edulis* leads to carbon starvation compared with the less-conservative *Juniperus osteosperma* (Breshears et al. 2009).

Maintenance of higher water potentials during drought is often explained by higher stomatal sensitivity, primarily in response to high VPD and/or declining soil water status. In the present study, stomata of *E. marginata* and *C. calophylla* were insensitive to increasing VPD over the day when soil water availability was at its maximum in spring/early summer (Farrington et al. 1996). As $\Psi$ declined over summer, $g_s$ decreased in response to increasing VPD over the day, indicating that stomata remained responsive to VPD; however, $g_s$ was primarily governed by leaf water status (Mott and Parkhurst 1991, Sasse and Sands 1996, Bhaskar and Ackerly 2006, Flexas et al. 2006) in *E. marginata* and *C. calophylla*. This is a common trend in eucalypts from seasonally dry environments (Doley 1967, Carbon et al. 1981, Pereira et al. 1986, Prior et al. 1997, Faria et al. 1998, MacFarlane et al. 2004, Wildy et al. 2004, Drake et al. 2009). Stomatal conductance was lowest in late summer/autumn when VPD was lower than in mid-summer and water status was lowest (lowest $\Psi$ and RWC), which indicates that by this time $g_s$ was limited by low water availability at both sites and for both species. Although conductance was at its lowest late in the dry season, it remained sensitive to diurnal variation in VPD; however, it was much less sensitive than in mid-summer. Bleby (2003) showed, in 6–9-year-old *E. marginata* saplings at restored bauxite mines, that transpiration ($E$) was positively correlated with VPD to a point, presumably a soil-moisture threshold, after
which E ‘decoupled’ from VPD and declined linearly, irrespective of further changes in VPD. This response has also been demonstrated for E. marginata in the glasshouse (Stoneman et al. 1994) and in the field with 1–2-year-old seedlings (Stoneman et al. 1995) and in mature forest (Doley 1967, Crombie 1992). The declining morning gs late in the dry season coupled with stomatal closure earlier in the day at relatively low VPDs in the present study agrees with the observed linear decrease in E shown by Bleby (2003).

Stomatal sensitivity has previously been described by correlating stomatal conductance with water potential (Pereira et al. 1987, White et al. 2000, Brodribb and Holbrook 2003, Franks et al. 2007) or RWC (Gulías et al. 2002). In the present study, the slope of the relationship between gs and Ψ for E. marginata was similar to that of Eucalyptus camaldulensis, which White et al. (2000) considered to have stomata highly sensitive to changes in Ψ. This high stomatal sensitivity did not stop the development of low water potentials, which was also reported by Warren et al. (2007) for 7-year-old E. marginata and by Franks et al. (2007) for E. gomphocephala from the coastal plain of south-western Australia. In the present study, the correlation between gs and Ψ was poor for C. calophylla, which suggests that it has a low stomatal sensitivity to declining plant water status and may indicate the presence of an alternative mechanism for stomatal regulation, such as release of abscisic acid from roots (Davies et al. 1990, Davies and Zhang 1991). The slope of morning gs and RWC, however, was similar for E. marginata and C. calophylla, indicating that their stomata were equally sensitive to decreases in RWC, despite the fact that the RWC of E. marginata was 7% lower than that of C. calophylla at the peak of the dry season. High stomatal sensitivity is a trait typical of eucalypts from environments with high water availability. For example, at a low-rainfall (480 mm year−1) site, White et al. (2000) found that two low-rainfall zone eucalypts (Eucalyptus leucoxylon and Eucalyptus platypus) had lower stomatal sensitivities to declining leaf water status than the riparian E. camaldulensis. Eucalyptus pauciflora (Körner and Cochrane 1985), E. regnans (Ashton and Sandiford 1988) and E. nitens (White et al. 1996) from high-rainfall mountain ranges in south-eastern Australia, along with Eucalyptus grandis (Fan et al. 1994) and Eucalyptus cloeziana (Ngugi et al. 2003) from the moist subtropics of southern Queensland, all demonstrate strong stomatal sensitivity to leaf water deficits.

Stomatal sensitivity to leaf water potential is influenced by leaf cell-wall elasticity (White et al. 2000, Carter et al. 2006). Leaves with high cell-wall elasticity can effectively maintain turgor as leaf water content declines, because the concentration of their solutes increases as a consequence of the reduced cell volume (Zimmermann and Steudle 1978). In the present study, E. marginata leaves had rigid cell walls in winter (high Emin); however, their elasticity increased (Emin decreased) in response to drought, suggesting an enhanced ability to maintain turgor at low RWC and Ψ during drought (White et al. 2000). This finding has not been presented for E. marginata previously and does not agree with that of Stoneman et al. (1994), who showed that...

Figure 6. Relationship between morning stomatal conductance (gs) and midday water potential (Ψ; a and b), and average daily RWC (c and d) for E. marginata (jarrah) and C. calophylla (marri) from both low-quality (black) and high-quality (white) restored bauxite mine sites. Each point represents data captured for each month between November 2003 and March 2005. Data for April–September, 2004, are omitted to avoid the complication of low gs as a result of low morning vapour pressure deficits (VPD < 1 kPa) and/or temperature (<15 °C). Bars represent mean standard error (n = 3–18).
E. marginata seedlings subjected to drought in the glasshouse showed no change in cell-wall elasticity. Corymbia calophylla leaves in the present study were highly elastic at both measurement times. High cell-wall elasticity tends to be a feature of drought-tolerant rather than drought-avoiding eucalypts (Clayton-Greene 1983, Prior and Eamus 1999, White et al. 2000). It must be noted that leaf age has a bearing on comparisons between summer and winter (Prior and Eamus 1999); however, the similar TW:DW ratio between seasons for both species indicates that the leaves used were not significantly different in structure. Abbott et al. (1989) describes E. marginata as producing new leaves from naked buds in late winter that expand until early summer, and then mature and harden over the summer months; thus it is unlikely that major structural leaf changes took place between March and August when pressure–volume curves were derived.

Elastic adjustment may explain how E. marginata was able to tolerate lower \( \Psi \) and RWC over the dry season; however, it does not explain how C. calophylla was able to maintain a higher water status than E. marginata at both sites. Pressure–volume curves showed that elastic adjustments in E. marginata were not accompanied by accumulation of solutes, which is consistent with previous studies on E. marginata saplings in the field (Crombie 1997, Bleby 2003, Warren et al. 2007) but not with studies on seedlings subjected to high water deficits in the glasshouse (Stoneman et al. 1994). The relatively rapid onset and high severity of the drought stress applied to seedlings in the glasshouse by Stoneman et al. (1994) may explain why osmotic adjustment was not observed. In contrast to E. marginata, C. calophylla leaves had a significantly lower \( \Pi_0 \) in summer than in winter, indicating osmotic adjustment. Corymbia calophylla leaves had similarly elastic cell walls in winter and summer; therefore, the higher \( \Pi_1 \) in summer was unlikely to be due to an increase in cell-wall elasticity. The magnitude of osmotic adjustment in C. calophylla was similar to levels recorded for most eucalypts exposed to seasonal drought, including Eucalyptus tetrodonta (Prior and Eamus 1999), Eucalyptus behriana, Eucalyptus microcarpa (Clayton-Greene 1983) and E. nitens (White et al. 1996). Seasonal patterns in \( \Pi \) confirmed that C. calophylla showed lower osmotic potentials than E. marginata during drought. The combination of a low \( \epsilon_{\text{max}} \) and active accumulation of solutes may contribute to turgor maintenance as leaf RWC declines in C. calophylla, and therefore make it better able to survive periods of low water availability than E. marginata. This potential advantage of C. calophylla is not supported by a higher productivity; however, ability to survive drought is rarely positively correlated with tree size in eucalypts (Merchant et al. 2006). The present study is the first evidence of osmotic adjustment in C. calophylla; although many eucalypts from a range of environments have previously shown osmotic adjustment in response to drought (Clayton-Greene 1983, Tuomela 1997, Li 1998, White et al. 2000, Merchant et al. 2007, Arndt et al. 2008).

Conclusions

The present findings show that seasonal physiology of E. marginata and C. calophylla at restored sites is heavily influenced by site quality and that mechanisms for coping with drought are enhanced under adverse soil conditions. Corymbia calophylla leaves maintain lower stomatal conductance and photosynthesis at a higher water status during drought compared with E. marginata, indicating that they have a more conservative physiological response to drought that may improve their ability to survive. This ability appears to be linked to the ability of C. calophylla leaves to osmotically adjust, rather than the result of a higher stomatal sensitivity to VPD. Forest restoration managers may consider the option of increasing the proportion of C. calophylla in restored areas where water availability is perceived to be sub-optimal. With regard to the wider implications of this study, it is clear that co-occurring species within the same functional group can possess different physiological strategies for coping with resource limitation. Future work exploring the nature of these differences should be encouraged as more natural systems are disturbed and as resource availabilities change in response to short- and long-term shifts in climate.

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