Relationships between growth, specific leaf area and water use in six populations of *Eucalyptus microtheca* seedlings from two climates grown in controlled conditions

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Summary

To gain a better understanding of those parameters that control growth and productivity of *Eucalyptus microtheca* populations from seasonally dry to semi-arid sites in Australia we measured their total plant biomass, allocation of dry matter to roots and shoots, specific leaf area (SLA) and water use. For this purpose the seedlings were raised up to five months under three different pot moisture conditions applied by cyclical watering regimes. Young seedlings of six provenances of *Eucalyptus microtheca* fell generally into two groups, the Rockhampton, Walgett and Maree provenances which evolved in evenly distributed, though erratic, rainfall conditions at semi-arid sites, and the Pilbara, West Kimberleys and Camooweal provenances which evolved in seasonally dry sites with a reliable monsoon. The former group had the greater leaf area and total plant biomass. Specific leaf area correlated positively with leaf area and total plant biomass at various times across the experimental period. However, as only minor differences in the average plant water use and carbon discrimination were detected among the populations, greater biomass production in semi-arid populations appears to stem from their capacity to produce a large total leaf area with thin leaves, which thus contributes to the total plant photosynthesis.

The root:shoot ratio was consistently higher in seedlings from seasonally dry sites compared to those from semi-arid sites. A higher ratio in seasonally dry sites is likely to be beneficial as it allows an adequate water supply for longer during the dry period. A greater allocation to roots apparently reduces the amount of leaf area produced, decreasing growth potential.

Key words: Biomass growth, *Eucalyptus microtheca*, root:shoot ratio, specific leaf area, water use

Introduction

Eucalyptus microtheca F. Muell., coolibah, occurs along water courses and on flood plains throughout seasonally dry and semi-arid Australia. The species has been planted in tropical drylands in Africa and the Middle East for fuel, poles, shade and fencing. (NAS 1980; FAO 1981; Armitage 1985). Results from the International Provenance Trial of *E. microtheca* launched in late 1970s by FAO have shown that populations from semi-arid sites are more productive than those from seasonally dry sites, whatever the water regime (Schiller 1995; Johansson and Tuomela 1996; Mustafa pers. comm. 1997). For example, in an irrigated plantation in Kenya, the MAI of tree populations from semi-arid sites is about three times higher than those from seasonally dry sites.

Seedlings and trees from semi-arid sites have more negative xylem leaf water potentials compared to those from seasonally dry sites (Tuomela 1993; Tuomela and Kanninen 1995) but they have less capacity for osmotic adjustment in response to water deficit (Tuomela 1997). Although these differences likely reflect differences in the climates to which populations are adapted, it is not clear how they are linked with processes that control productivity. In recent studies specific leaf area (SLA) is found to be closely linked with productivity in a range of species (Reich *et al*. 1997; Atkin 1998).

In this study we measured the total plant biomass growth, allocation of dry matter to roots and shoots, SLA, water use and long term water use efficiency (Δ) of *Eucalyptus microtheca* seedling from seasonally dry to semi-arid sites in Australia to gain a better understanding of those parameters that control their growth. For this purpose, the seedlings were raised up to five months under three different pot moisture conditions applied by cyclical watering regimes.

Material and method

Table 1 and Figure 1 give the geographic and climatic data for three sites in semi-arid Australia, Rockhampton, Walgett and Maree, and three in seasonally dry Australia, Pilbara, West Kimberleys and Camooweal. The seasonally dry sites have higher mean annual maximum temperatures (33 to 36°C) than the semi-arid sites (27 to 28°C). Monthly summer temperatures are between 35 and 40°C at all sites. Monthly winter temperatures are lower at the semi-arid sites (20 to 25°C) than at the seasonally dry sites (25 to 30°C).

Rainfall ranges from 380 to 517 mm per annum at the seasonally dry sites; it is summer dominant with nil to minimal rain in the winter and early spring. There is a much wider rainfall range at the semi-arid sites (196 mm per annum at Marree, 469 mm at Walgett and 961 mm at Rockhampton). Rainfall is more or less evenly distributed at Marree and Walgett although there is a tendency towards more winter rain at the former and summer rain at the latter. While rainfall at Rockhampton is summer dominant, some rain falls during the rest of the year (5 to 15 mm per month).

Table1. Origin of six provenances of *Eucalyptus microtheca* used in the study (Climatic Averages Australia, 1998)

Locality	Latitude	Longitude	Annual rainfall (mm)	Mean annual maximum temperature (°C)	Mean annual humidity (%)
Pilbara (WA)	22° 40'	118° 05'	394	32.7	22
West Kimberleys (WA)	18° 00'	125° 00'	517	36.0	27
Camooweal (NT)	19° 40'	135° 15'	380	33.2	24
Marree (SA)	28° 30'	136° 40'	196	28.6	26
Walgett (NSW)	29° 20'	148° 35'	469	27.2	37
Rockhampton (Qld)	23° 22'	150° 31'	961	28.2	47

Note: SA, South Australia;WA, Western Australia; NT, Northern Territory; NSW, New South Wales; Qld, Queensland. Seedlot codes and the number of mother trees (in parentheses) for the studied populations: Pilbara, 15070 (8), West Kimberleys, 15073 (8), Camooweal, 15075 (11), Marree, 15077 (20), Walgett, 15084 (15) and Rockhampton, 15944 (not available).

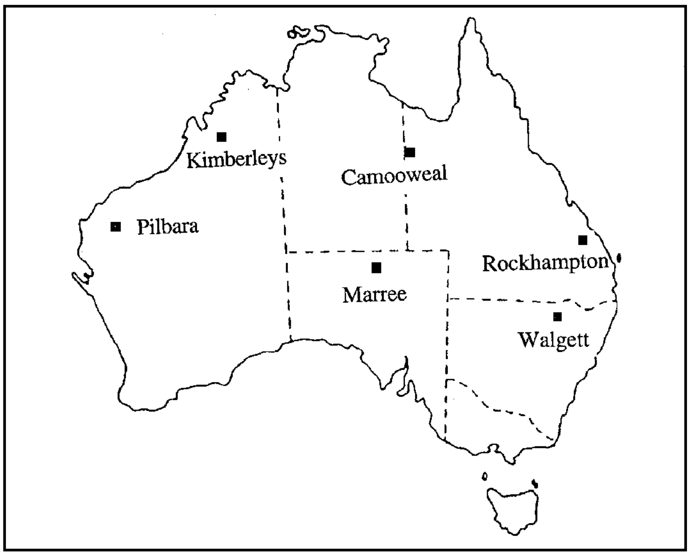

Figure 1. Location of six seed sources of *Eucalyptus microtheca*. Rockhampton, Walgett and Marree are semi-arid sites. Pilbara, Kimberley and Camooweal are seasonally dry sites.

Thus *E. microtheca* has a particularly wide environmental range in essentially dry areas of the continent.

Seed from each provenance was germinated on wet tissue paper, and the germinants were transferred to plastic pots. At four weeks, 45 seedlings of each provenance were transplanted separately into commercial peat-sand mixture packed at about 0.4 g cm^{-3} in 2L pots. One m^3 of substrate contained 0.7 kg fertilizer (10% N, 8% P, 16% K) and 8 kg Mg-rich limestone powder. The pots were in a naturally lit glasshouse in Helsinki in which the temperature (minimum temperature set at 17°C) and vapour pressure deficit fluctuated with the weather over the five months experimental period as shown in Figure 2. Temperature and relative humidity were recorded daily at 14:00 h and vapour pressure deficit was calculated using the model presented by Dilley (1968).

All pots were watered to field capacity, determined gravimetrically, for one month after which the three watering treatments were applied. For the seedlings in the control treatment the average water consumption was assessed from five seedlings per provenance. In the control treatment, pots were watered to field capacity at dusk every two days. Pots in the other treatments (50% and 25%) received one half or one quarter of the volume of water required to bring the control pots of each provenance to field capacity on the two-day cyclical watering regime. The seedlings were fertilised with a 0.3%

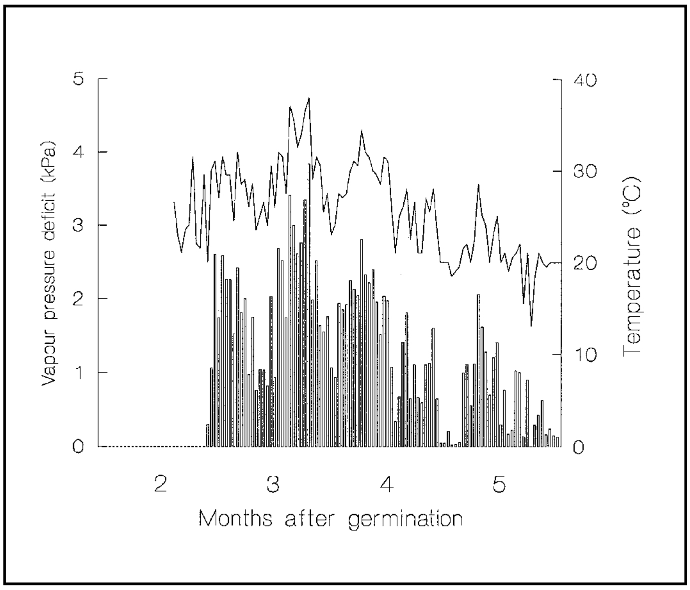

Figure 2. Daily temperature (solid line) and calculated vapour pressure deficit (bars) at 14:00 h in the greenhouse during the experiment. June 28 to October 28 1995

solution (Kekkila Superx-10; 7% N, 5% P, 26% K) twice during the experiment. Pesticides (Applaud, Pirimor and Vertimec) and fungicides (Benlate and Saprol) were used as necessary.

The layout was a completely randomised design with two factors using six provenances and three watering treatments (270 seedlings in total). Each watering treatment consisted of 15 seedlings per provenance and the pots were circulated twice during the experimental period, 9 June – 8 October 1995.

Growth measurements

Four seedlings in each provenance and watering treatment were harvested when three months old. Measurements were made of height, xylem (sapwood) diameter above root collar, total leaf area using a Li-COR 3000A and total leaf, shoot and root (>2 mm diameter) dry weights after drying for 16 h in an oven at 18°C. These measurements were repeated for three and two seedlings from each provenance and watering treatment when four and five months old respectively.

Carbon isotope discrimination

Two to three mature leaves of five-month-old seedlings in each provenance and watering treatment were oven dried for 24 h at 70°C, ground in a ball mill and combusted with copper oxide (Cu(II)O) at 550°C. The CO_2 formed was purified cryogenically and the carbon isotope composition relative to VPDB standard was determined with a mass spectrometer (Finnegan MAT Delta-E) by the Radio Dating Laboratory, University of Helsinki. The obtained δ -values were used to determine Δ. The overall precision in δ -values was better than 0.1% determined by repetitive samples.

Statistical analyses

Analysis of variance was used to test the effects of watering treatment and provenance on the measured variables. The relationships among the average water use (for the control), the total plant biomass, and leaf area were analysed by linear regression analysis.

Results

Growth of seedlings from all provenances was largest in the well-watered control treatment. Whilst growth in height was similar in all provenances, seedlings from the semi-arid provenances – Rockhampton, Maree and, to a lesser extent, Walgett - produced significantly larger biomass at all levels of water than seedlings from the seasonally dry provenances – Pilbara, Western Kimberleys and Camooweal - shown inTable 2 for the five-month-old seedlings. At three months of age, differences were not significant and, at four months, they were as for the five-month-old seedlings (data not shown).

While total leaf area increased in all provenances with increasing water (Table 2), differences in mean leaf size (Table 3) and in SLA (Table 2) were not related to watering treatments. Seedlings from the semi-arid sites had the smallest mean leaf sizes and largest SLAs, while those from the seasonally dry sites had larger mean leaf sizes and smaller SLAs. Overall, SLA decreased with decreasing water supply.

Table 2. Means and standard errors (in parentheses) for growth traits of six provenances of *E. microtheca* at five months of age (n=2). Sites listed with the same letter are not statistically significant at the 5% level

Site	Watering treatment	Total plant biomass (g)	Leaf area (m^2)	Specific leaf area ($m^2\ kg^{-1}$)	Height (m)
Pilbara	25%	5.50 (1.39)	0.033 (0.009)	10.88 (0.72)	0.38 (0.02)
	50%	9.00 (0.56) B	0.045 (0.002) B	9.62 (0.03) AB	0.75 (0.09) A
	Control	11.50 (3.65)	0.061 (0.017)	10.90 (0.35)	0.92 (0.09)
West Kimberleys	25%	5.21 (1.10)	0.026 (0.005)	8.02 (0.02)	0.44 (0.14)
	50%	9.00 (0.40) B	0.037 (0.002) B	7.09 (0.10) B	0.53 (0.03) A
	Control	13.17 (-)	0.078 (-)	10.87 (-)	0.60 (-)
Camooweal	25%	5.14 (1.90)	0.025 (0.006)	7.76 (0.69)	0.35 (0.08)
	50%	8.99 (0.19) B	0.050 (0.002) B	8.66 (0.65) B	0.57 (0.09) A
	Control	19.20 (0.08)	0.103 (0.006)	9.23 (0.56)	0.71 (0.09)
Marree	25%	6.36 (0.01)	0.034 (0.002)	9.55 (0.76)	0.45 (0.03)
	50%	16.42 (1.67) A	0.073 (0.008) A	7.94 (1.19) B	0.56 (0.09) A
	Control	30.84 (2.57)	0.142 (0.013)	10.46 (0.74)	0.88 (0.12)
Walgett	25%	6.04 (0.15)	0.029 (0.002)	10.16 (0.29)	0.37 (0.07)
	50%	9.67 (2.38) AB	0.053 (0.007) AB	12.62 (1.97) A	0.67 (0.14) A
	Control	24.5 (1.20)	0.124 (0.013)	11.66 (1.97)	0.98 (0.06)
Rockhampton	25%	6.42 (0.27)	0.039 (0.003)	12. 18 (1.18)	0.47 (0.07)
	50%	13.48 (1.00) A	0.073 (0.002) A	11.93 (0.32) A	0.72 (0.04) A
	Control	32.54 (10.8)	0.154 (0.036)	11.95 (1.51)	1.15 (0.10)

Root/shoot ratios increased with decreasing water in all provenances, with the ratio being smallest in the semi-arid provenances. Statistical differences in leaf area: sapwood ratios could not be ascribed to provenances or watering treatments (Table 3).

Average daily water consumption increased similarly in all provenances with increasing total biomass (r^2=0.58, p=0.001) (Fig. 3) and leaf area (Fig. 4). Water use efficiency (Δ) increased with increasing water in all provenances (Table 3).

There were significant positive relationships, at 3 and 4 months of age, between SLA and leaf area (r^2=0.50, p=0.001; r^2=0.52, p=0.001) and between SLA and total plant biomass (r^2=0.21, p=0.053; r^2=0.39, p=0.006) with a less significant relationship at 5 months.

Table 3. Means and standard errors (in parentheses) for morphological traits of six provenances of *E. microtheca* (n=9). Sites listed with the same letter are not statistically significant at the 5% level

Site	Watering treatment	Leaf size (cm^2)	Leaf area :sapwood area ratio ($m^2\ cm^{-2}$)	Root/shoot -ratio ($g\ g^{-1}$)	Δ (‰)*
Pilbara	25%	10.5 (1.9)	0.39 (0.03)	0.81 (0.08)	19.3 (0.4)
	50%	11.2 (1.6) B	0.37 (0.02) AB	0.68 (0.06) B	20.6 (0.7) A
	Control	9.6 (0.9)	0.38 (0.05)	0.64 (0.08)	22.9 (0.5)
West Kimberleys	25%	17.0 (2.6)	0.39 (0.04)	0.93 (0.11)	18.6 (0.3)
	50%	18.2 (1.4) A	0.37 (0.03) B	0.91 (0.07) A	19.5 (0.3) A
	Control	17.1 (1.8)	0.49 (0.05)	0.58 (0.04)	22.6 (0.4)
Camooweal	25%	9.7 (1.2)	0.48 (0.05)	0.74 (0.06)	18.8 (0.4)
	50%	23.9 (8.0) A	0.50 (0.06) C	0.63 90.06) B	19.9 (0.4) A
	Control	12.6 (1.2)	0.46 (0.03)	0.52 (0.03)	22.3 (0.4)
Maree	25%	10.8 (2.9)	0.50 (0.03)	0.56 (0.06)	18.8 (0.5)
	50%	9.1 (1.1) B	0.37 (0.04) B	0.42 (0.03) C	19.8 (0.2) A
	Control	13.3 (1.9)	0.37 (0.03)	0.48 (0.04)	22.7 (0.2)
Walgett	25%	5.5 (1.3)	0.31 (0.02)	0.73 (0.09)	18.9 (0.4)
	50%	7.9 (2.1) B	0.31 (0.03) A	0.58 (0.02) C	21.0 (0.9) A
	Control	9.2 (1.6)	0.36 (0.03)	0.42 (0.03)	22.9 (0.5)
Rockhampton	25%	6.1 (1.1)	0.34 (0.03)	0.56 (0.03)	18.5 (0.3)
	50%	6.7 (0.4) B	0.43 (0.05) B	0.45 (0.04) C	19.2 (0.2) A
	Control	11.0 (1.2)	0.41 (0.05)	0.48 (0.04)	22.5 (0.4)

Note: * (n=5)

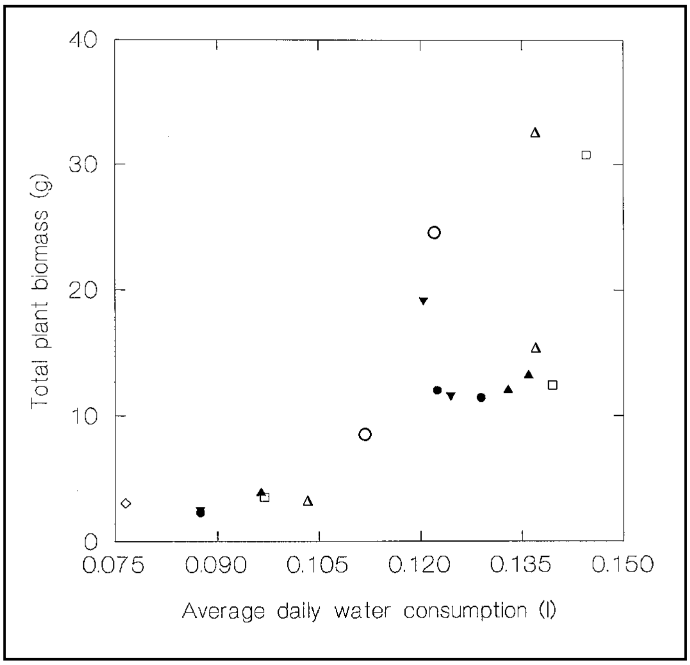

Figure 3. The relationship between total plant biomass (g) and the average daily water consumption of the control plants in six provenances of *E. microtheca*: Rockhampton (Δ), Walgett (❍), Maree (❑) Camooweal (▼), West Kimberlies (▲) and Pilbara (●). Solid symbols refer to provenances from seasonally dry sites and open symbols to those from semi-arid sites.

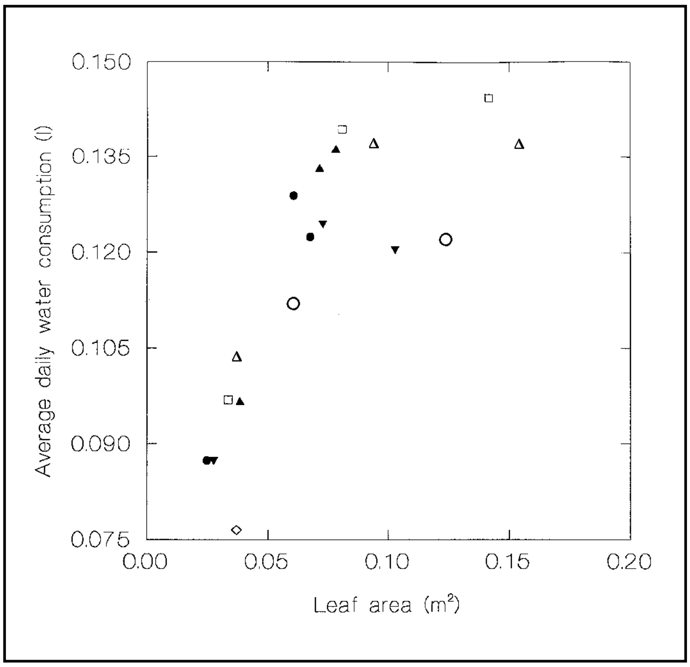

Figure 4. The relationship between the average daily water consumption (L) of the control plants and leaf area (m^2) in six provenances of *E. microtheca*: Rockhampton (Δ), Walgett (❍), Maree (❑), Camooweal (▼), West Kimberlies (▲) and Pilbara (●). Solid symbols refer to provenances from seasonally dry sites and open symbols to those from semi-arid sites

Discussion

Young seedlings of six provenances of *Eucalyptus microtheca* tested in this study fell generally into two groups. The Rockhampton, Walgett and Maree provenances which evolved in evenly distributed, though erratic, rainfall conditions at semi-arid sites, had greater leaf area and total plant biomass than the Pilbara, West Kimberleys and Camooweal provenances which evolved in reliable monsoon conditions at seasonally dry sites (Table 2). These results are consistent with the findings of *E. microtheca* provenance variation in plantations overseas (Schiller 1995; Johansson and Tuomela 1996). The average plant water use explained rather well the total plant biomass, which was apparently related to leaf area development (Figs 3 and 4).

SLA correlated positively with leaf area, significantly at 3 and 4 months, which is in agreement with other studies of woody species (Saverimuttu and Westoby 1996; Cornelissen 1997). Positive correlation between the growth rate and SLA is known in a range of species (Reich *et al.* 1997). Atkin *et al.* (1998) found that the low SLA of *Acacia* species from dry sites was a prime factor in their slow growth compared to those from more mesic sites in Australia. Lambers and Poorter (1992) suggested that a lower SLA of slow-growing species results from a relatively thick cell structure and high concentration of secondary compounds, leading to a lower photosynthetic rate per unit leaf dry weight. They proposed that a lower SLA, mainly due to secondary compounds, reflects the need for protection against detrimental abiotic and biotic factors. We did not find large differences in average water use, nor in Δ, among the populations despite distinct differences in their SLA, biomass and leaf area. Therefore, greater biomass in semi-arid populations may stem from higher total plant photosynthesis due to greater leaf area.

Although increasing in water deficit, root:shoot ratio was consistently higher in seedlings from seasonally dry sites compared to those from semi-arid sites (Table 3). A higher root:shoot ratio in seasonally dry sites is likely to be beneficial as it allows an adequate water supply longer during the dry season. A greater allocation to roots apparently reduces the amount of leaf area produced and thus decreases the total photosynthesis and growth potential.

Despite differences in the leaf area:sapwood area ratio among provenances, we did not find a consistent trend in the ratio between the seedlings from semi-arid and seasonally dry sites. In general, this ratio depends on vapour pressure deficit at a site, and thus tends to decrease towards drier sites since trees avoid damaging effects of low water potentials by reducing leaf area (Whitehead *et al.* 1984; Mencuccini and Grace 1993). As the ratio is environmentally determined, higher leaf area:sapwood ratios in these seedlings from seasonally dry sites, classified drier sites based on their annual vapour pressure deficit, appear to reflect their intrinsically slower growth.

The average leaf size, largely independent of water level, increased from semi-arid to seasonally dry sites, supporting the results of Tuomela and Gibson (unpubl. data) from native sites of *E. microtheca* populations. Although our results indicate that the average leaf size is to a large extent under genetic control, it is not clear how thicker and larger leaves reflect adaptation to alternating rainy and hot, dry seasons at seasonally dry sites, and smaller and thinner leaves reflect adaptation at semi-arid sites where rainfall is more evenly distributed but erratic and winters are cooler.

Fast early growth seems to relate to differences in carbon allocation patterns in young seedlings of *E. microtheca* provenances. Seedlings from seasonally dry sites had a bigger leaf size and a lower SLA and they invested more carbon to roots. These adaptations appear to result in slower innate growth and reflect their low productivity in plantations overseas compared to those from semi-arid sites.

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