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Anatomical variation in juvenile *Eucalyptus* leaves accounts for differences in specific leaf area and CO$_2$ assimilation rates

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Introduction

Eucalyptus L'Her. species occur in diverse habitats ranging from tropical to semi-arid Australia, and vary in their growth rate and productivity (Boland *et al.* 1984). Specific leaf area (SLA), the leaf area per unit leaf dry mass, is a key leaf attribute representing the trade-offs between resource acquisition and conservation strategies. Faster growing species tend to maximise light and CO$_2$ capture by having leaves with a high specific leaf area SLA (e.g. Atkin *et al.* 1996). However, a high SLA may be costly in resource poor environments where water and nutrient retention, and protection from herbivores are more important (Poorter and Remkes 1990). Seedling SLA may also be an important indicator of ecological strategy (Westoby 1998; Wilson *et al.* 1999). Species variation in SLA is not commonly examined in terms of both leaf anatomy and photosynthetic capacity, despite fundamental relationships between the effects of changing leaf anatomy on photosynthetic resource (light and CO$_2$) capture (Evans 1999). Variation in SLA resulting from changes in the proportion or arrangement of photosynthetic tissue should reflect photosynthetic characteristics of the leaf. We expected that *Eucalyptus* species of contrasting habitat and growth form would differ in SLA, and we tested the hypothesis that this variation was expressed as distinct leaf anatomy that has important implications for photosynthetic function of the leaf. The questions were addressed in three species; *E. grandis* W. Hill ex Maiden, *E. camaldulensis* Dehnh. and *E. occidentalis* Endl.

Materials and methods

Seedlings were grown in pots containing topsoil amended with nutrients. The four genotypes were arranged in a randomised block design and grown for 12 weeks in a naturally illuminated glasshouse (25°C/18°C day/night, average RH 48%) with adequate water.

At week ten, gas exchange characteristics, chlorophyll, leaf anatomy and SLA were determined on the youngest fully expanded leaf as follows. Maximum light saturated photosynthetic rates (A_{max}) were determined at 360 µL L^{-1} CO_2 (C_a) and 1200 µmol quanta m^{-2} s^{-1} with an open portable photosynthesis system (LI-6400, Li-Cor, USA). The maximal Rubisco carboxylation (Vc_{max}) and electron transport (J_{max}) rates were estimated using the model of von Caemmerer and Farquhar (1981). Instantaneous water use efficiency (WUE) was calculated as the ratio of A_{max} to transpiration. Chlorophyll was determined using a Minolta Chlorophyll Meter (SPAD 302). The size and proportion of cell layers was analysed by ImagePro computer software from one µm thick transverse sections of the leaf lamina.

Six replicates $genotype^{-1}$ were harvested 2, 4, 6, 8, 10, 12 weeks after transplantation. Dry mass of each plant fraction and leaf area was determined. Leaf nitrogen concentration (LNC) was determined by CHN-900 analyser (Leco, USA). Photosynthetic nitrogen use efficiency (A/N) ($µmol$ CO_2 $(mmol$ $N)^{-1}$ s^{-1}) was determined as the ratio of A_{max} and LNC of that leaf. Instantaneous relative growth rate (RGR; mg g^{-1} day^{-1}), leaf area ratio (LAR; cm^2 g^{-1}), and leaf nitrogen productivity (LNP; mg dry mass $(mmol$ $N)^{-1}$ day^{-1} were calculated at the common plant dry mass of 2.1 g.

Results

SLA varied between species due to differing numbers of palisade layers contributing to leaf thickness. Hence, the greater layering of photosynthetic tissue (palisade) in thick leaves increased leaf N concentration and A_{max} (Table 1).

Table 1. Variation in leaf anatomy and thus SLA, was due to different numbers of palisade layers, affecting leaf N concentration and A_{max}.

Superscripts indicate significant differences between species P<0.001

	E. grandis	*E. camaldulensis*	*E. occidentalis*
SLA (m^2 kg^{-1})	21.8[c]	17.6[b]	14.8[a]
No. palisade layers	1.0[a]	2.0[b]	3.7[c]
Leaf nitrogen (mmol m^{-2})	57.3[a]	95.3[b]	165.1[c]
A_{max} (µmol m^{-2} s^{-1})	13.7[a]	18.6[b]	25.8[c]

Fig. 1. The relationship of SLA and A/N in *E. grandis*, *E. camaldulensis* and *E. occidentalis* leaves.
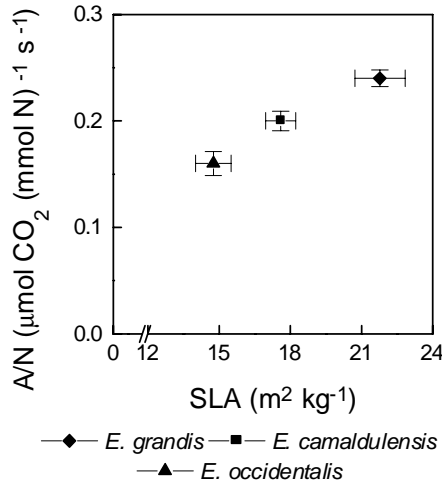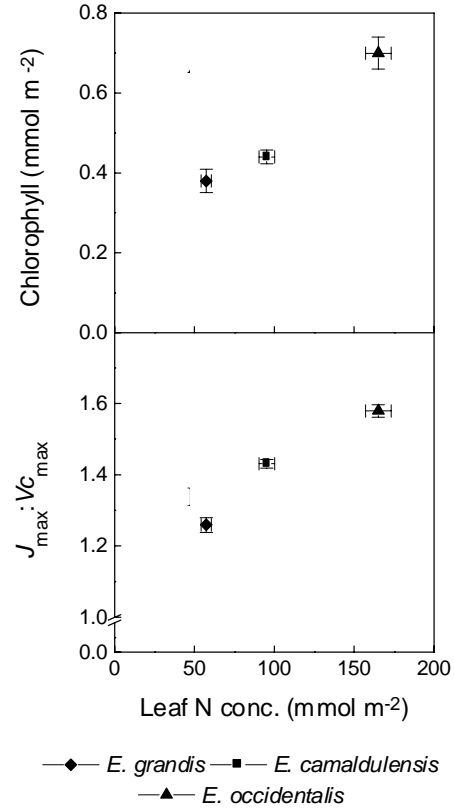
Values are means ± SE of 6 replicates.

Fig. 2. The relationship of leaf N concentration and chlorophyll concentration (A), and $J_{max}:Vc_{max}$ (B) in *E. grandis*, *E. camaldulensis* and *E. occidentalis* leaves.

Values are means ± SE of 6 replicates (A) or 3 replicates (B).

Fig. 1: A/N (μmol CO_2 (mmol N)$^{-1}$ s^{-1}) vs SLA (m^2 kg^{-1})
—◆— *E. grandis* —■— *E. camaldulensis*
—▲— *E. occidentalis*

Fig. 2: Chlorophyll (mmol m^{-2}) and $J_{max}:Vc_{max}$ vs Leaf N conc. (mmol m^{-2})
—◆— *E. grandis* —■— *E. camaldulensis*
—▲— *E. occidentalis*

Despite having more leaf N per leaf area, low SLA leaves (*E. occidentalis*) had reduced nitrogen use efficiency (A/N) (Fig. 1). Higher $J_{max}:Vc_{max}$ in *E. occidentalis* reflects greater allocation of resources to photosynthetic tissue with consequently greater chlorophyll concentrations (Fig. 2). Leaf scale nitrogen use efficiency indicated whole-plant nitrogen use efficiency and growth potential, while WUE increased as SLA decreased (Table 2).

Table 2. Resource use efficiency and RGR in *E. grandis*, *E. camaldulensis* and *E. occidentalis* seedlings.

Superscripts indicate significant differences between species P<0.01

	E. grandis	*E. camaldulensis*	*E. occidentalis*
WUE (μmol CO_2 (mol H_2O)$^{-1}$)	2.26[a]	2.74[b]	3.25[c]
RGR (mg g^{-1} day^{-1})	111[b]	114[b]	76[a]
LNP (mg (mmol N)$^{-1}$ day^{-1})	182[b]	205[b]	121[a]
Leaf area (cm^2)	4019[c]	2546[b]	665[a]

Discussion

SLA was a key attribute determining photosynthetic capacity at both leaf- and canopy-scale of three *Eucalyptus* species from contrasting habitats. Consistent with our results, Garnier *et al.*(1997) found that between closely related species leaf N concentration decreases with increasing SLA. This is because SLA varied due to changes in photosynthetic tissue (high N) rather than structural tissue (low N). Leaf anatomy thus contributed to the efficiency of N use. High chlorophyll concentration associated with more palisade should not result in a proportionally large increase in light absorptance (Evans 1996). Therefore, the large difference in chlorophyll concentration between *E. occidentalis* and *E. grandis* leaves (84%) did not proportionally increase light absorptance. Hence, excess chlorophyll in *E. occidentalis* contributed to its low N use efficiency. Although A/N was reduced in thicker leaves, WUE was increased clearly as a result of higher A_{max}, but also probably due to reduced C_i. Large leaf area offset reduced A_{max} in thin, palisade-poor *E. grandis* leaves, permitting faster growth. This strategy is advantageous for seedlings colonising gaps in mesic forests where water is not limiting. *E. occidentalis* leaves had typical sun-leaf anatomy that reflects adaptation to high-light and low-water environments characteristic of this woodland species.

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