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GROWTH RESPONSE OF *EUCALYPTUS MICROTHECA* PROVENANCES TO WATER STRESS

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LI, C. 1998. Growth response of *Eucalyptus microtheca* provenances to water stress. Growth variation was observed under three watering regimes in a greenhouse experiment among four *Eucalyptus microtheca* provenances whose natural habitats represented a gradient of different climatic conditions. Compared with provenances from northern and central Australia, southeastern Australian provenances showed a faster early seedling growth in the different treatments. There were significant correlations between shoot biomass, total biomass or total leaf area and driest-quarter rainfall of the natural habitats of the provenances. The pattern of shoot height growth for each provenance in relation to the watering regime indicated a linear regression in the control treatment, whereas the growth response followed a curvilinear regression under water stress. In addition, statistically significant differences in leaf stomatal length were detected between the provenances. These results were interpreted as suggesting that there are different adaptive strategies in response to water deficit among the provenances of this eucalypt species which are manifested when the provenances are grown outside their natural Australian range. It is suggested that the northern and central provenances would not be able to take advantage of favourable hydrological conditions, to the extent the southeastern Australian provenances would do in a similar situation.

Key words: Early seedling growth - *Eucalyptus microtheca* - provenance variation - water stress

LI, C. 1998. Tindak balas pertumbuhan provenans *Eucalyptus microtheca* terhadap tekanan air. Variasi pertumbuhan dicerap di tiga regim penyiraman dalam uji kaji di rumah hijau bagi empat provenans *Eucalyptus microtheca* yang habitat semula jadinya mewakili kecerunan pada keadaan iklim yang berbeza. Provenans dari tenggara Australia menunjukkan pertumbuhan awal anak benih yang lebih cepat dalam rawatan yang berbeza berbanding dengan provenans dari bahagian utara dan tengah Australia. Terdapat korelasi yang bererti di antara biojisim pucuk, jumlah biojisim atau luas kawasan daun dan bahagian hujan paling sedikit bagi habitat semula jadi provenans tersebut. Kaitan pola pertumbuhan dan ketinggian pucuk setiap provenans dengan regim penyiraman menandakan regresi linear dalam rawatan yang dikawal, manakala tindak balas pertumbuhan mengikuti regresi lengkung linear di bawah tekanan air. Di samping itu, panjang stomata daun berbeza dengan bererti di antara provenans. Keputusan ini mengesyorkan bahawa terdapat perbezaan strategi penyesuaian dalam tindak balas terhadap kekurangan air dengan provenans spesies eucalypt ini yang dibuktikan apabila provenans ini ditanam di luar kawasan semula jadi Australia. Disyorkan supaya provenans di kawasan utara dan kawasan tengah tidak dapat mengambil kesempatan daripada keadaan hidrologi yang menguntungkan sehinggalah provenans di tenggara Australia melakukannya dalam situasi yang serupa.

Introduction

Different eucalypt species in Australia occur over a wide variety of habitats and thus obviously reflect adaptation to different environments. Within a given eucalypt species there also seems to be genetically controlled differentiation among populations or ecotypes in relation to the environment (Gibson *et al.* 1991, 1994). In particular, site variation related to soil moisture may contribute to the genetic differentiation of eucalypt species and populations, since rainfall is the most distinctly varying environmental factor within the distribution range of eucalypts in Australia.

Plant growth characteristics are indicators of adaptation to local conditions. Seedling establishment and stand development in a dry environment require specific adaptation mechanisms in the plant which enable the growth to continue under limited water availability (Turner & Kramer 1980, Kramer 1983, Dickmann *et al.* 1992, Tuomela 1997). The variation in the mechanisms of drought adaptation and in water-use strategies has been studied among and within different species of eucalypts (Pereira & Kozlowski 1979, Sinclair 1980, Bachelard 1986a, 1986b, Myers & Neales 1986, Wang *et al.* 1988, Davidson & Reid 1989, Myers & Landsberg 1989, Lemcoff *et al.* 1994). However, the provenance variation in early seedling growth under contrasting water regimes has been relatively little studied.

Eucalyptus microtheca F. Muell. (coolibah) has a wide geographic range, mainly within the arid and semi-arid zones of Australia. It has been successfully grown in plantations in dry tropical and subtropical regions. It can tolerate heavy-textured, calcareous and gypseous soils, and has grown well in the Sudan (Mustafa 1989), Israel (Schiller 1995) and Kenya (Johansson & Tuomela 1996). In these countries, its main use is for fuel and poles, shelterbelts and soil stabilisation.

The aim of this investigation was to study the main seedling growth characteristics during the initial phase of seedling development in some provenances of *E. microtheca* under water stress. A specific aim was to develop methods for the selection of suitable genotypes of this species for growing under different hydrological conditions. Particular attention was given to finding traits that are as simple as possible for such screening.

Materials and methods

Seeds

Four provenances of *Eucalyptus microtheca* were selected for the study (Table 1). Seeds were obtained from the Australian Tree Seed Centre, CSIRO, Division of Forest Research, Canberra. All seeds were stored in air-tight containers.

Table 1. Origin of four *Eucalyptus microtheca* provenances (with seedlot numbers indicated) used in the study

Provenance	Parent trees	Locality [1]	Mean annual rainfall (mm)	Driest-quarter rainfall (mm)	Mean annual maximum temperature (°C)	Mean annual minimum temperature (°C)
15074	10	Newcastle Waters (NT)	494	8	34.2	19.5
15076	16	Central Austral. (NT)	263	27	28.7	13.8
15081	20	South West Qld. (QLD)	360	57	28.2	14.3
15085	8	Western NSW (NSW)	233	52	23.7	12.0

[1]NT, Northern Territory; QLD, Queensland; NSW, New South Wales.
Climatic data from CSIRO (1979).

Growth conditions and experimental design

Seeds of each provenance were sown in Helsinki, Finland, on wet tissue paper in Petri dishes. After germination, the seedlings were transplanted into small plastic pots (180 cm^3); the pots were kept wet by daily watering. After one month, the seedlings were transplanted into 2-liter pots. A commercial peat-sand mixture was used as growth medium. One cubic metre of this mixture contained 0.7 kg of fertiliser (10% N, 8% P and 16% K) and 8 kg of Mg-rich limestone powder. The substrate was packed into each pot with a density of about 0.4 g cm^{-3}. The seedlings were grown in a greenhouse within a temperature range of 17.0 - 35.0 °C, according to the prevailing weather conditions.

For field capacity measurement, the 2-litre pots were thoroughly watered and immersed partially in a water basin. On the following day, the pots were removed from the water basin and allowed to drain. At this stage, the pots were assumed to be at field capacity and weighed.

A completely randomised design with two factors (four provenances: 15074, 15076, 15081 and 15085; and three watering regimes) was used. For each provenance, there were 45 seedlings equally divided among 3 watering regimes, i.e. control treatment (100% field capacity), and water stress treatments (50% and 25% of field capacity respectively). One seedling was planted in each pot, giving a total of 180 seedlings.

A two-day watering cycle was applied throughout the experiment. To determine the amounts of added water, five randomly selected pots from the control treatment were weighed every second day to estimate the average water loss for each provenance. In the control and the two water stress treatments, 100%, 50% and 25% of this average water loss were compensated for respectively.

Seedling measurements

The seedlings were measured for shoot height every two weeks until the end of the experiment. At harvest (after five months), the seedlings were measured for shoot height, basal diameter, leaf shape (length/width), total leaf area, leaf, stem and root fresh and dry weights, and specific leaf area (total leaf area/leaf dry weight). The total leaf area was determined with a LI-COR 300A device. For stomatal analysis, the fifth leaf counted from the tip of the stem was sampled for five randomly selected seedlings per provenance and watering treatment at the age of three months. The samples were taken using colourless nail varnish and analysed under a light microscope. The stomatal index was calculated by multiplying the average stomatal frequency and length to estimate the total stomatal pore area per unit leaf area exposed to gas diffusive processes.

Analysis of data

Data were subjected to analysis of variance, and Tukey's test was used to detect differences between the provenances. The relationships between early seedling growth and climatic data of the natural habitats of the provenances studied were determined using correlation analysis. The models of shoot height growth were built with regression analysis. Statistical analyses were done with the SYSTAT statistical software package.

Results

At harvest, shoot biomass (Sb), total biomass (Tb), leaf area (La) and specific leaf area (Sl) in the southeastern provenances (No. 15081 and 15085) were larger than those in the northern or central provenances (No. 15074 and 15076) in all comparable treatments, indicating a higher inherent growth rate in the southeastern provenance seedlings (Table 2). In all provenances, the early seedling growth decreased significantly under water stress ($p<0.01$). The watering x provenance interaction was significant ($p<0.05$) in Sb, Tb and La. However, leaf shape was not affected significantly by watering.

Correlations between early seedling growth and climatic data (mean annual rainfall, MAR; driest-quarter rainfall, DQR; mean annual maximum temperature, MAXT; mean annual minimum temperature, MINT; and mean annual raindays, RDAY) of the natural habitats of the provenances were studied, and it was found that there were significant correlations mainly between Sb, Tb or La and DQR in all treatments (Table 3).

The shoot height growth in eucalypt seedlings indicated different models under three different watering regimes; this variation was similar in all four provenances. In the control treatment (100% field capacity), the model of height growth was described by a linear equation ($y = a+bx$), but in the water stress treatments (50% or 25% of field capacity), the model was better described by a curvilinear regression ($y = a+b\ln x*\ln x$) (Table 4).

Stomatal characteristics were also studied under different watering regimes in the four provenances (Table 5). There were no statistically significant overall differences between the provenances in stomatal frequency or stomatal index (total stomatal length per unit leaf area), whereas such differences were confirmed in stomatal length. The effect of watering on stomatal characteristics was significant ($p<0.05$). In contrast to the case of biomass or leaf area, no watering x provenance interaction was found in the stomatal characteristics.

Table 2. Early seedling growth characteristics (means and standard errors) in four *Eucalyptus microtheca* provenances as affected by three watering regimes (25%, 50% and 100% water loss compensation)[1)]

Provenance	Watering (%)	Shoot biomass (g)	Total biomass (g)	Leaf area (dm^2)	Specific leaf area (dm^2 g^{-1})	Leaf shape (length/width)
15074	25	2.64(0.10)	4.94(0.22)	2.46(0.10)	1.37(0.02)	4.44(0.11)
	50	5.87(0.11) A	10.56(0.29) A	4.82(0.08) A	1.54(0.01) A	4.34(0.08) A
	100	9.38(0.15)	15.94(0.36)	7.29(0.18)	1.66(0.01)	4.49(0.13)
15076	25	3.29(0.06)	5.49(0.18)	2.59(0.11)	1.43(0.07)	3.75(0.08)
	50	7.92(0.15) B	12.87(0.17) B	5.95(0.05) B	1.56(0.02) AB	3.82(0.08) B
	100	12.31(0.31)	18.32(0.21)	9.12(0.41)	1.67(0.01)	3.63(0.08)
15081	25	4.51(0.11)	6.80(0.12)	3.73(0.22)	1.42(0.01)	3.37(0.10)
	50	10.11(0.13) C	15.43(0.19) C	8.06(0.07) C	1.63(0.02) BC	3.26(0.08) C
	100	16.08(0.31)	22.57(0.32)	11.32(0.43)	1.71(0.01)	3.16(0.16)
15085	25	4.75(0.07)	6.91(0.10)	3.36(0.07)	1.43(0.01)	3.49(0.09)
	50	10.55(0.13) C	15.35(0.28) C	8.21(0.17) C	1.67(0.01) C	3.28(0.08) C
	100	17.25(0.32)	24.45(0.49)	12.52(0.45)	1.74(0.01)	3.26(0.09)

[1)] Capital letters refer to differences between the provenances; values followed by the same letter(s) are not statistically different at $p<0.05$.

Table 3. Correlations between early seedling growth and climatic indicators of the natural habitats of four *Eucalyptus microtheca* provenances. Correlation coefficients are shown in bold, p values in italics type

Watering	Seedling traits	MAR	DQR	MAXT	MINT	RDAY
25%	Sb	**- 0.642**	**0.979**	**- 0.888**	**- 0.821**	**- 0.334**
		0.358	*0.021**	*0.112*	*0.179*	*0.666*
	Tb	**- 0.597**	**0.984**	**- 0.858**	**- 0.790**	**- 0.283**
		0.403	*0.016**	*0.142*	*0.210*	*0.717*
	La	**- 0.349**	**0.955**	**- 0.652**	**- 0.596**	**- 0.038**
		0.651	*0.045**	*0.348*	*0.404*	*0.962*
	Sl	**- 0.949**	**0.748**	**- 0.877**	**- 0.973**	**- 0.868**
		0.051	*0.252*	*0.123*	*0.027**	*0.132*
50%	Sb	**- 0.717**	**0.984**	**- 0.916**	**- 0.880**	**- 0.439**
		0.283	*0.016**	*0.084*	*0.120*	*0.561*
	Tb	**- 0.687**	**0.995**	**- 0.881**	**- 0.862**	**- 0.419**
		0.313	*0.005***	*0.119*	*0.138*	*0.581*
	La	**- 0.626**	**0.990**	**- 0.870**	**- 0.814**	**- 0.323**
		0.374	*0.010**	*0.130*	*0.186*	*0.677*
	Sl	**- 0.611**	**0.914**	**- 0.889**	**- 0.774**	**- 0.280**
		0.389	*0.086*	*0.111*	*0.226*	*0.720*
100%	Sb	**- 0.701**	**0.975**	**- 0.920**	**- 0.864**	**- 0.408**
		0.299	*0.025**	*0.080*	*0.136*	*0.592*
	Tb	**- 0.669**	**0.955**	**- 0.915**	**- 0.833**	**- 0.358**
		0.331	*0.045**	*0.085*	*0.167*	*0.642*
	La	**- 0.723**	**0.955**	**- 0.939**	**- 0.873**	**- 0.427**
		0.277	*0.045**	*0.061*	*0.127*	*0.573*
	Sl	**- 0.612**	**0.885**	**- 0.891**	**- 0.764**	**- 0.280**
		0.388	*0.115*	*0.109*	*0.236*	*0.720*

Table 4. Best-fit models of shoot height growth under three watering regimes in four *Eucalyptus microtheca* provenances

Provenance	Watering (%)	Height growth model[1)]	r^2 [1)]	p
15074	25	$h = 6.755 + 3.161 \ln t * \ln t$	0.990	< 0.001
	50	$h = 5.300 + 4.939 \ln t * \ln t$	0.998	< 0.001
	100	$h = 4.582 + 4.498\, t$	0.945	< 0.001
15076	25	$h = 5.024 + 3.202 \ln t * \ln t$	0.976	< 0.001
	50	$h = 8.242 + 5.288 \ln t * \ln t$	0.951	< 0.001
	100	$h = 5.476 + 4.742\, t$	0.939	< 0.001
15081	25	$h = 5.814 + 4.392 \ln t * \ln t$	0.945	< 0.001
	50	$h = 5.782 + 7.862 \ln t * \ln t$	0.962	< 0.001
	100	$h = 4.327 + 5.091\, t$	0.947	< 0.001
15085	25	$h = 3.035 + 5.332 \ln t * \ln t$	0.955	< 0.001
	50	$h = 4.804 + 8.158 \ln t * \ln t$	0.986	< 0.001
	100	$h = 4.092 + 5.943\, t$	0.978	< 0.001

[1)] Symbol h indicates shoot height (cm), t the time after transplanting (weeks), and r the coefficient of determination.

Table 5. Stomatal characteristics (means and standard errors) under three watering regimes in four *Eucalyptus microtheca* provenances [1)]

Provenance	Watering (%)	Stomatal frequency (No. mm^{-2})	Stomatal length (µm)	Stomatal index (µm mm^{-2})
15074	25	136.2(8.0)	12.5(0.3)	1704.7(101.9)
	50	132.9(4.2) A	14.1(0.4) A	1875.4(82.4) A
	100	146.5(5.7)	13.4(0.4)	1951.4(40.1)
15076	25	139.3(8.5)	12.7(0.6)	1791.6(150.6)
	50	131.4(3.1) A	14.0(0.2) AB	1845.5(61.6) A
	100	142.3(7.1)	14.3(0.3)	2013.5(71.1)
15081	25	127.7(6.9)	13.6(0.3)	1727.6(80.0)
	50	139.4(5.0) A	14.2(0.3) BC	1949.0(64.3) A
	100	145.2(3.1)	14.2(0.3)	2058.8(70.2)
15085	25	132.1(8.3)	14.2(0.3)	2034.1(81.2)
	50	142.5(5.0) A	14.4(0.2) C	1911.0(116.2) A
	100	143.2(3.0)	13.9(0.3)	1998.0(60.2)

[1)] Capital letters refer to differences between the provenances; values followed by the same letter(s) are not statistically different at $p<0.05$.

Discussion

In this study, statistically significant differences were found among the provenances in shoot biomass, total biomass, total leaf area, specific leaf area and leaf shape at all watering regimes. The substantial variation found in early seedling growth and in growth adjustment following water stress suggests that differences in growth characteristics are related to the variation in drought resistance mechanisms among the provenances. An analysis of the relationships between early seedling growth and climatic data of the natural habitats of the provenances indicated that there were significant correlations between shoot biomass, total biomass, or total leaf area and driest-quarter rainfall in all watering treatments. This suggests that the growth capacity of eucalypt provenances can be attributed to the hydrological conditions of their natural habitats in Australia. A clear conclusion was that the seedlings of the southeastern provenance had the highest inherent growth rate under the present experimental conditions.

Since growth strategies are adaptations to the environment in which trees have evolved (Gibson *et al.* 1994), differences in growth adjustment may explain specific adaptation mechanisms within the species under diverse conditions of water supply. The slowest-growing, northern Australian provenance (No. 15074) used in this study was native to a region with a high temperature and a low driest-quarter rainfall; trees from this region could be assumed to possess a high drought tolerance which is achieved at the expense of the growth rate. In contrast, the fastest-growing, southeastern provenances (No. 15081 and 15085) were associated with a low temperature and a high driest-quarter rainfall; trees from this region could be assumed to utilise abundant soil water supplies and to therefore grow rapidly.

Drought adaptation of tree species is related to leaf stomatal frequency and length (Carpenter & Smith 1974, Abrams 1986, 1988) and to the total stomatal pore area per unit leaf area (Cowan 1977, Tuomela *et al.* 1993), because, at the leaf level, stomatal characteristics can regulate the transpiration rate under water deficit. However, in the present study, the total stomatal length per unit leaf area (stomatal index) or the stomatal frequency was not an indicator of variation in drought adaptation, although some statistically significant differences in stomatal length between the provenances were found.

The present findings are in agreement with earlier observations on the adaptation of trees to the water supply. Under a limited water supply, trees have evolved towards two contrasting water-use strategies that depend on the degree of water deficit in their natural habitats (Passioura 1982, Johansson & Tuomela 1996). According to this ecophysiological explanation, *E. microtheca* provenances from northern and central Australia have a conservative water-use strategy, whereas the provenances from southeastern Australia show lavish water use. Further studies are needed to improve our understanding of the relationships between environmental conditions and the mechanisms of drought resistance in eucalypts and to allow the use of seedling characteristics related to drought adaptation in eucalypt provenance selection.

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