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Leaf inorganic phosphorus as a potential indicator of phosphorus status, photosynthesis and growth of *Eucalyptus grandis* seedlings

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Abstract

In eucalypts the reduction in CO_2 assimilation and total leaf area at low phosphorus (P) supply is not associated with lower leaf total P concentrations. We tested the hypothesis that the leaf concentration of inorganic phosphorus ($[P_i]$) may be a better indicator of P nutrition status in *Eucalyptus grandis* W. Hill ex Maiden by growing seedlings in P deficient soil supplemented with P supplies ranging from 3 to1000 mg kg^{-1}. Height, biomass accumulation, gas exchange, chlorophyll fluorescence and concentrations of total P ($[P_t]$), organic P ($[P_o]$) and $[P_i]$ of the last fully expanded leaves were measured when harvested at 19 weeks. All parameters of growth increased with larger applications of soil P with most becoming saturated at additions of 500 mg P kg^{-1} soil. Soil P supply had larger effects on biomass and canopy leaf area, by both increased in leaf initiation and expansion, than on CO_2 assimilation (*A*). Leaf $[P_t]$ and $[P_o]$ concentrations were largely invariant to soil P supply and were not correlated with any of the measured growth and photosynthetic parameters. By contrast, leaf $[P_i]$ increased from 171 to 398 mg kg^{-1} with increasing soil P supply. Furthermore, number of leaves, total leaf area and seedling biomass increased exponentially with leaf $[P_i]$, while individual leaf area and *A* increased linearly with leaf $[P_i]$, and quantum yield of photosystem II similarly increased, and non-photochemical quenching decreased, with increasing leaf $[P_i]$. The response of *A* to internal CO_2 concentration (C_i) indicated that at lower P supplies *A* became increasingly restricted by limitations associated with Rubisco and RuBP regeneration. Stomatal limitation may in part be masking the full effect of P supply on *A* as C_i declined with either increasing soil P or leaf $[P_i]$ supply. We conclude that leaf $[P_i]$ was a potentially better indicator than $[P_t]$ or $[P_o]$ for correlating the effects of soil P supply on growth and photosynthesis of *E. grandis*. Furthermore, as *A* achieved at saturating C_a increased with increasing P supply leaf $[P_i]$, these findings suggest that a greatly increased rate of canopy assimilation could be achieved at higher P supply in response to the expected increase in global CO_2 levels.

Keywords: CO_2 assimilation; Canopy growth; Eucalypt; Homeostasis; Leaf growth; Stomatal limitation

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1. Introduction

Phosphorus (P) impoverished soils limit the growth and commercial yield of crops, grasses and forests throughout the world (Conroy et al., 1990; Herbert and Fownes, 1995; Seneweera and Conroy, 1997; Fujita et al., 2003; Thomas et al., 2006). The problem of low soil P is widespread in Australia and is a key issue for plantation forestry because tree growth and wood production from species such as eucalypts is severely restricted at sites where soil P concentrations are low (Cromer et al., 1981; Birk and Turner, 1992; Herbert and Fownes, 1995; Keith et al., 1997; Xu et al., 2002). The use of chemical foliar analysis has successfully been employed to determine P deficiency and manage P fertilizer supply of many crops (Huett et al., 1997; Reuter et al., 1997). Curiously, this has not always been possible for some eucalypts, including *Eucalyptus grandis* W. Hill ex Maiden, because total phosphorus concentration $[P_t]$ of leaves of trees and seedlings are generally, although not always, unaffected by decreasing soil P supply in spite of reductions in tree and seedling growth (Hingston et al., 1979;

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Cromer et al., 1981; Kirschbaum and Tompkins, 1990; Birk and Turner, 1992; Kirschbaum et al., 1992; Keith et al., 1997; Xu et al., 2002). For example, while foliar concentrations of P were invariant at 1280 mg kg^{-1}, height growth of 6-year-old *E. grandis* × *E. urophylla* hybrid saplings doubled and biomass quadrupled as P fertiliser application increased from 13 to 312 kg P ha^{-1} (Xu et al., 2002). Webb et al. (2000) suggest the use of [P_t] in the rachis and petioles may offer an alternative test because this concentration of [P_t] was correlated with volume growth. An alternative prospect is the use of leaf [P_i] in determining the P nutrition of eucalypt species.

While the consequences of low soil P concentrations for tree growth are well documented, the underlying physiological factors have received less attention, especially in eucalypts. Studies have demonstrated that plants respond to low P supplies by reducing total leaf area and leaf CO_2 assimilation rate (*A*). Consequently, canopy CO_2 assimilation rates are lower, less photosynthate is available for transport to sinks such as grain or fruit resulting in lower yields (Kirschbaum et al., 1992; Chiera et al., 2002). The reduction in total leaf area due to low P supply is caused by both a reduction in number of leaves and area of individual leaves (Kirschbaum et al., 1992; Mason et al., 2000; Chiera et al., 2002). The reduction in *A* is thought to be associated with both stomatal limitations (e.g. Kirschbaum and Tompkins, 1990; Jacob and Lawlor, 1991) and the light and dark reactions of photosynthesis (e.g. Jacob and Lawlor, 1993a, 1993b; Barrett and Gifford, 1995; Loustau et al., 1999). Whether similar responses to low P supplies occur in leaves of eucalypts is not known.

We hypothesize that concentration of P_i ([P_i]) in the youngest fully expanded leaves (YFEL) may be a potentially better indicator of photosynthetic function and growth. P_i constituted 29% of total leaf P in leaves of *E. grandis* irrigated with effluent containing high concentrations of P (Hawkins and Polglase, 2000). The P_i is located either in the cytosol where it is a key regulator of *A* or in the vacuole from which it can be translocated to meet the demand of growing sinks (Bieleski, 1973; Brady, 1973; Marschner, 1995). In contrast, the organic phosphorus (P_o) fraction must be hydrolysed to P_i before it can be retranslocated and this only occurs during leaf senescence. Therefore, it is likely that [P_i] in the YFEL may be a potentially better indicator of growth than [P_t].

In this paper we describe the effects of different soil P supplies on leaf [P_i] and leaf [P_t] of *E. grandis*. We relate leaf [P_i] to total leaf area, stem volume and seedling dry mass. Furthermore, we study the relationship between leaf [P_i] and *A* using leaf gas exchange and chlorophyll fluorescence techniques.

2. Materials and methods

2.1. Soil and growth conditions

A randomised design with 10 levels of P supply and five replicates was used. The differing P supply treatments were created by the addition of increasing amounts of P, to an inherently low P soil (Belanglo State Forest, New South Wales). This soil is podsolic derived from Triassic shales and sandstones. Total N is less than 0.09% and exchangeable Ca less than 0.4 me% (Turner, 1982). The *A* horizon used had very low available P, less than 1 mg P kg^{-1} soil (Bray method 1, Bray and Kurtz, 1954). Different soil P supplies were obtained by addition of P, as $CaHPO_4$, to the soil at 3, 8, 18, 38, 70, 100, 175, 250, 500 and 1000 mg P kg^{-1} soil. This equated to between 0.0225 and 7.5 g P per pot. Soil had previously been adjusted to a pH of 6.7 (pH, 1:5 w/v in 0.01 M $CaCl_2$) with $CaCO_3$ (7 g kg^{-1}) and $MgCO_3$ (1.8 g kg^{-1}). Basal nutrients were added to the soil (mg kg^{-1} dry soil): K (90 + 360 as $K_2SO_4 + K_2CO_3$ respectively); B (5 as H_3BO_3); Cu (5 as $CuSO_4$); Zn (10 as $ZnSO_4$); Mo (0.1 as Na_2MoO_4); Mn (50 as $MnSO_4$); Fe (50 as $FeSO_4$). Nitrogen was added weekly at the rate of 67 mg kg^{-1} as KNO_3; $Ca(NO_3)_2$; $Mg(NO_3)_2$; $(NH_4)_2SO_4$ and NH_4NO_3 in the ratio (by weight of salts) of 1:2:1:1:1.

Pots were placed in a naturally sunlit temperature controlled glasshouse located at the University of Western Sydney, Hawkesbury campus, Richmond, NSW (latitude 150°45′E, longitude 33°36′S.). *E. grandis* W. Hill ex Maiden seeds obtained from the Australian Tree Seed Centre were sown directly into 7.5 kg amended soil contained in the 6.9 L pots (PVC pipe diameter 15 cm, height 39 cm). Approximately 40 seedpieces (seeds + chaff) were sown per pot which resulted in between one and seven germinating seedlings per pot. The seedlings were thinned to two seedlings per pot 5 weeks after sowing, and to one seedling per pot 13 weeks after sowing. The remaining seedling was grown for a total of 19 weeks before it was harvested.

Temperature and relative humidity were monitored with microprocessor data loggers (TGP-1500 Tiny-Tag plus, Gemini data loggers, Chichester, UK). Average maximum day temperature was 28 °C and minimum night temperature was 18 °C throughout the 19-week experimental period. Maximum vapour pressure deficit averaged 2.0 kPa during the daylight periods. Throughout the experimental period the pots were watered to field capacity on a daily basis.

2.2. Leaf CO_2 assimilation rates and chlorophyll fluorescence

A and stomatal conductance was measured between 10:00 and 16:00 h on a YFEL of all plants 17–18 weeks after sowing using an open portable photosynthesis system (LI6400 portable, Li Cor Inc., Lincoln, NE, USA) equipped with a light source (6400-02B LED, Li Cor). During measurements of *A* the photosynthetic photon flux density at the leaf surface was maintained at 1200 μmol m^{-2} s^{-1} and leaf temperature was maintained at 27 °C. Ambient humidity of the incoming air to the leaf chamber was left at that of the external glasshouse environment. *A* was measured when ambient CO_2 concentration (C_a) of the incoming gas stream was maintained at 360 μmol mol^{-1} (A_{360}), a value close to those present during plant growth. Similar conditions for measuring leaf gas exchange were maintained during responses of *A* when C_a was increased to 525 μmol mol^{-1} (A_{525}) and to 1500 μmol mol^{-1} (A_{1500}). In addition the response of *A* to