

Growth and Physiological Characteristics of Seedlings, Coppices, and Intact Adults of *Eucalyptus camaldulensis* under Saline Conditions in Western Australia

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Abstract: In Australia, a rise in the water table usually causes soil salinization, for which afforestation has been encouraged to manage the groundwater level. In this study, the growth and physiological characteristics of afforested trees of *Eucalyptus camaldulensis* were measured to evaluate the ability of this species to grow and regenerate under saline conditions. The photosynthetic capacity of seedlings at predawn in winter was higher than those of coppices and intact adults. However, the maximum rate of photosynthesis in winter did not differ among them, and in summer, the lowest values were observed in seedlings. The lowest stomatal conductance and the highest ratio of carbon stable isotopes were also observed in seedlings, thus indicating that the seedlings were exposed to severe water stress in summer. The water stress affected the growth and survival of each seedling. The biomass growth of coppices and intact adults were significantly larger than that of seedlings, and the survival rates of coppices and intact adults were higher than that of seedlings. The survival rate of seedlings was only 33%, as the severe water stress in summer would cause high seedling mortality. When comparing the growth forms of coppices, seedlings, and adults, only seedlings have small root systems. A well-developed root system contributes to a larger water supply and more successful growth in semiarid saline areas. Our results suggest the importance of taking countermeasures to avoid summer water stress for successful regeneration by planting seedlings.

Key Words: Afforestation, Dry land, *Eucalyptus camaldulensis*, Regeneration, Salinity.

1. Introduction

Soil salinization had been reported in many regions of the world (Rengasamy, 2006). Soil salinity reduces the area available for agriculture and the productivity of affected farmland. This problem is quite severe in Australia, where dryland salinity affects more than 2.5 million ha, and with the area affected expanding at an annual rate of about 3 to 5% (Marcar and Crawford, 2004). Moreover, the National Land and Water Resources Audit (NLWRA) predicted that areas with a high potential to develop dryland salinity will cover 17 million ha by 2050 (NLWRA, 2001a). As a result, the development of countermeasures against salinity is now considered an urgent need.

In many cases, salinization results from a rise in the water table (Barrett-Lennard *et al.*, 2003). A rising water table mobilizes the salts stored in unsaturated soil horizons, and transports the salts to the ground surface (Bell, 1999). A change from forest vegetation to farmland can disturb a catchment's water balance. Therefore, in the wheat belt of

Western Australia, afforestation is being encouraged to improve the water balance by increasing the amount of water extracted from the soil by plants. Previous research has shown that one potential approach to the treatment of secondary salinization is through the management of water use by vegetation (Greenwood and Beresford, 1979). In Australia, many researchers have studied tree-water relations (e.g. Boomsma and Hunter, 1990; Whitehead and Beadle, 2004). Some studies focused on the amount of water used by afforested stands, and assessed the ability of afforestation to manage shallow water tables that can lead to salinization (Cramer *et al.*, 1999; White *et al.*, 2002; Aikawa *et al.*, 2009).

The management of planted trees would be also important for the long-term management of shallow water tables. Several approaches have been taken in southwestern Australia to integrate environmental restoration and the use of carbon stored on such plantations (Harper *et al.*, 2011). After the use of forest carbon, the felled trees should be regenerated by coppicing or planting new seedlings. Therefore, the growth and physiological characteristics of coppices, seedlings, and adult trees were measured to reveal their growth and any

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factors that may limit that growth.

2. Materials and Methods

2.1. Study site and species

The study was conducted at an afforestation site near Calingiri, Western Australia (31°12'S, 116°28'E). The average annual rainfall between 2000 and 2010 was 315.7 mm, and most of the rainfall had observed in winter. The stand was afforested in 2001 by planting *Eucalyptus camaldulensis* in a flat marginal area of abandoned saline farmland. The trees were spaced 2.0 m apart within the rows and 3.0 m between the rows, and the resulting stand density was 1667 trees ha⁻¹. Calingiri was considered to be at moderate risk of salinization in 2000, but is now considered to be at high risk after 2020 (Short and McConnell, 2000). The dominant soil type in this area was a Sodosol (NLWRA, 2001b). This is a specific kind of sodic soil with a clear or abrupt textural horizon that is not strongly acidic in its sandy or loamy surface soil. The subsoil is usually clayey, with restricted hydraulic conductivity essentially caused by the dispersive nature of sodic clay. The depth to the water table was less than 2.0 m, and the electrical conductivity of the groundwater ranged from 25 to 50 dS m⁻¹ around the site (Short and McConnell, 2000). The electrical conductivity of the surface soil was less than 0.5 dS m⁻¹ at our study site (Aikawa *et al.*, 2009). As is often the case in lands at risk of salinization, waterlogging destroyed the vegetation in low-lying areas of the catchment.

Eucalyptus camaldulensis is one of the most widely distributed *Eucalyptus* species in Australia (Boland *et al.*, 2006). The species is called "River red gum" due to its abundance near rivers, and shows both a high tolerance for waterlogging and a fast growth rate. These traits make *E. camaldulensis* a promising species for afforestation and environmental restoration.

2.2. Measurements

In August 2009, a 96 × 15 m plot was established in the afforestation stand. Four 12 × 15 m sub-plots were also established in the plot, and in the two of the sub-plots all planted trees were cleared to measure coppice regeneration. At the same time, 158 seedlings were planted beside the plot. The heights (*H*) and diameters at 0.3 m above the ground (*D*_{0.3}) were then measured in all stems of 60 intact adults, 60 coppices, and 158 seedlings twice a year. We calculated the aboveground biomass (dry weight) of the trees by using allometric equations obtained from the felled trees (Table 1).

The photosynthetic capacity of the adults, coppices, and seedlings were measured by using a portable photosynthesis system (Li-6400, LI-COR Inc., USA). In September 2010

Table 1. Allometric equations developed from felled trees.

	organ (unit)	Coefficient		R ²	P
		a	b		
Adults	stem (kg)	107.760	0.963	0.907	<0.05
	branch (kg)	48.943	1.096	0.980	<0.01
	leaf (kg)	8.697	0.643	0.830	<0.05
Coppices	stem (g)	0.570	0.808	0.933	<0.01
	branch (g)	0.127	0.951	0.755	<0.05
	leaf (g)	1.493	0.648	0.915	<0.01
Seedlings	stem (g)	8.0 × 10 ⁻⁶	3.014	0.964	<0.01
	branch (g)	2.0 × 10 ⁻⁶	3.166	0.760	<0.05
	leaf (g)	4.0 × 10 ⁻⁴	2.276	0.836	<0.01

The dry weight in each organ was estimated by the power function (Dry wight = a x^b). The independent variable (x) was *D*_{0.3}²*H* (cm³) in adults and coppices, and was *H* (cm) in seedlings.

(winter) and in January 2011 (summer), the maximum rate of Rubisco carboxylase activity (*V*_{cmax}) and the maximum rate of photosynthetic electron transport (*J*_{max}) were measured to compare the photosynthetic capacity among adults, coppices, and seedlings. *V*_{cmax} and *J*_{max} are key parameters used to describe photosynthetic capacity (Farquhar *et al.*, 1980; Fan *et al.*, 2011). These parameters measured in winter and summer were converted to normalized values at 25°C by using a method described by Kosugi *et al.* (2003). The normalized *V*_{cmax} and *J*_{max} are represented as *V*_{cmax25} and *J*_{max25}, respectively. The maximum rate of photosynthesis (*A*_{max}) and stomatal conductance (*g*_s) were also measured in winter and in summer.

The stable carbon isotope composition of leaves was measured by using a system that combined an elemental analyzer (Flash 2000, Thermo Fisher Scientific Inc., USA) and a mass spectrometer (DELTA V, Thermo Fisher Scientific Inc., USA). The ¹³C/¹²C isotope ratios (δ¹³C) were calculated relative to the Pee Dee Belemnite international standard.

3. Results and Discussion

3.1. Physiological characteristics

In winter, the mean (± standard error) values of *V*_{cmax25} for the leaves of coppices, seedlings, and adults were 86.0 ± 4.7 μmol m⁻² s⁻¹, 91.4 ± 5.7 μmol m⁻² s⁻¹, and 70.5 ± 6.2 μmol m⁻² s⁻¹, respectively (Fig. 1). The *V*_{cmax25} values in seedlings were significantly higher than those of adults in winter (Tukey test, *P* < 0.05). In summer, the *V*_{cmax25} values of coppices and adults were 58.9 ± 1.2 μmol m⁻² s⁻¹, and 64.0 ± 7.7 μmol m⁻² s⁻¹, respectively. The summer *V*_{cmax25} values in coppices were significantly lower than those of winter values (ANOVA, *P* < 0.001), however, the winter *V*_{cmax25} values were not significantly differed between coppices and adults (ANOVA, *P* > 0.05). In winter, the *J*_{max25} values for the leaves of coppices, seedlings, and adults were 160.9 ± 7.6 mol m⁻² s⁻¹, 196.5 ± 10.1

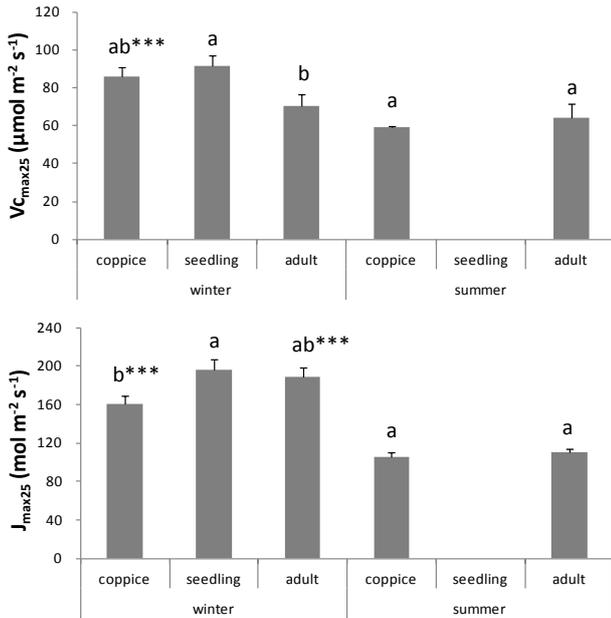


Fig. 1. Maximum rate of Rubisco carboxylase activity ($V_{c_{max}}$) and maximum rate of photosynthetic electron transport (J_{max}) of seedlings, coppices, and adults. The summer values in seedlings could not be measured due to the severe water stress throughout the day. Different alphabets indicate significant difference between the values in each season (Tukey test, $P < 0.05$). Asterisks indicate significant difference between winter and summer (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

mol m⁻² s⁻¹, and 188.2 ± 10.1 mol m⁻² s⁻¹, respectively. The J_{max25} values in seedlings were significantly higher than those of coppices in winter (Tukey test, $P < 0.05$). In summer, the J_{max25} values of coppices and adults were 105.0 ± 5.3 mol m⁻² s⁻¹ and 110.1 ± 3.9 mol m⁻² s⁻¹, respectively. There was no significant difference in the summer J_{max25} values of coppices and adults (ANOVA, $P > 0.05$), although the summer values were significantly lower (ANOVA, $P < 0.001$). The summer values of $V_{c_{max}}$ and J_{max} in seedlings could not be measured due to severe water stress throughout the day.

The maximum rates of photosynthesis (A_{max}) and stomatal conductance (g_s) were also measured in winter and summer (Fig. 2). The A_{max} values of coppices, seedlings, and adults in winter were 17.9 ± 0.6 µmol m⁻² s⁻¹, 16.3 ± 1.5 µmol m⁻² s⁻¹, and 14.9 ± 1.1 µmol m⁻² s⁻¹, respectively, with no significant difference being observed (Tukey test, $P > 0.05$). The A_{max} values of coppices, seedlings, and adults in summer were 10.8 ± 0.4 µmol m⁻² s⁻¹, 7.6 ± 1.5 µmol m⁻² s⁻¹, and 14.7 ± 2.3 µmol m⁻² s⁻¹, respectively, with a significant difference between the A_{max} values of seedlings and adults (Tukey test, $P < 0.05$). The winter values were significantly higher in coppices and seedlings (ANOVA, $P < 0.001$). The g_s values of coppices, seedlings, and adults in winter were 0.47 ± 0.05 mol m⁻² s⁻¹, 0.49 ± 0.07 mol m⁻² s⁻¹, and 0.34 ± 0.06 mol m⁻² s⁻¹, respectively, with no significant difference being observed

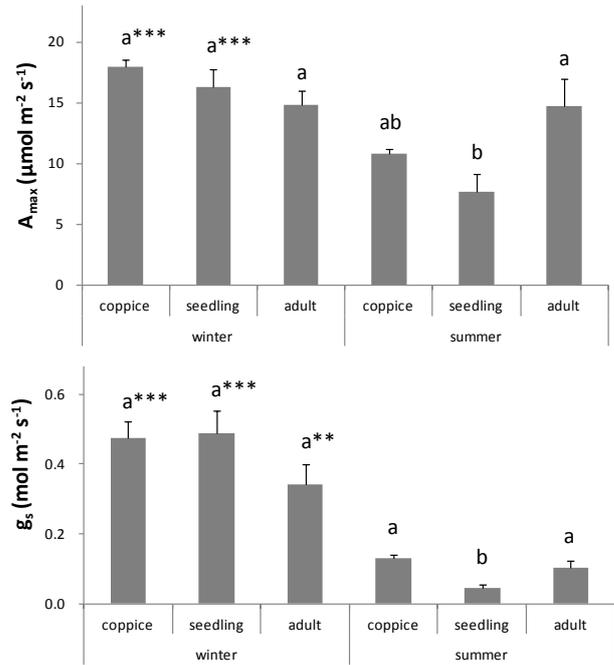


Fig. 2. Maximum rate of photosynthesis (A_{max}) and stomatal conductivity (g_s) of seedlings, coppices, and adults. Different alphabets indicate significant difference between the values in each season (Tukey test, $P < 0.05$). Asterisks indicate significant difference between winter and summer (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Table 2. Rate of carbon stable isotope in summer leaves.

	organ	Mean	SE	
Adults	leaf	-27.5 ± 0.29	0.29	a
Coppices	leaf	-27.2 ± 0.27	0.27	a
Seedlings	leaf	-25.4 ± 0.57	0.57	b

Different alphabets indicate significant difference between the values (Tukey test, $P < 0.05$).

(Tukey test, $P > 0.05$). In contrast, the g_s values in summer were 0.13 ± 0.01 mol m⁻² s⁻¹, 0.05 ± 0.01 mol m⁻² s⁻¹, and 0.10 ± 0.02 mol m⁻² s⁻¹, respectively, with seedlings showing a significantly lower value relative to the coppices and adults (Tukey test, $P < 0.05$).

The ratio of stable carbon isotopes represented as ($\delta^{13}C$) indicated the intensity of a plant's water stress, because the $\delta^{13}C$ value is related to the long-term stomatal conductance of leaves. The $\delta^{13}C$ values of coppices, seedlings, and adults in summer were -27.2 ± 0.3‰, -27.5 ± 0.3‰, and -25.4 ± 0.6‰, respectively (Table 2). The $\delta^{13}C$ value in seedlings was significantly higher than the $\delta^{13}C$ values in coppices and adults (Tukey test, $P < 0.05$).

The results of $\delta^{13}C$ and stomatal conductance results indicated severe water stress in summer, especially in seedlings. The decline in stomatal conductance in seedlings had also been

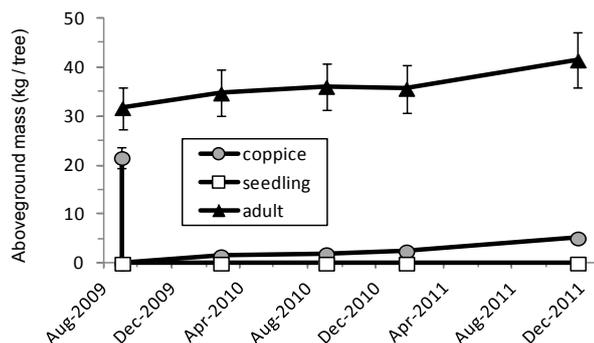


Fig. 3. Growth in aboveground biomass of seedlings, coppices, and adults.

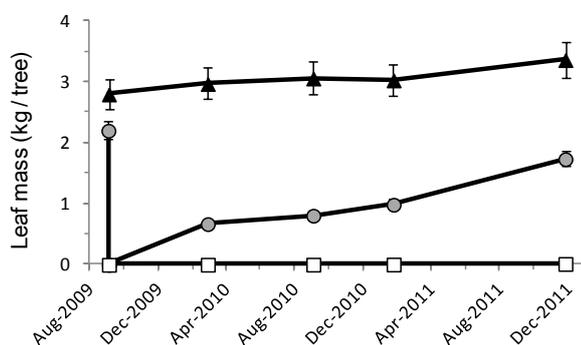


Fig. 4. Growth in leaf mass of seedlings, coppices, and adults.

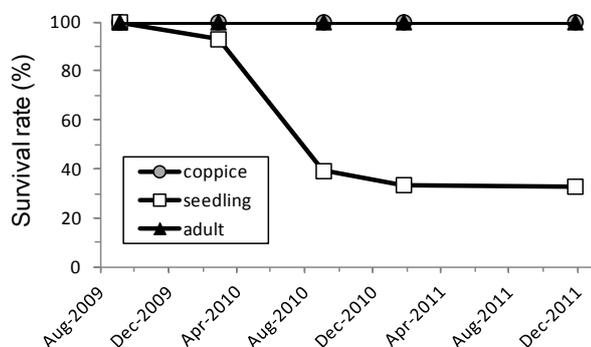


Fig. 5. Survival rate of seedlings, coppices, and adults.

reported by a previous study (Blake, 1980). When comparing the growth forms of coppices, seedlings, and adults, only seedlings have small root systems. A well-developed root system in adults and coppices would contribute to a larger water supply and higher photosynthetic rate in summer.

3.2. Growth and survival rates

At the end of our study period (i.e., 27 months after felling and planting), the aboveground biomass of coppices, seedlings, and adults derived from the equations (Table 1) became $5.1 \pm 0.4 \text{ kg tree}^{-1}$, $0.026 \pm 0.009 \text{ kg tree}^{-1}$, and $41.5 \pm 5.6 \text{ kg tree}^{-1}$, respectively (Fig. 3). The increases in the aboveground biomass of coppices, seedlings, and adults were $2.3 \pm 0.2 \text{ kg}$

$\text{tree}^{-1} \text{ y}^{-1}$, $0.011 \pm 0.004 \text{ kg tree}^{-1} \text{ y}^{-1}$, and $4.3 \pm 0.6 \text{ kg tree}^{-1} \text{ y}^{-1}$, respectively. The aboveground biomass and growth rate were thus significantly different among coppices, seedlings, and adults (Tukey test, $P < 0.05$). The leaf mass of coppices, seedlings, and adults at the end of our study period were $1.7 \pm 0.1 \text{ kg tree}^{-1}$, $0.014 \pm 0.004 \text{ kg tree}^{-1}$, and $3.4 \pm 0.3 \text{ kg tree}^{-1}$, respectively (Fig. 4), thus showing significant differences in leaf mass (Tukey test, $P < 0.05$).

The survival rates of coppices and adults during the study period were 100%, but only 32.9% for seedlings (Fig. 5). Most seedlings died after the first summer.

The growth rate of seedlings was less than one-hundredth of that for coppices and adults. Despite the small amount of leaves in seedlings, the leaves of seedlings were exposed to severe water stress. This indicated that the seedlings had poor water absorption ability. The poor water supply in seedlings raised water stress - the cause of high mortality. It had been reported that the development of the root system plays an important role in the growth of coppices in the arid region of Western Australia (Aikawa *et al.*, 2013). Our results indicated that a well-developed root system was also important in semiarid saline areas.

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