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Effect of fertiliser and water supply on the growth, nutrient status and photochemical efficiency of *Eucalyptus pilularis* seedlings in a phosphorus-deficient soil

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Summary

A factorial pot trial, using a clay loam P-deficient soil, was conducted with two fertiliser rates (a) unfertilised and (b) fertilised with N, P, K and Mg, and with water supply within the range of 20% to 95% field-capacity (FC). Within the growth period of 180 days, plants of *Eucalyptus pilularis* Smith were exposed to increasing drought. Growth and photochemical efficiency were measured under these conditions and leaves and stems were analysed for biomass and nutrients.

As judged by photochemical efficiency, fertilised plants were more stressed than unfertilised plants, where exposed to severe drought. As pre-conditioned plants displayed greater tolerance to drought than non-conditioned plants, it is recommended that where seedlings are to be planted in drought-prone areas, fertiliser application should be reduced and drought periods imposed during the nursery stage.

Both stem and leaf nutrient concentrations increased with fertiliser application. On the other hand, leaf nutrient concentration decreased with increasing water supply, but stem concentration did not. It follows that the stem nutrient concentration may be the better indicator of the nutrient status of *E. pilularis* seedlings and this should be subject to further investigation.

Keywords: nutrition; nutrient deficiencies; assessment; indicators; fertilizers; drought; phosphorus; *Eucalyptus*

Introduction

The eucalypt plantation program in northern NSW has been expanding, but increasing land prices close to the coast have forced Forests NSW to establish plantations on land with rainfall below 1000 mm y^{-1}, considered marginal for *Eucalyptus pilularis* Smith. *Eucalyptus* productivity is strongly influenced by nutrient and water availability (Fabião *et al.* 1995; Misra *et al.*1998; Whitehead and Beadle 2004) and the current expansion of plantations to sites with rainfall near the limits of species' ability to survive and grow makes resistance to water stress an important attribute of seedlings (Whitehead and Beadle 2004). Fertiliser application at plantation establishment is a common practice (Attiwill and Adams 1996) but there is little information on the effect of fertiliser on susceptibility of trees to drought (Fisher and Binkley 2000). In Australia generally, 10–25 g nitrogen (N) and 10–30 g phosphorus (P) per tree are added at planting, irrespective of local climatic conditions. The effect of fertiliser application on seedlings exposed to drought can vary with soil type. Graciano *et al.* (2005) showed that nitrogen fertiliser reduced osmotic adjustment of *E. grandis* exposed to drought in both sandy and clay loam soil, whereas P fertiliser reduced it in sandy soil but increased it in clay loam soil.

Several studies with eucalypts have shown that plants react to drought periods with morphological adjustments such as a reduction in specific leaf area (Wang *et al.* 1988; Myers and Landsberg 1989; Rhizopoulou and Davies 1993) and osmotic adjustment (Guarnaschelli *et al.* 2003; Ngugi *et al.* 2003). Rolando and Little (2003) proposed the status of the plants' photosynthetic apparatus is a good indicator of stress and stress adaptation. The chlorophyll *a* fluorescence parameter F_v/F_m (the ratio of maximum variable to maximum total fluorescence) is a direct quantitative measure of the efficiency of the photosynthetic apparatus for trees under stress (Mohammed *et al.* 1995), and has been used to assess the effect of drought on the photosynthetic apparatus in tree leaves including those of *E. grandis* (Ögren 1990; Rolando and Little 2003). This non-destructive tool can be used when plants have ceased growth due to drought.

While fertiliser applications may potentially affect the survival of seedlings under drought due to morphological (specific leaf area) and physiological (osmotic) adjustments, they should also aim to ensure best plant growth once water conditions have improved. Initial fertiliser applications may not be sufficient to sustain optimum growth over the first years after establishment, and leaf analysis is often necessary to establish the nutritional status of the trees (Olsen and Bell 1990; Gregoire and Fisher 2004). However, leaf-P concentration in eucalypts is often little affected by nutrient P supply. Shortcomings of leaf analysis in reflecting the P status of eucalypt trees have been noted by various authors (Lamb 1976; Schönau 1981; Dell *et al.* 1983, 1987; Grove 1990; Olsen and Bell 1990; Dighton and Jones 1992). Stem and bark P concentrations have been found to change according to P supply in eucalypt seedlings (Dell *et al.* 1987; Olsen and Bell 1990), while Grove (1990) found analysis of twigs more indicative than leaf analysis of the P status of mature plants. Thomas *et al.* (2006) found the inorganic-P concentration of leaves to be indicative of

the P status of *E. grandis* seedlings, but the necessary immediate freeze drying of leaf material for sample preparation may be difficult under field conditions.

Given the limitation of leaf-P concentration in indicating the P status of eucalypts, it is important to establish which tissue best reflects the nutritional status of the plant, and to what degree it can be influenced by temporary fluctuations in water supply. Nutrient demand and water supply are to some degree related because the absolute nutrient demand of a better-growing plant will always be higher than that of a plant struggling under water limitations. In areas with fluctuating and marginal rainfall, however, there is no reliable method to assess potential P fertiliser needs covering both humid and dry periods of the year.

Our pot experiment had two aims. Firstly, to assess the effect of water supply and fertiliser application on the growth and photochemical efficiency of *E. pilularis*. Secondly, to determine whether plant nutrients in leaf and stem tissues are affected by fertiliser and water supply, and to identify the part of the plant most indicative of the P status of eucalypt seedlings.

Methods

Soil description and preparation

A clay loam soil from Cumberland State Forest, derived from Ashfield Shale and Hawkesbury Sandstone, was used for the pot experiment. Selected soil attributes are listed in Table 1. The soil has a low organic matter content of 3.6% and a very low total soil P concentration of 110 mg kg^{-1} P as well as low soil available P (Colwell-P). Soil was sieved, air-dried and the field capacity (FC) determined. Each pot contained 2.5 kg of air dry soil, 24 g of vermiculite and 12 g of perlite.

General conditions

The pot trial was conducted in a glasshouse (day/night temperature 20–36°C/14–28°C) for 180 days (planted 10 March 2005, harvested 5 September 2005), using a range of *E. pilularis* clones derived from seedlings of the same family. Plants, including their root plug, were transferred to pots when plants were about 20 cm tall and were kept at 80% field capacity (FC) for the first two weeks. From day 14 onwards water treatments were imposed and at day 25 all pots reached their respective fraction of the field capacity (see below).

Table 1. Selected soil attributes of the Cumberland State Forest topsoil

Attribute	Value
pH (H_2O)	5.3
pH ($CaCl_2$)	4.5
EC	<0.05 dS m^{-1}
Organic carbon	1.9%
Organic matter	3.6%
Total N	0.06%
Total P	110 mg kg^{-1}
Colwell P	<4 mg kg^{-1}
Hot $CaCl_2$–boron	0.4 mg kg^{-1}
Exchangeable Ca	<0.1 meq 100 g^{-1}
Exchangeable Mg	0.8 meq 100 g^{-1}
Exchangeable Na	<0.1 meq 100 g^{-1}
Exchangeable K	0.2 meq 100 g^{-1}
Exchangeable Al	140 meq 100 g^{-1}

Fertiliser treatments

The two-factorial trial consisted of two fertiliser treatments (unfertilised and fertilised) and three water regimes (incorporating a range of water supply treatment) and four replicates for each treatment. The pots were lined with plastic (thus undrained) and contained one plant. The fertiliser treatment consisted of a slow-release fertiliser, formulated as a single tablet of 2.55 g, which was placed under the root plug. Slow-release fertilisers are generally designed to release the components within 1–2 y (Jacobs *et al.* 2005), depending on water supply. The fertiliser tablet, produced by Bayer, weighed 2.55 g and contained 5.6% N (2/3 slow release, 1/3 ammonium), 5.4% P (high analysis fertiliser-P and Di-Cafos), 4.2% K and 0.85% MgO.

Water regimes

The following watering regimes (WR) were applied:

- WR I — Days 25–71: treatments maintained at constant (c.) 95%, c.70% and c.45% of field capacity (FC), respectively; plants were watered every second day to weight and water additions recorded.
- WR II —Days 72–123: watering levels reduced by 25%, that is to c.70%, c.45% and c.20% of FC, respectively.
- WR IIIa — Days 124–144: plants exposed to greater water stress: the c.45% and c.20% treatments were now watered once weekly — designated as w.45% and w.20% of FC. The c.70% treatment was maintained throughout the experiment.
- WR IIIb — Days 145–180: water addition was further reduced in one of the treatments, that is, treatment w.45%. FC was reduced to w.30% FC.

Photochemical efficiency

The photochemical efficiency of seedlings (chlorophyll fluorescence ratio of F_v/F_m) was measured using a fluorescence induction monitor. Photochemical efficiency was recorded from week five onwards at a mature leaf, located just below the uppermost mature branching of the plant. The measurements were taken on similar leaves or leaf positions throughout the experiment. Measurements were taken initially weekly and, after day 127, every third day. After day 127 two leaves per plant were measured, with the second leaf being a mature, expanded leaf just above the uppermost mature branching of the plant. A minimum of 30 minutes' dark exposure was imposed prior to chlorophyll fluorescence measurement.

Plant measurement

During the course of the experiment plant heights and diameters were recorded on 15 and 4 occasions, respectively. Stems and leaves were harvested at day 180 and dried at 70°C for 48 h. Leaves which had more than 70% of necrotic area were sampled

separately. Leaves shed three days prior to harvest were also collected. Dry weights were determined for stems, healthy leaves, necrotic leaves and dropped leaves. Leaves (healthy plus necrotic) and stems were analysed for plant nutrients. Plant parts were dried at 70°C for 48 h and digested in concentrated nitric and hydrochloric acid. Element concentrations in digest solutions were determined by Radial CIROS inductively coupled plasma atomic emission spectroscopy (ICP–AES).

Data analysis

Data were analysed using the ANOVA procedure in the SAS statistical package.

Results

Responses to the treatments are presented in terms of plant height, plant dry weight production, the photochemical efficiency of leaves, and stem and nutrient concentrations.

Responses in plant height

Figure 1 shows plant response to treatments in terms of plant height for WR I (c.95%, c.70% and c.45% FC) and part of WR II (c.70%, c.45% and c.20% FC). Growth of plants in all treatments increased exponentially in the first 60 days after planting and slowed thereafter, partly due to cooler temperatures after day 75. At the end of WR I the fertilised plants had grown significantly more than unfertilised plants. Fertilised plants grown at 45% FC were equal in height to the unfertilised plants at 95% FC.

Water regime II was imposed between days 72 and 123. By day 90 (Fig. 1) the fertilised plants now held at c.70% and c.45% FC were maintaining their height growth, while growth of plants in other treatments was slowing down. The growth of unfertilised plants at c.20% FC was now more or less stagnant.

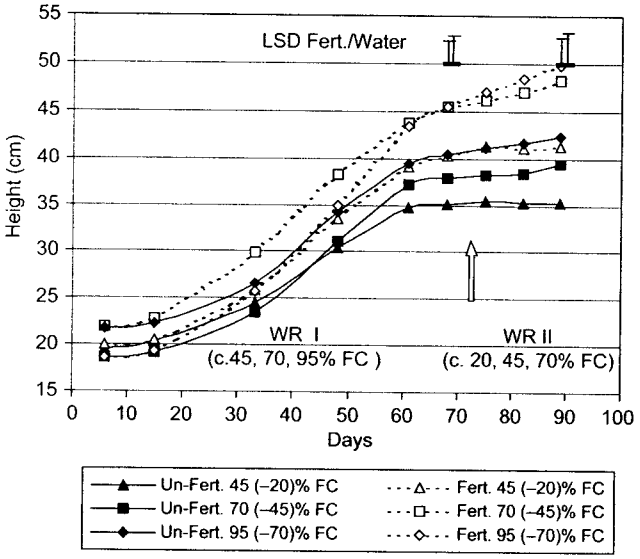

Figure 1. Height growth during water regime (WR) I and II (Table 2), as affected by fertiliser and water application (arrow indicates the change in WR). (LSD $P < 0.05$ for fertiliser and water regime is shown).

Figure 2 illustrates the continuing response in height to day 124 (WR II) and, from day 124, the subsequent imposition of more severe droughting associated with weekly watering (WR IIIa and IIIb). At the end of WR II there was a clear distinction between two sets of treatments — (i) the fertilised c.70%, fertilised c.45%, unfertilised c.70% treatments and (ii) the unfertilised c.45%, unfertilised c.20% and fertilised c.20% FC treatments. These patterns continued through WR IIIa and b, with the latter treatments now being more or less stagnant.

The height growth patterns in Figure 1 and 2 suggest that in this soil, a high level of water supply may be compensating in some way for low (unfertilised) nutrient status. It is possible from these patterns to identify the field capacity which becomes limiting for growth of *E. pilularis* in this soil. Under water regimes IIIa and b, soil water content in the w.20% FC treatment fell as low as 10–13% FC and in the w.45% FC treatment water content fell below 25% FC (Fig. 3). In WR IIIb it fell from 30% to 13% FC. Based on response patterns, plants could not access water below 10–15% FC.

The effect of fertiliser application on height growth was not significant when weekly drought was imposed during WR IIIa and b (Table 2). Moreover, there was no significant response to fertiliser in the c.70% FC treatment during the same period. Leaf symptoms of P deficiency were obvious mainly in the c.70% FC treatment (both fertilised and unfertilised), with P deficiency potentially restricting further growth.

Both water and or fertiliser application significantly enhanced growth of plants. However, fertiliser application significantly

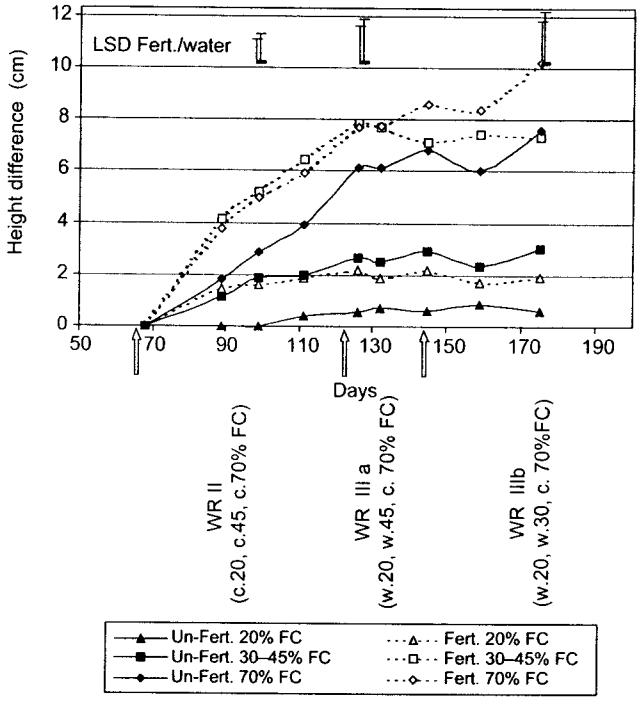

Figure 2. Height increment during water regime II, IIIa and IIIb, as affected by fertiliser and water application (arrows indicate changes in water regime). (LSD $P < 0.05$ for effect of fertiliser and water regime is shown for day 98, day 126 and day 175).

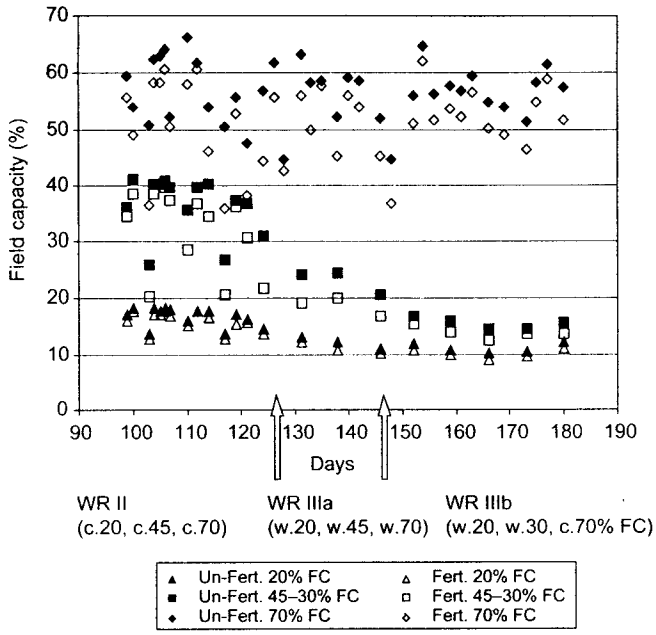

Figure 3. The minimum field capacity (FC) reached in the pots prior to re-watering to the respective FC in each treatment

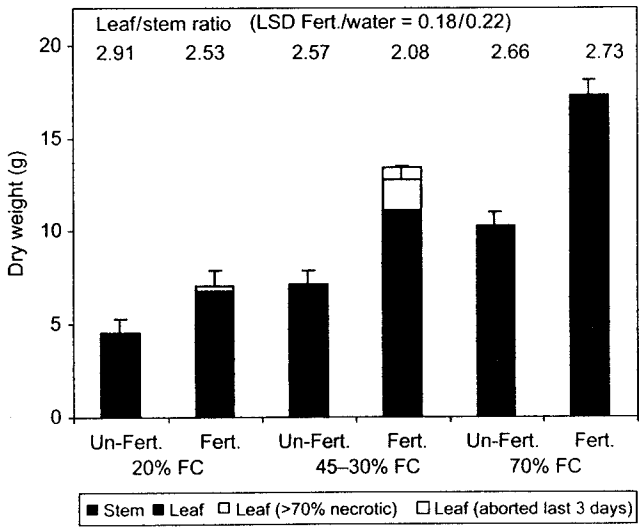

Figure 4. Dry weight of fertilised (Fert.) and unfertilised (Un-Fert.) plants as affected by water regime. (LSD P <0.05 fertiliser)

enhanced growth only when field capacity was above 30% (Fig. 3 treatment c.45% FC) in this clay loam soil. Also, water supply enhanced growth measurably only when the field capacity was above 20–30% FC. Plants held at FC 45% and above showed improved growth when fertilised. Under severe drought, growth ceased.

Response in plant dry weight

During the whole growth period, which included the initial 74-day period (WR I), seedling dry weight increased with both fertilisation and higher water treatments (Fig. 4). The increases were larger with fertilisation, and, in all treatments, larger in leaves than in stems.

Increasing stress of fertilised plants was shown in increased leaf necrosis and abortion. Leaf necrosis and leaf abortion was shown in fertilised plants where drought stress was increased — notably, where water supply was suddenly reduced from w.45% to w.30% FC. Leaf abortion was much more extensive in this treatment than in plants which had experienced a comparable but stable water supply over a longer period.

Photochemical efficiency

The leaf photochemical efficiency (F_v/F_m) during WR II and IIIa, b is shown in Figure 5. The F_v/F_m value was largely unaffected by fertilising or the amount of water supplied during WR I (not shown) and WR II. The F_v/F_m declined in all treatments from day 124 onwards, possibly due to reduced night temperature. At constant and sufficient water supply (c.70% FC) there were some fluctuation in F_v/F_m values in fertilised plants during WR IIIa and b, though values for unfertilised and fertilised plants were essentially similar. Under moderate droughting (w.45% FC) the F_v/F_m values were also similar in fertilised and unfertilised treatments, but when weekly watering was reduced to w.30% FC (WR IIIb), values for the fertilised plants declined sharply. Similarly when water was applied weekly at w.20% FC, there was also a sharp decline in the F_v/F_m values in fertilised plants.

Photochemical efficiency also decreased somewhat in unfertilised plants where subject to sudden drought (from w.45% to w.30% FC), compared with plants in continuous drought (w.20% FC). This suggests a preconditioning effect, that is, the effect of drought is less severe when plants are grown under a low water regime for a prolonged period, than where water supply is reduced suddenly.

Table 2. Significance of the effect of water and fertiliser application on height increments during different phases of growth and severity of water stress (c = constant, w = weekly)

Water treatment (% FC)	Day	Water regime	Fertiliser	Water	Water × fertiliser	Replicate
c.95–c.70–c.45 (7 wks)	25–71	I	***	***	ns	ns
c.70–c.45–c.20 (7 wks)	72–123	II	***	***	ns	ns
c.70–w.45–w.20 (3 wks)	124–144	IIIa	ns	**	ns	ns
c.70–w.30–w.20 (5 wks)	145–180	IIIb	ns	***	ns	ns
c.70–w.30/45–w.20 (8 wks)	124–180	IIIa+b	ns	***	ns	ns

***F-Prob < 0.001; **F-Prob < 0.01; *F-Prob < 0.05; ns = not significant

Foliar and stem nutrient concentrations

Leaf and stem nutrient analysis shows the distribution of nutrients under various water regimes. In general the K, Ca, Mg, Na, B, S, Mn and Fe concentrations were higher in the leaf than in the stem, whereas the P, Zn, Cu and Al concentrations were higher in the stem than in the leaves of plants.

Phosphorus (P)

Phosphorus concentration and uptake in leaf and stem are shown in Figure 6 and statistical analysis of the data in Table 3. The stem-P concentration was higher than the leaf-P concentration for most of the treatments (Fig. 6). Leaf and stem-P concentration increased after fertiliser application. The water regime affected leaf-P but not stem-P concentration. Leaf-P concentration was significantly decreased by increased water supply, in both unfertilised and fertilised plants, although P uptake of plants increased or remained constant. In contrast, the stem-P concentration was not significantly affected by the water application rate. Stem-P uptake increased with increased water supply in fertilised plants but remained constant in unfertilised plants. An interaction between water and fertiliser application was apparent (Table 3),

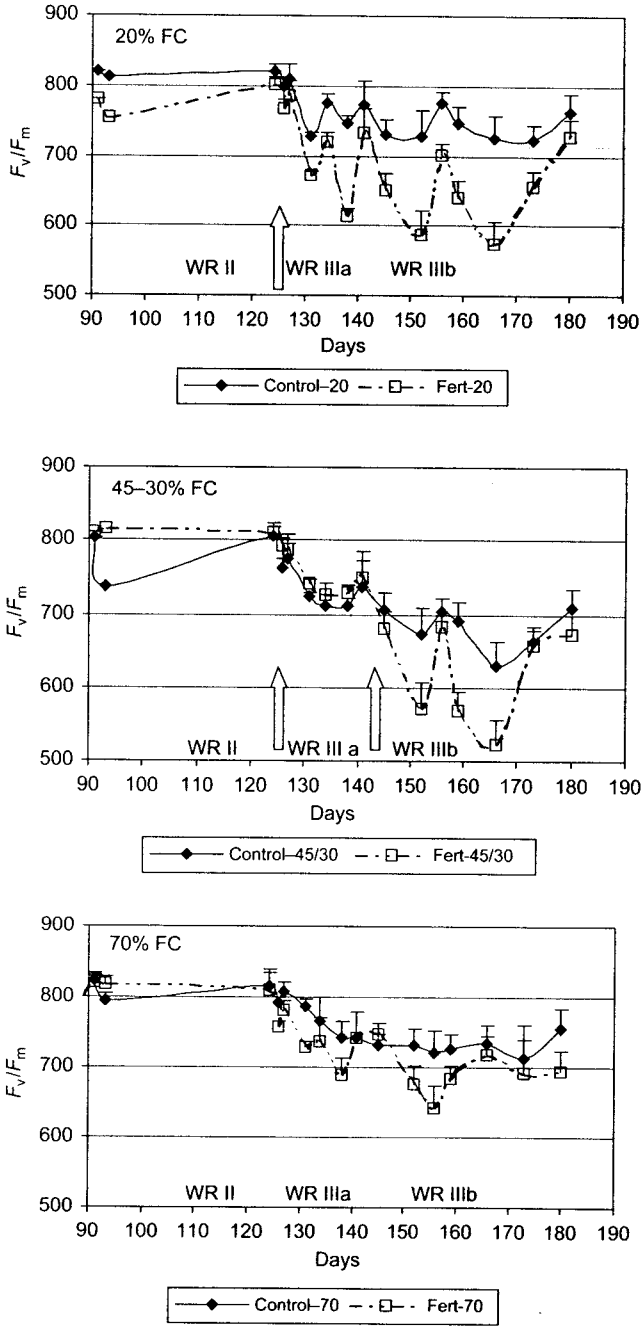

Figure 5. Photochemical efficiency (F_v/F_m) of plant leaves over time as affected by fertiliser application and water regime (arrows indicate a change in water regime). (LSD $P < 0.05$)

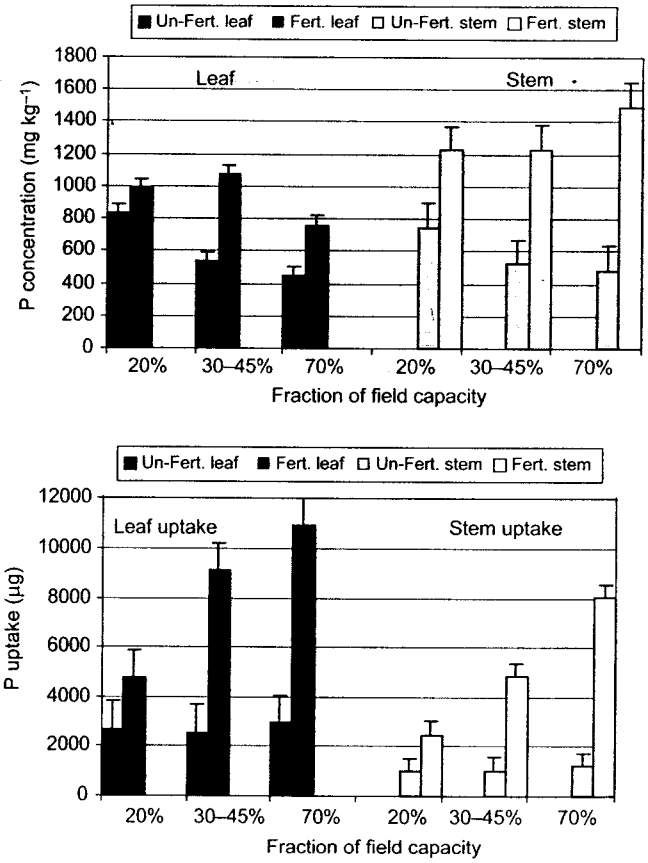

Figure 6. Effect of fertiliser application and water supply on leaf and stem-P concentration and uptake. (LSD-Fertiliser $P < 0.05$)

Table 3. Significance of the effect of fertiliser application and water supply on P, K and Mg concentration in the leaf and stem of *Eucalyptus pilularis*

Factor	Leaf			Stem		
	P	K	Mg	P	K	Mg
Fertiliser	***	***	*	***	**	***
Water	***	***	*	ns	*	ns
Fertiliser × water	***	***	ns	*	ns	ns
Replicate	ns	ns	ns	ns	ns	*

*F-Prob < 0.05; **F-Prob < 0.01; ***F-Prob < 0.001; ns = not significant

with unfertilised plants' stem-P concentration tending to decrease with increasing water supply, whereas the reverse was true for fertilised plants. Leaf-P concentrations were comparably low. Leaf symptoms of P deficiency, such as purple interveinal blotches in mature leaves, were obvious in the last two weeks of the trial, in the medium and well-watered plants (w. 45/30% FC and c.70% FC). This was the case in both fertilised and unfertilised plants, but not consistently across treatments.

Potassium (K) and magnesium (Mg)

The fertiliser tablet contained K and Mg. The K concentration in both leaf and stem was enhanced following fertiliser application (Fig. 7, Table 3), with changes in leaf-K concentration being more pronounced. With increasing water supply, both leaf and stem-K concentrations were reduced in fertilised and unfertilised plants (Fig. 7) but K-uptake in leaf and stem was increased with biomass increase (data not shown). The effect of water supply on stem-K concentration was more gradual than on leaf-K-concentration.

Following fertiliser application, there was an increase in leaf and stem Mg concentration (Fig. 8) and even a more significant increase in leaf-Mg uptake (data not shown). Water supply did affect leaf-Mg concentration but not stem-Mg concentration (Fig. 8, Table 3).

Calcium (Ca), sodium (Na), boron (B) and sulphur (S)

Water or fertiliser application affected the concentration of Ca, Na, B and S in stem or leaves. Fertiliser application increased leaf- and stem-S concentrations and decreased leaf- and stem-B concentration (Table 4). Increased water application decreased leaf- and stem-S concentration, whereas it increased stem Ca and Na concentrations. Stem-B concentration was not affected by water application.

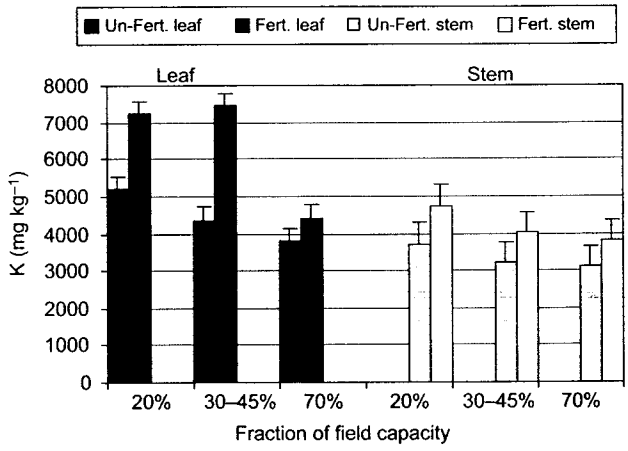

Figure 7. Effect of water supply and fertiliser application on leaf and stem K concentration. (LSD-Fertiliser $P < 0.05$)

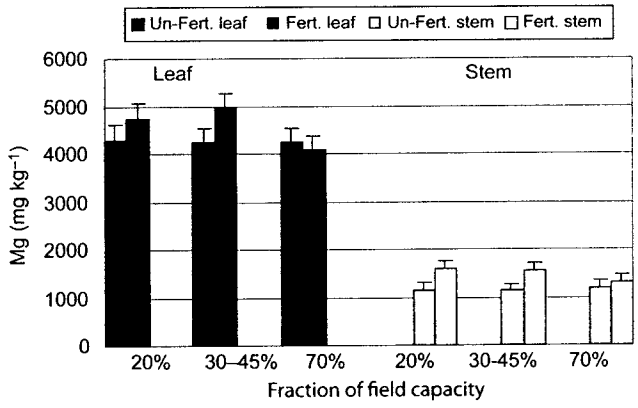

Figure 8. Effect of water supply and fertiliser application on leaf and stem Mg concentration. (LSD-Fertiliser $P < 0.05$)

Table 4. Effect of fertiliser and water supply on leaf nutrient concentration in *Eucalyptus pilularis* seedlings

Factor or treatment	Leaf (significance and concentration, mg kg^{-1})				Stem (significance and concentration, mg kg^{-1})			
	Ca	Na	B	S	Ca	Na	B	S
Fertiliser	ns	ns	***	***	*	ns	**	**
Water	ns	***	*	***	*	**	ns	***
Fertiliser × water	ns	ns	*	***	*	ns	*	*
Replicate	ns	ns	*	ns	ns	ns	ns	ns
Fert 0	4500	4733	34.3 a	960 b	2600 a	2240	13.8 a	335 b
Fert 1	4542	4475	26.6 b	1332 a	2385 b	2064	11.9 b	405 a
20% FC	4775	4800 a	30.5 ab	1313 a	2277 b	1937 b	12.5	446 a
45% FC	4425	4937 a	27.8 b	1240 b	2500 ab	2145 ab	12.5	380 b
70% FC	4362	4075 b	32.9 a	886 c	2700 a	2375 a	13.6	285 c

*F-Prob < 0.05; **F-Prob < 0.01; ***F-Prob < 0.001; ns = not significant
a, b and c: values with similar letters are not significantly different at F-Prob < 0.05

Table 5. Effect of fertiliser and water supply on leaf and stem nutrient concentration in *Eucalyptus pilularis* seedlings

Factor or treatment	Leaf (significance and concentration, mg kg $^{-1}$)					Stem (significance and concentration, mg kg $^{-1}$)				
	Zn	Cu	Mn	Al	Fe	Zn	Cu	Mn	Al	Fe
Fertiliser	ns	***	ns	*	ns	ns	ns	**	***	ns
Water	***	***	***	**	***	***	ns	***	***	ns
Fertiliser × water	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Replicate	ns	ns	ns	ns	*	ns	ns	ns	*	ns
Fert 0	13.7	4.3 b	427	45.6 b	46.5	32.9	8.5	196 a	161 a	23.8
Fert 1	14.8	5.8 a	434	55.1 a	46.8	30.8	7.4	158 b	105 b	23.3
20% FC	18.9 a	6.2 a	545 a	60.1 a	57.8 a	41.3 a	9.1	229 a	186 a	25.5
45% FC	13.8 b	5.3 b	403 b	48.5 b	49.4 b	29.1 b	7.5	149 b	117 b	25.6
70% FC	10.0 c	3.7 c	344 c	42.4 b	32.7 c	25.3 b	7.3	152 b	96 b	19.7

*F-Prob <0.05; **F-Prob <0.01; ***F-Prob <0.001; ns = not significant

a, b and c: values with similar letters are not significantly different at F-Prob <0.05

Zinc (Zn), copper (Cu), manganese (Mn), aluminium (Al) and iron (Fe)

The leaf concentrations of Zn, Cu, Mn, Al and Fe were all reduced with increasing water application (Table 5). Stem concentrations of Zn, Mn and Al were also significantly reduced due to increased water supply, and for Cu and Fe a similar tendency appeared (Table 5). Fertiliser application decreased the Al and Mn concentration in stems, whereas it increased concentration of Cu and Al in leaves. There was no interaction between water supply and fertiliser application for any of these elements.

Discussion

Plant growth

Plant growth increased with increasing water supply from 20% to 95% FC in unfertilised plants. Fertiliser application enhanced growth significantly and its effect increased with water supply. However, for fertilised plants, medium water application (c.45% FC) appeared sufficient for maximum growth. It appears as if fertiliser application could, to some degree, substitute for water supply, at least in this P-deficient soil. The P supply to roots is mainly influenced by diffusion (Barber 1962), which in turn is highly dependent on soil moisture. Increased water supply to the unfertilised plants most likely improved P diffusion and thus P supply to roots, whereas in the fertilised plants P supply was improved by the fertiliser addition. Fabião *et al.* (1995) similarly found that fertiliser and or water application increased eucalypt growth and could partly be substituted where biomass production is considered. This does not apply when plants are under severe water stress.

At very low water supply, the effect of fertiliser application on growth decreased, but was still significant when field capacity was fluctuating between 25% and 45%. Even at c.20% FC the effect of fertiliser application was measurable, but only when plants had slowly adapted to the lower water supply (c.20% FC, not in w.45–30% FC). At a field capacity below 20%, however, growth ceased and fertiliser application did not change this. Reduced growth is the first drought response by plants, potentially followed by osmotic adjustment, change in leaf tissue elasticity and relative water partitioning (Ngugy *et al.* 2003).

Photochemical efficiency

The change in photochemical efficiency of mature leaves with time can be examined in terms of three observations:

1. Effect of drought and fertiliser application

Most importantly, the fertilised plants showed lower photochemical efficiency than unfertilised plants under severe drought conditions (treatments w.20% FC and w.30% FC with actual 12–20% FC and 14–30% FC, respectively). A number of authors have shown the functioning of photosystem II, as measured by the photochemical efficiency (F_v/F_m parameter), to be relatively insensitive to medium drought stress (Ögren 1990; Jefferies 1994; Giardi *et al.* 1996; Lu and Zhang 1998) although it changes under more severe water stress (Ögren and Öquist 1985; Methy *et al.* 1996; Pukacki and Kaminska-Rozek 2005). Rolando and Little (2003) have used photochemical efficiency to detect water and light stress in *E. grandis*, and it has been shown to correlate with stem volume increment of black spruce after planting following heat exposure of seedlings (Mohammed *et al.* 1997).

The current data show that the lowest water regime caused severe drought stress in fertilised plants, as measured by photochemical efficiency, but only low drought stress in unfertilised plants. While it has to be recognised that unfertilised plants had less biomass to sustain than fertilised plants, the fertilised plants showed a significantly increased number of necrotic leaves. The considerable daily variation in photochemical efficiency of fertilised plants (Fig. 5) was presumably due to daily variation in heat and drought stress on the plants. It has been shown that chloroplast function, including photochemical efficiency, can be restored when plants experience more favourable conditions after a stress period (Pukacki and Kaminska-Rozek 2005).

Other research also found that fertiliser application under drought condition can reduce drought adaptation, such as affecting osmotic adjustment of *E. grandis*, with P fertiliser having an increasing effect and N fertiliser a decreasing one in a clay loam soil (Graciano *et al.* 2005).

2. Drought preconditioning of seedlings

The effect on photochemical efficiency of maintaining plants at a lower level of water supply differed from that of rapidly reducing water supply. Plants raised under long-term low water supply (e.g. treatment w.20% FC) showed a higher photochemical efficiency than plants raised under greater water supply but subsequent drought (e.g. reducing water supply for w.45% to w.30% FC). These results suggest that preconditioned seedlings may display greater tolerance to water stress than non-conditioned plants, and thus perform better during early establishment in drought-prone areas. Guarnaschelli *et al.* (2003) found that drought-preconditioned seedlings had reduced specific leaf area and increased osmotic adjustment and showed higher productivity and lower mortality in a subsequent growth test.

3. The effect of temperature change

The photochemical efficiency of well-watered plants (those raised at 70% FC, both fertilised and unfertilised) may have been reduced when the glasshouse temperature was reduced. Close *et al.* (2004) have shown that a reduction in average minimum temperature reduced the photochemical efficiency of photosystem II (F_v/F_m) and increased the anthocyanin concentration. Increased anthocyanin concentration was observed in our plants, although mainly in young leaves not measured for photochemical efficiency.

Practical implications

The first two observations have practical implications for seedling production. Where fertiliser increases the drought-susceptibility of seedlings, its application might be reduced in drought-prone areas. This may apply more to N than to P fertiliser, as Graciano *et al.* (2005) have shown that under drought P fertiliser proved beneficial on medium to heavy-textured soil, whereas N fertiliser affected osmotic adjustment in water-stressed plants. Furthermore, drought cycles during seedling production in the nursery appear to improve seedlings' resistance to drought and should be applied.

Water treatments in the current trial are comparable with the soil water status in eucalypt plantations in Australia, although directly comparable data are limited. Soil water content of around 8% (g water g^{-1} soil) has been measured for half the year, and 30–40% in the humid half of the year, in a eucalypt plantation in Tasmania (Paul *et al.* 2003). Those values are approximately comparable to 25% and 95% of field capacity (assuming clay loam soil). Field capacity is dependent on soil texture, and comparisons of field observations with current water treatments are indicative only. In northern NSW, soil water contents of 18–38% (g water g^{-1} soil) with an average of 28% for sandy clay loam soils were measured during eucalypt plantation establishment (D. Thomas, Forests NSW, 2005, *pers. comm.*). Values would approximately compare to 30–100% of field-capacity and 45–60% of field-capacity, respectively, in the current trial. Soil water content during plantation establishment appears to be in a range where plant response to fertiliser application occurred in our study. The severe drought treatment applied will occur under rainfall shortages post planting, when follow-up rains are unreliable.

Nutrient analysis

Phosphorus

It is important to know whether leaf-P or stem-P concentration is the better indicator of the P status of eucalypt plants and which might be used to assess fertiliser needs. In our two-factorial trial the first factor, fertiliser application, significantly enhanced both leaf- and stem-P concentration compared to unfertilised plants. This is consistent with the findings of Dell *et al.* (1987), who also suggested that stem-P concentration was more responsive to P supply. The second factor, increased water supply, decreased leaf-P concentration significantly, particularly for the fertilised plants, whereas stem-P concentration remained mainly unchanged.

Probably due to increased leaf expansion, leaf-P concentration in the fertilised c.70% FC plants was quite low. Stem-P concentration (1492 mg kg^{-1}) and leaf-P concentration (760 mg kg^{-1}) in these plants differed significantly. The question arises as to which plant part is a reliable indicator of the P status of the plant. If it is assumed that substantial translocation of stem-P to leaves will occur during severe P deficiency, then our data suggest that stem-P is a more reliable indicator of the nutritional status of eucalypts, because it reflects previous P supply and is little affected by changes in water supply — in contrast to leaf-P concentration.

The use of stem analysis as an indicator of the P status of a plant needs to be further developed. Previous research suggests that eucalypts are particularly efficient in translocating P within the plant, for example by translocating P from wood during heartwood formation (Hingston *et al.* 1990; Grove *et al.* 1996) and in a form that is readily mobilised (Mulligan 1988). However, Grove *et al.* (1996) considered the temporary seasonal storage of mobile nutrients in the sapwood and bark of eucalypts has not been adequately investigated, and this needs attention when further addressing stem-P analysis.

Potassium (K) and magnesium (Mg)

Stem nutrient analysis also appears to be a useful indicator for the K and Mg status of plants. Leaf- and stem-K concentration were increased by fertiliser and decreased by water application. Due to increases in water supply, there was a gradual decrease in K concentration in leaf *and* stem of unfertilised plants. However, those changes were abrupt in leaves of fertilised plants but remained gradual in stems of those plants.

As a result, interpretations of leaf and stem-K concentration do not agree for the fertilised plants in the higher water treatments (45% FC, 70% FC), with stem analysis suggesting a medium K status of plants and leaf analysis suggesting a high and low K status of plants respectively. The gradual changes in stem-K concentration due to fertiliser and water supply suggest that stem analysis allows a more sensible assessment of the K status of plants.

This also applies to the interpretation of the Mg status of *E. pilularis* seedlings. Changes in stem-Mg concentration were more gradual than those in leaf-Mg concentration. The Mg concentration in both leaf and stem increased in response to

fertilisation, and leaf-Mg concentration decreased in response to water supply. Hence there was a discrepancy in the interpretation of stem and leaf analysis for the fertilised c.70% FC treatment. As the leaf-Mg concentration is most likely prone to growth dilution effects, arguably it appears that stem-Mg concentration shows the Mg status of plants more reliably.

Conclusion

This study has shown that while fertiliser application enhanced seedling growth under medium and high water supply, it also increased the sensitivity of eucalypt seedlings to drought. Sensitivity to drought was less where seedlings were grown at a constant low water supply than where exposed to drought following growth at a higher level of water supply. Drought preconditioning of seedlings and reduced application of fertiliser in the nursery are recommended for seedlings intended for planting on more drought-prone sites.

There was a contrast in the way leaf and stem nutrient concentrations responded to increasing fertiliser and water supply respectively: P application increased both leaf and stem-P concentrations, *but* stem-P concentration was not affected by water supply — in contrast to leaf-P concentration. Hence stem-P concentration may be a better indicator of the P status of seedlings than leaf-P. Similarly the stem concentrations of K and Mg appeared to indicate the nutritional status of *E. pilularis* seedlings more reliably than leaf analysis. It is concluded that the development of stem, bark or phloem nutrient analysis might be further investigated.

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