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The influence of nitrogen and phosphorus supply and genotype on mesophyll conductance limitations to photosynthesis in *Pinus radiata*

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Summary Mesophyll conductance, g_m, may pose significant limitations to photosynthesis and may be differentially affected by nutrition and genotype in *Pinus radiata* D. Don. Simultaneous measurements of gas exchange and chlorophyll fluorescence were made to determine g_m, using the constant *J* method (Harley, P.C., F. Loreto, G. Di Marco and T.D. Sharkey. 1992. Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. Plant Physiol. 98:1429–1436), in a fast- and a slow-growing clone of *P. radiata* grown in a greenhouse with a factorial combination of nitrogen (N) and phosphorus (P) supply. Values of g_m increased linearly with the rate of photosynthesis at saturating irradiance and ambient CO_2 concentration, A_{sat} ($g_m = 0.020A_{sat}$, $r^2 = 0.25$, $P < 0.001$) and with stomatal conductance to CO_2 transfer, g_s ($g_m = 1.16g_s$, $r^2 = 0.14$, $P < 0.001$). Values of g_m were greater than those of stomatal conductance, g_s, and the ratio (g_m/g_s) was not influenced by single or combined N and P additions or clone with a mean (±SE) value of 1.22 ± 0.06. Relative limitations to mesophyll conductance, L_m (16%) to photosynthesis, were generally greater than those imposed by stomata, L_s (13%). The mean (±SE) CO_2 concentration in the intercellular air spaces (C_i) was 53 ± 3 μmol mol^{-1} lower than that in the atmosphere (C_a). Mean (±SE) CO_2 concentration in the chloroplasts (C_c) was 48 ± 2 μmol mol^{-1} lower than C_i. Values of L_s, L_m and CO_2 diffusion gradients posed by g_s ($C_a - C_i$) and g_m ($C_i - C_c$) did not significantly differ with nutrient supply or clone. Mean values of V_{cmax} and J_{max} calculated on a C_c basis were 15.4% and 3.1% greater than those calculated on a C_i basis, which translated into different slopes of the J_{max}/V_{cmax} relationship (C_c basis: $J_{max} = 2.11V_{cmax}$, $r^2 = 0.88$, $P < 0.001$; C_i basis: $J_{max} = 2.43V_{cmax}$, $r^2 = 0.86$, $P < 0.001$). These results will be useful for correcting estimates of V_{cmax} and J_{max} used to characterize the biochemical properties of photosynthesis for *P. radiata*.

Keywords: chloroplastic CO_2 concentration, electron transport, nutrient limitation, Rubisco carboxylation, stomatal limitation.

Introduction

The model of photosynthesis originally proposed by Farquhar et al. (1980) is widely used for analysing measurements of photosynthesis for individual leaves and in models to scale carbon exchange for canopies. In this model, photosynthesis is limited by the maximal rate of ribulose-1,5-bisphosphate (RuBP) carboxylase–oxygenase (Rubisco) carboxylation, V_{cmax}, and by the maximal electron transport rate driving the regeneration of RuBP, J_{max} (Farquhar et al. 1980, von Caemmerer and Farquhar 1981, von Caemmerer 2000). These parameters are fitted to the response of photosynthesis, A, to the CO_2 concentration in the intercellular spaces, C_i, known as A/C_i curves. Values of C_i are estimated from stomatal conductance to CO_2 transfer, g_s, and the ambient CO_2 concentration external to the leaf, C_a.

In most studies, the mesophyll conductance of CO_2 from the intercellular spaces to the sites of carboxylation in chloroplasts, g_m, has been considered to be large enough to be negligible (Farquhar et al. 1980). However, there is a growing realization that the significance of g_m in limiting photosynthesis can be similar to the limitation imposed by stomata (Harley et al. 1992, Loreto et al. 1992, von Caemmerer 2000, Warren et al. 2003, Warren and Adams 2006, Flexas et al. 2008). As a result, C_i is greater than the CO_2 concentration at the chloroplasts, C_c, and values of V_{cmax} and J_{max} are underestimated when fitted to estimates of C_i rather than to those of C_c.

Mesophyll conductance has been widely measured in herbaceous plants and angiosperm tree species, but there are few measurements for conifers (e.g., De Lucia et al. 2003, Warren et al. 2003, Warren 2006). While comparisons of g_m between species and plant functional groups (Loreto et al. 1992) have been focused upon, fewer studies have investigated the effects of environmental variables on mesophyll conductance in tree species (e.g., Warren et al. 2004, Grassi and Magnani 2005). Nitrogen (N) and phosphorus (P) supply to leaves affect Rubisco activity (Evans and Terashima 1988) and photosynthesis, and strong relationships between leaf nutrient concentrations and A, V_{cmax} and J_{max} are well established (Conroy et al. 1986, Walcroft et al. 1997, Loustau et al. 1999, Grassi et al. 2002, Bown et al. 2007). However, little is known about the response of g_m to nutrient supply and the implications for the regulation of photosynthesis and the determination of correct values for V_{cmax} and J_{max} for use in models that scale photosynthesis from leaves to canopies. Further, the significance of the regulation of photosynthesis by g_m to account for the differences in growth rate by different genotypes has been largely unexplored in woody plants. To test this possibility, we chose to work on two clones, A and B, of *Pinus radiata* D. Don with known differences in growth rate. Our previous measurements had shown that the rates of diameter, height and foliage area growth were 22, 17 and 29% higher for clone A than for clone B (Bown et al. 2007).

In this study, we used a factorial experiment to measure g_s, g_m, V_{cmax} and J_{max} in two clones of *P. radiata* with known differences in growth rate that were supplied with two rates of N and P. Our objectives were to (i) investigate the effects of N and P supply on g_m, (ii) determine the relative magnitude of limitation to photosynthesis by g_s and g_m, and biochemical processes, (iii) investigate differences in g_m between the two clones with known differences in tree growth rate and (iv) quantify differences in V_{cmax} and J_{max} when calculated on the basis of C_i and C_c.

Materials and methods

Plant material

The experiment was conducted using a factorial design with two clones of *P. radiata*, irrigated with two levels of nitrogen (N_0 = 1.43 and N_1 = 7.14 mol m^{-3}) and phosphorus (P_0 = 0.084 and P_1 = 0.420 mol m^{-3}) supply. Ingestad (1979) suggested that N should be provided at concentrations of 100 ppm (7.14 mM) and P at 13 ppm (0.420 mM) for optimum growth of *Pinus pinaster* Ait. These concentrations were chosen as the high-N and high-P supply regimes. The low-N (1.43 mM) and low-P (0.084 mM) supply regimes were chosen as one-fifth of the high-N and high-P concentrations, respectively. Nitrogen was provided as NH_4NO_3, while P was provided as KH_2PO_4. Nutrients other than N and P were provided at concentrations of 0.51 mol m^{-3} K, 0.25 mol m^{-3} Ca, 0.41 mol m^{-3} Mg, 0.28 mol m^{-3} S, 12.53 mmol m^{-3} Fe, 0.45 mmol m^{-3} Zn, 0.47 mmol m^{-3} Cu, 7.28 mmol m^{-3} Mn, 0.073 mmol m^{-3} Mo, 18.50 mmol m^{-3} B, 0.85 mmol m^{-3} Cl and 0.13 mmol m^{-3} Na, following Ingestad (1979). The clones with a fast (clone A) and a slow (clone B) rate of growth were selected from a set of 400 genotypes planted in the Purokohukohu Experimental Basin (Beets et al. 2004). Further details of the clones have been described previously by Bown et al. (2007).

One-year-old *P. radiata* cuttings from clones A and B were raised under standard nursery conditions and transplanted to 4.3-l pots containing silica sand during the first year of growth. The roots of all plants were artificially inoculated with the spores of *Rhizopogon rubescens* Tul. and the presence of mycorrhizae was confirmed either by the visual inspection of roots or by the presence of fruiting bodies. At the end of the first year, plants were transplanted to 42-l pots and were grown for another year. Nutrient treatments were allocated randomly to the plants and applied for 24 months. All plants received 0.5 l of nutrient solution per week during the first year, and this amount was doubled during the second year. All plants were supplied with water daily with the same amounts in excess of requirements, and the pots were allowed to drain freely. Plants were grown in a thermostatically controlled greenhouse where mean (±SE) air temperature during the day was 18 ± 4 °C and at night 15 ± 4 °C. The plant material was the same as that used in our earlier investigation of the effects of nutrient supply and clone on photosynthesis parameters for *P. radiata* (Bown et al. 2007).

Gas exchange measurements

Simultaneous measurements of gas exchange and chlorophyll fluorescence were carried out on six trees per treatment for each clone (48 plants) 18 months after the experiment was started. Trees were moved from the greenhouse to a thermostatically controlled room set at 20 °C the day before the measurements were done. All measurements were done with a portable photosynthesis system equipped with an integrated chlorophyll fluorescence and gas exchange chamber (Model LI-6400-40, Li-Cor, Lincoln, NE).

For each tree, three fascicles were placed across the 200-mm^2 cuvette to avoid shading between needles. Temperature in the cuvette was maintained at 20 °C, while the leaf-to-air vapour pressure deficit was maintained below 1 kPa. The needles were left to equilibrate for 10 min at a CO_2 concentration of 360 μmol mol^{-1} and saturating irradiance (1500 μmol m^{-2} s^{-1}), before measuring the response of photosynthesis, A, to intercellular CO_2 concentration, C_i. External CO_2 concentration, C_a, was supplied in steps at the set points 360, 300, 200, 150, 125, 100, 75, 50, 360, 450, 600, 800, 1000, 1200 and 1500 μmol mol^{-1}, with a saturating irradiance, Q (400–700 nm), maintained at

1500 μmol m^{-2} s^{-1}. Measurements were recorded after the values of A, C_i and g_s were stable, but with a minimum waiting time of 4 min at each step.

At each value of C_i, measurements of fluorescence for the light-adapted foliage were done simultaneously, and the measurements of F' and F_m' were used to calculate the photochemical efficiency of photosystem II, Φ_{PSII}.

Values of the rate of mitochondrial respiration in the light, R_d, and the chloroplastic CO_2 compensation point, Γ^*, were estimated for each sample using the Laisk method (von Caemmerer 2000). The A/C_i response was measured at three levels of low irradiance (Q = 50, 100 and 300 μmol m^{-2} s^{-1}) for seven decreasing values of C_a from 200 to 0 μmol mol^{-1}. Linear relationships between A and C_i were fitted, and the point of intersection of the three lines allowed the estimation of R_d and Γ^*.

The photosynthesis model described by Farquhar et al. (1980) was fitted to the A/C_i and A/C_c curves by non-linear least-squares regression (SigmaPlot, Version 7.1, SPSS, Chicago, IL). Values of V_{cmax} and R_d were estimated from the lower part of the A/C_i or A/C_c curve (C_i < 220 μmol mol^{-1}), and these values were then used to estimate J_{max} over the entire range of measured C_i or C_c. The Michaelis–Menten constants of Rubisco for CO_2 and O_2, K_c and K_o, used in the fitting (at a temperature of 20 °C) were 231.2 μmol mol^{-1} and 213.9 mmol mol^{-1}, respectively (Bernacchi et al. 2001).

Calculations of mesophyll conductance

Mesophyll conductance, g_m, was estimated using the 'constant J' method (Harley et al. 1992, Loreto et al. 1992), where J is the rate of electron transport. This method uses the data in the RuBP-regeneration limited portion of the A/C_i curve, where the rates of electron transport are constant. Within this region, further increases in photosynthesis with increasing C_i are due to the suppression of photorespiration as the rate of carboxylation progressively substitutes the rate of oxygenation. Thus, photosynthesis is related to C_c and the relative CO_2/O_2 specificity of Rubisco, normally described by the chloroplastic CO_2 compensation point, Γ^*. The constant J method is sensitive to errors in both the rate of mitochondrial respiration in the light, R_d, and Γ^*, and the approach assumes that both J and g_m are constant across the range of C_a concentrations used for the measurements (Harley et al. 1992, Pons et al. 2009). The relationship of g_m with Γ^*, R_d and intercellular CO_2 compensation point in the absence of day respiration, C_i^*, is given by $\Gamma^* = C_i^* + R_d/g_m$ (von Caemmerer 2000, Peisker and Apel 2001).

The photochemical efficiency of PSII (Φ_{PSII}) was estimated from chlorophyll fluorescence measurements as $(F_m' - F)/F_m'$, where F and F_m' are the steady and maximal fluorescence in the light-adapted sample, respectively (Schreiber et al. 1994). The values of Φ_{PSII} are directly proportional to the rate of electron transport through PSII, and therefore can be used to determine the portion of the A/C_i curve where the rate of electron transport is constant (Genty et al. 1989). Optimal values for g_m were resolved iteratively from three or more measurements of photosynthesis at high values of C_i that correspond with constant rates of electron transport (Singsaas et al. 2003, Warren 2006). This was done using the solver add-in for Microsoft Excel with measurements of A, C_i, Γ^* and R_d to resolve the value of g_m that best explained changes in photosynthesis with changes in C_i indicated by a minimum variance in J (Harley et al. 1992, Singsaas et al. 2003, Warren 2006).

Stomatal and mesophyll limitations to photosynthesis

The A/C_i response and the values for g_s and g_m were used to partition stomatal, mesophyll and biochemical limitations to photosynthesis. Values of stomatal conductance to CO_2 transfer were calculated by dividing values for water vapour transfer by 1.64 (Jones 1992), accounting for the difference in rates of diffusion of water vapour and CO_2 in air. Relative stomatal limitations were calculated using the Farquhar and Sharkey method (1982) as $L_s = 1 - A_{a\text{-}gs}/A_{i\text{-}gs}$, where $A_{a\text{-}gs}$ and $A_{i\text{-}gs}$ are the actual value of A and the value estimated when g_s is infinite, respectively. Similarly, following Bernacchi et al. (2002), relative limitation to photosynthesis imposed by g_m was calculated as $L_m = 1 - A_{a\text{-}gm}/A_{i\text{-}gm}$, where $A_{a\text{-}gm}$ and $A_{i\text{-}gm}$ are the actual value of A and the value estimated when g_m is infinite, respectively. The CO_2 concentration in the chloroplasts, C_c, was calculated from $C_c = C_i - A/g_m$.

Foliage surface area and nutrient concentrations

All measurements are presented on a hemi-surface area basis. Following the completion of A/C_i curves, the foliage surface area of the needles used for each measurement was calculated for fascicles consisting of three needles from $[nld(1 + \pi/3)]/2$, where d is the fascicle diameter, l is the fascicle length and n is the number of needles in the sample. Foliage samples were dried at 70 °C to constant dry mass and were used to calculate the foliage area to mass ratio, S. Foliage samples were finely ground, acid-digested by the Kjeldahl method, and the N and P concentrations were determined colorimetrically (Blakemore et al. 1987). Foliage concentrations are expressed on a hemi-surface area basis (N_a, P_a) and photosynthetic nitrogen-, E_N and phosphorus-, E_P use efficiencies were defined as A/N_a and A/P_a, respectively.

Data analysis

All analyses were undertaken at the tree level using SAS software (2000; SAS Institute, Cary, NC). Variables were tested for normality and homogeneity of variance, and transformations were made as necessary to meet the underlying statistical assumptions of the models used. The main and interactive effects of N and P supply and genotype

on g_m and the associated variables were examined by analysis of variance. Tukey's least significant difference test was used to distinguish among individual mean values where applicable with a confidence level of $P \leq 0.05$. Differences in the slopes and the intercepts in the relationships between mesophyll conductance and both light-saturated photosynthetic rates and stomatal conductance were tested for significance between the clones using analysis of covariance. The analysis of covariance was also applied to the J_{max}/V_{cmax} linear relationship to test the influence of nutrient treatment and genotype.

Results

Treatment effects on foliar nutrient concentrations

Foliage N and P concentrations increased in relation to the rates of supply ($F_{3,40} > 40$, $P < 0.001$) (Table 1). At the tree level, observed N_a ranged almost fivefold from 38 to 184 mmol m^{-2}, whereas P_a ranged almost eightfold from 1.2 to 9.4 mmol m^{-2}. The N_a/P_a ratio ranged 18-fold from 5 to 91 (mole basis). Values of N_a/P_a did not differ significantly between the N_0P_0 and N_1P_1 treatments with a mean ($\pm$SE) value of 26.3 $\pm$ 1.1. However, the N_a/P_a ratios for trees in the N_0P_1 and N_1P_0 treatments differed significantly with all other treatments with mean ($\pm$SE) values of 8.7 $\pm$ 0.8 and 71.5 $\pm$ 2.6, respectively (data not shown). Values of N_a, P_a and their ratio were not influenced by the clone ($F_{1,40} < 0.38$, $P > 0.54$).

Treatment effects on rates of photosynthesis

The rate of photosynthesis at saturating irradiance and ambient CO_2 concentration, A_{sat}, increased significantly with N additions ($F_{3,40} = 9.2$, $P < 0.001$) from 6.9 μmol m^{-2} s^{-1} in low-N treatments to 9.6 μmol m^{-2} s^{-1} in high-N treatments, independent of clone ($F_{1,40} = 3.2$, $P = 0.08$). Phosphorus additions only slightly enhanced the rate of photosynthesis (Table 1).

Photosynthetic nitrogen-, E_N and phosphorus-, E_P use efficiencies were strongly influenced by nutrient supply ($F_{3,40} > 9.9$, $P < 0.001$) not differing between clones ($F_{1,40} < 0.12$, $P > 0.73$) (Table 1). Values of E_N were similar in nutrient treatments other than the low value for the N_1P_0 treatment. Values of E_P were similar in the balanced treatments N_0P_0 and N_1P_1, and were intermediate between values for the imbalanced treatments N_0P_1 and N_1P_0 (Table 1).

Stomatal and mesophyll conductances

Mesophyll conductance (g_m) increased significantly with nutrient supply ($F_{3,40} = 5.49$, $P = 0.003$) (Table 2), being 26% higher in the high-N high-P supply regime than in the low-N low-P supply treatment. Although they did not differ significantly, values of g_m for trees in the N_1P_0 treatment were 13% higher than the g_m values for trees in the N_0P_1 treatment.

Although stomatal conductance to CO_2 transfer, g_s, was not significantly influenced by nutrient treatment, clone or their interaction, g_s increased with increasing nutrient supply (Table 2). Values of g_s for trees growing in the N_1P_0 treatment were 18% higher than those for trees growing in the N_0P_1 treatment. Mean values of g_m were greater than those of g_s, and the mean ratio (g_m/g_s) was ($\pm$1 SE) 1.22 $\pm$ 0.06, not being significantly affected by nutrient treatment ($F_{3,29} = 0.68$, $P = 0.57$) or clone ($F_{1,29} = 0.22$, $P = 0.65$) (Table 2).

Table 1. Foliage area to mass ratio, S, N and P concentrations on an area basis (N_a and P_a), light-saturated rate of photosynthesis, A_{sat}, and photosynthetic nitrogen- E_N and phosphorus-use efficiency, E_P across nutrient treatments and clones. Nutrient treatments comprised two N supply regimes (N_0 = 1.43 and N_1 = 7.14 mol m^{-3}) and two P supply regimes (P_0 = 0.084 and P_1 = 0.420 mol m^{-3}). Values are presented as mean values ($\pm$1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) is shown as ns, non-significant; ***, significant at $P < 0.001$. Separation of mean values was determined by a Tukey test. Different letters within treatments or clones indicate that the mean values are significantly different at $P < 0.05$.

	S (m^2 kg^{-1})	N_a (mmol m^{-2})	P_a (mmol m^{-2})	A_{sat} (μmol m^{-2} s^{-1})	E_N (μmol mol^{-1} s^{-1})	E_P (μmol mol^{-1} s^{-1})
Treatments						
N_0P_0	9.3 ± 0.6 a	57 ± 3 a	2.4 ± 0.2 a	6.8 ± 0.4 a	125 ± 12 a	2910 ± 245 a
N_0P_1	8.8 ± 0.4 a	58 ± 5 a	6.8 ± 0.4 b	7.0 ± 0.4 a	119 ± 6 a	1027 ± 95 b
N_1P_0	9.5 ± 0.7 a	141 ± 9 b	2.0 ± 0.2 a	9.4 ± 0.6 b	70 ± 7 b	4894 ± 398 c
N_1P_1	8.7 ± 0.7 a	85 ± 5 c	3.0 ± 0.2 a	9.8 ± 0.4 b	117 ± 4 a	3335 ± 176 a
Clones						
A	8.1 ± 0.3 a	82 ± 9 a	3.6 ± 0.4 a	7.8 ± 0.4 a	108 ± 7 a	3001 ± 349 a
B	10.0 ± 0.5 b	90 ± 8 a	3.4 ± 0.4 a	8.8 ± 0.4 a	107 ± 7 a	3172 ± 325 a
ANOVA						
T	ns	***	***	***	***	***
C	***	ns	ns	ns	ns	ns
C × T	ns	ns	ns	ns	ns	ns

Table 2. Comparison of mesophyll, g_m, and stomatal, g_s, conductances to CO_2 diffusion, and their ratio (g_m/g_s), across nutrient treatments and clones. Nutrient treatments comprised two N supply regimes (N_0 = 1.43 and N_1 = 7.14 mol m^{-3}) and two P supply regimes (P_0 = 0.084 and P_1 = 0.420 mol m^{-3}). Values are presented as mean values (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) is shown as ns, non-significant; **, significant at $P < 0.01$. Separation of mean values was determined by a Tukey test. Different letters within treatments or clones indicate that the mean values are significantly different at $P < 0.05$.

	g_m (mmol m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	g_m/g_s
Treatments			
N_0P_0	160 ± 10 a	132 ± 14 a	1.12 ± 0.13 a
N_0P_1	156 ± 10 a	122 ± 14 a	1.29 ± 0.19 a
N_1P_0	176 ± 6 ab	144 ± 10 a	1.24 ± 0.08 a
N_1P_1	202 ± 8 b	172 ± 12 a	1.21 ± 0.08 a
Clones			
A	164 ± 6 a	142 ± 6 a	1.20 ± 0.07 a
B	182 ± 8 a	142 ± 12 a	1.24 ± 0.11 a
ANOVA			
T	**	ns	ns
C	ns	ns	ns
C × T	ns	ns	ns

Relationships between mesophyll conductance and photosynthesis

There were significant positive relationships between g_m and A_{sat} (r^2 = 0.25, $P < 0.001$), g_s (r^2 = 0.14, $P < 0.001$), but not with foliage N ($F_{3,43}$ = 1.33, P = 0.27) or P ($F_{3,43}$ = 1.87, P = 0.15) concentrations. Slopes ($F_{1–3,\ 40–44} < 1.96$, $P > 0.14$) and intercepts ($F_{1–3,\ 40–44} < 0.77$, $P > 0.46$) of the linear relationship of g_m against A_{sat} and g_s were not influenced by nutrient treatment or clone (Figure 1).

Stomatal and mesophyll limitations to photosynthesis

Relative stomatal, L_s, and mesophyll, L_m, limitations to photosynthesis were not significantly influenced by main or interactive effects of nutrient treatment or clone ($F_{7,40} < 1.03$, $P > 0.43$) (Table 3). The mean (±SE) relative limitation imposed by g_m (L_m) was 0.16 ± 0.01 (range 0.07–0.25) and was generally greater than the stomatal limitation (L_s), which was 0.13 ± 0.01 (range 0.03–0.20).

Parameters describing photosynthesis

The mitochondrial CO_2 compensation point in the light, Γ^*, was very similar across all nutrient treatments and clones ($F_{7,40}$ = 0.52, P = 0.81) with a mean (±SE) value of 49.4 ± 1.4 μmol mol^{-1} (Table 4). In contrast, the rate of mitochondrial respiration, R_d, was more variable and tended to increase with nutrient supply ($F_{3,40}$ = 1.87, P = 0.15), independent of clone ($F_{1,40}$ = 2.71, P = 0.06).

The maximum rate of Rubisco carboxylation (V_{cmax}) calculated on a C_c basis increased significantly with nutrient supply ($F_{3,40}$ = 6.65, $P < 0.001$), being 65% greater in the high-nutrient supply treatment (N_1P_1) than in the low-nutrient supply treatment (N_0P_0). Similarly, the rate of electron transport driving RuBP regeneration on a C_c basis (J_{max}) increased significantly with nutrient supply ($F_{3,40}$ = 4.94, P = 0.005) in the low- compared with the high-nutrient supply treatment (55% increase). The values of V_{cmax} and J_{max} in the N_0P_1 and N_1P_0 treatments were

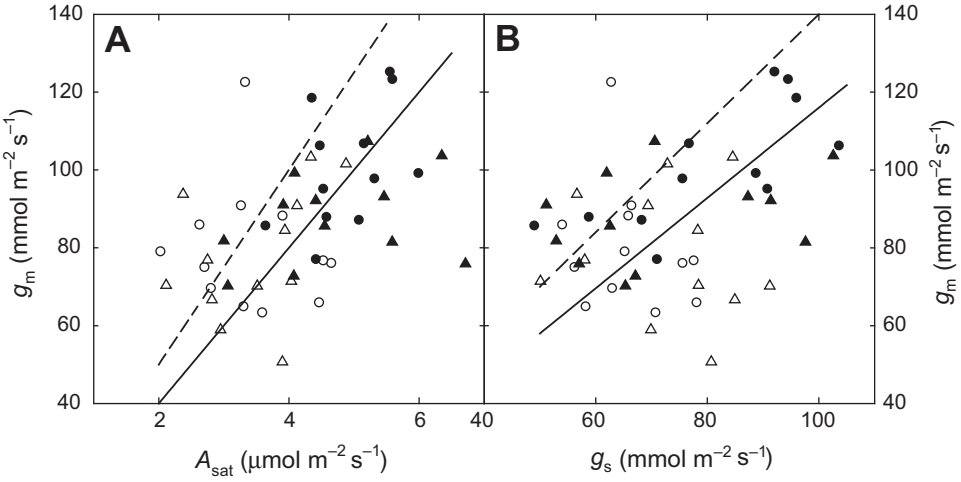

Figure 1. The response of mesophyll conductance, g_m, to (A) the rate of photosynthesis at constant air temperature of 20 °C, saturating irradiance (1500 μmol m^{-2} s^{-1}) and ambient CO_2 concentration, A_{sat} and (B) stomatal conductance, g_s. Fitted (solid) lines are (a) $g_m = 0.020A_{sat}$, r^2 = 0.25, $P < 0.001$ and (b) $g_m = 1.16g_s$, r^2 = 0.14, $P < 0.001$. Slopes and intercepts of the g_m/A_{sat} and g_m/g_s linear relationships did not differ significantly between nutrient treatments or clones ($P > 0.14$). Dashed lines are given by (a) $g_m = 0.025A_{sat}$, r^2 = 0.76 and (b) $g_m = 1.4g_s$, r^2 = 0.80, determined for 15 angiosperm species by Loreto et al. (1992). Nutrient treatments are shown as ○, N_0P_0; Δ, N_0P_1; ▲, N_1P_0 and ●, N_1P_1.

Table 3. Relative stomatal, L_s, and mesophyll, L_m, limitations to photosynthesis across nutrient treatments and clones. Treatments comprised a combination of two N supply regimes (N_0 = 1.43 and N_1 = 7.14 mol m^{-3}) and two P supply regimes (P_0 = 0.084 and P_1 = 0.420 mol m^{-3}). Values are presented as mean values (±1 SE) for each treatment and clone. Differences between clones and treatments were non-significant (ns).

	Relative limitation (%)	
	L_s	L_m
Treatments		
N_0P_0	12 ± 2 a	13 ± 2 a
N_0P_1	13 ± 3 a	16 ± 2 a
N_1P_0	12 ± 1 a	18 ± 1 a
N_1P_1	13 ± 1 a	15 ± 1 a
Clones		
A	11 ± 1 a	15 ± 1 a
B	14 ± 2 a	17 ± 1 a
Mean	13 ± 1	16 ± 1
ANOVA		
T	ns	ns
C	ns	ns
C × T	ns	ns

intermediate between the N_0P_0 and N_1P_1 treatments. Single N additions exerted a greater influence on the values of V_{cmax} (29% increase) and J_{max} (24% increase) than single P additions. Mean values of V_{cmax} and J_{max} were 15% and 20% higher in the slow- (clone B) than in the fast-growing clone (clone A) (data not shown). However, genotype did not influence the values of V_{cmax} and J_{max} when we included the leaf area to mass ratio (M) as a covariate in the analysis (Table 4).

Single or combined N and P additions and genotype did not influence the ratio J_{max}/V_{cmax} ($F_{7,40}$ = 1.16, P = 0.35) with a mean value of 2.15 ± 0.03. Neither slopes ($F_{3,40}$ < 0.24, P > 0.86) nor intercepts ($F_{3,40}$ < 0.23, P > 0.87) of the linear J_{max}/V_{cmax} relationships significantly differ between nutrient treatments and clones (Figure 2). Mean values of V_{cmax} and J_{max} calculated on a C_c basis were 15.4% and 3.1% greater than those calculated on a C_i basis, respectively. As a consequence, the slope of the relationship between J_{max}/V_{cmax} was 13.2% lower when calculated on a C_c rather than a C_i basis.

Discussion

Consistent with our earlier work on *P. radiata* (Bown et al. 2007), we have shown that V_{cmax} and J_{max} increase with increasing foliage N and P concentrations. However, there were no significant effects of foliage N and P concentrations on g_m, even when nutrient supplies were very low. Evans and Terashima (1988) showed that g_m decreased with low N availability in spinach. Our data show that the average increase in foliage N concentration between the lowest (N_0P_0) and highest (N_1P_1) treatment of 49% resulted in an average increase in mesophyll conductance of 26%. This increase in g_m per unit N concentration is very similar to the values for a range of conifers and perennial angiosperms, but less than that for *Eucalyptus globulus* Labill. and much less than the values for annual angiosperms (Warren 2004). In rice, the increased rates of photosynthesis for leaves with

Table 4. The chloroplastic CO_2 compensation concentration in the absence of mitochondrial respiration, Γ^*, the rate of mitochondrial respiration in the light, R_d, the maximal rate of Rubisco carboxylation, V_{cmax}, the maximal rate of electron transport driving regeneration of RuBP, J_{max}, and the ratio J_{max}/V_{cmax} across nutrient treatments and clones. The leaf area to mass ratio was used as a covariate in the analysis of V_{cmax} and J_{max}. Measurements were done at a constant air temperature of 20 °C, and values of V_{cmax} and J_{max} were obtained from A/C_i curves at saturating irradiance (1500 μmol m^{-2} s^{-1}). Nutrient treatments comprised two N supply regimes (N_0 = 1.43 and N_1 = 7.14 mol m^{-3}) and two P supply regimes (P_0 = 0.084 and P_1 = 0.420 mol m^{-3}). Values are presented as mean values (±1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) is shown as ns, non-significant; **, significant at P < 0.01; ***, significant at P < 0.001. Separation of mean values was determined by a Tukey test where applicable. Different letters within treatments or clones indicate that the mean values are significantly different at P < 0.05.

	Γ^* (μmol mol^{-1})	R_d (μmol m^{-2} s^{-1})	V_{cmax} (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})	J_{max}/V_{cmax}
Treatments					
N_0P_0	47.6 ± 2.9 a	1.08 ± 0.06 a	26.8 ± 1.6 a	60.2 ± 4.2 a	2.24 ± 0.08 a
N_0P_1	49.7 ± 2.5 a	1.22 ± 0.18 a	30.8 ± 2.6 ab	66.2 ± 5.2 a	2.15 ± 0.03 a
N_1P_0	50.2 ± 2.8 a	1.22 ± 0.12 a	39.8 ± 4.2 bc	82.2 ± 8.8 ab	2.07 ± 0.04 a
N_1P_1	50.1 ± 3.6 a	1.4 ± 0.18 a	44.2 ± 3.2 c	93.4 ± 7.2 b	2.12 ± 0.05 a
Clones					
A	50.3 ± 2.0 a	1.04 ± 0.07 a	36.4 ± 2.8 a	75.8 ± 5.8 a	2.11 ± 0.05 a
B	48.5 ± 2.1 a	1.42 ± 0.11 a	34.4 ± 2.2 a	75.4 ± 4.8 a	2.18 ± 0.04 a
ANOVA					
T	ns	ns	***	**	ns
C	ns	ns	ns	ns	ns
C × T	ns	ns	ns	ns	ns

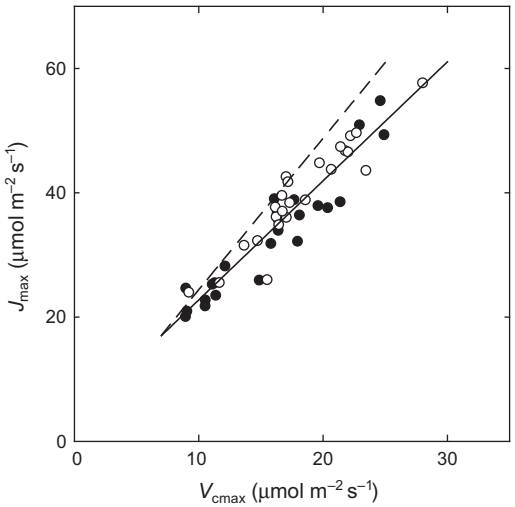

Figure 2. Relationship between the maximum rate of electron transport driving regeneration of RuBP, J_{max}, and the maximum rate of Rubisco carboxylation, V_{cmax}, calculated on the basis of chloroplastic CO_2 concentration, C_c (solid line) and intercellular CO_2 concentration, C_i (dotted lines). On a C_c basis: $J_{max} = 2.11V_{cmax}$, $r^2 = 0.88$, $P < 0.001$; on a C_i basis: $J_{max} = 2.43 V_{cmax}$, $r^2 = 0.86$, $P < 0.001$. Measurements were done at a constant air temperature of 20 °C and saturating irradiance (1500 μmol m^{-2} s^{-1}). Mean values of V_{cmax} and J_{max} calculated on a C_c basis were 15.4% and 3.1% greater than those on a C_i basis. Slopes and intercepts of the linear relationships between V_{cmax} and J_{max} did not differ significantly between clones ($P > 0.87$). Open symbols represent clone A and closed symbols represent clone B for V_{cmax} and J_{max} calculated on a C_c basis (symbols on a C_i basis not shown).

high-N concentration compared with those at low-N concentration were attributed to higher values of C_c at the chloroplasts, resulting from higher values of g_m because of increased chloroplast size (Li et al. 2009).

The limitation to photosynthesis of g_m was small (16%) and similar to the stomatal limitation (13%). The magnitude of these limitations to photosynthesis was also independent of foliage nutrient status. These observations together suggest that the limitations to photosynthesis resulting from low nutrient availability in *P. radiata* are dominantly associated with biochemical processes and much less dependent on stomatal and mesophyll conductances. Further, the lack of differences in V_{cmax}, J_{max}, g_m and limitations to photosynthesis by stomatal and mesophyll conductances between the two clones suggests that differences in growth rate were associated with processes other than photosynthesis, possibly the additional amount of light intercepted by the larger amount and size of needles (Bown et al. 2007).

The values we observed for g_m are consistent with those reported in other studies. The mean value across all treatments was 170 mmol m^{-2} s^{-1}, similar to the value of 153 mmol m^{-2} s^{-1} for *P. radiata* reported by De Lucia et al. (2003). There are few other measurements of g_m in woody species, especially conifers. Warren et al. (2003) measured the values of g_m between 140 and 200 mmol m^{-2} s^{-1} throughout a 33-m tall *Pseudotsuga menziesii* (Mirb.) Franco canopy, and similar values varying from 120 to 170 mmol m^{-2} s^{-1} for needles of different ages were measured in a *P. pinaster* plantation (Warren 2006). Values of g_m were much lower for the slow-growing native conifers *Dacrydium cupressinum* (32 mmol m^{-2} s^{-1}) and *Prumnopitys ferruginea* (G. Benn. ex. D. Don.) de Laub. (52 mmol m^{-2} s^{-1}) in a New Zealand rainforest (De Lucia et al. 2003). Overall, these data are consistent with the values obtained from nine coniferous and 10 broadleaved (Manter and Kerrigan 2004) and a wider range of 122 species (Flexas et al. 2008), showing that g_m is the lowest in evergreen conifers compared with other woody angiosperms and herbaceous plants.

Our mean value for the ratio of g_m/g_s was 1.22, which is close to the value of 1.1 for *P. radiata* reported by De Lucia et al. (2003). The limitation of photosynthesis attributable to g_m in our study was 16% and only slightly larger than the limitation attributable to g_s (13%), with no differences between clones or nutrient supply. Measurements of the limitations to photosynthesis also using the Farquhar and Sharkey method (1982) on mature *P. menziesii* trees showed that the limitation attributable to mesophyll conductance (20%) was less than the stomatal limitation (30%) in well-watered conditions (Warren et al. 2003). In our study, these limitations to photosynthesis are equivalent to a decrease in the CO_2 concentration gradient between C_i and C_c of 48 μmol mol^{-1}, which is within the range given for *P. menziesii* (30–88 μmol mol^{-1}), although this differs substantially between species (Warren and Adams 2006).

An increase in g_m with increasing rate of photosynthesis is consistent with previous findings from a wide range of species (von Caemmerer and Evans 1991, Loreto et al. 1992, Singsaas et al. 2003, Warren et al. 2003). Using the variable J method and carbon isotopes to estimate g_m for 15 angiosperm species, Loreto et al. (1992) estimated the slopes of the relationships between g_m (mmol m^{-2} s^{-1}) and rate of photosynthesis, A_{sat} (μmol m^{-2} s^{-1}), and stomatal conductance, g_s (mmol m^{-2} s^{-1}), to be 1.4 and 0.025, respectively. Our data for *P. radiata* superimposed on those of Loreto et al. (1992) showed very similar slopes of 1.16 for g_s and 0.020 for A_{sat}, at an air temperature of 20 °C, ambient CO_2 concentration, saturating irradiance and air saturation deficit below 1 kPa (Figure 1). However, Warren and Adams (2006) pointed out that the values of g_m at the same rates of photosynthesis can be quite variable and that plants may have low values of g_m, even in conditions of adequate water and nutrient supply. This causes differences in the magnitude of the gradient between C_i and C_c and results in an imperfect scaling between g_m and A_{sat}.

During biochemical limitation of photosynthesis, the differences in nutrient supply (Warren 2004) or illumination in canopies (Warren et al. 2003) are much larger than the differences in g_m, which results in changes in the degree of limitation of photosynthesis by g_m. We were not able to resolve such differences between the two clones and with differences

in nutrient supply, in part likely due to low precision in measurements (Warren and Adams 2006).

Harley et al. (1992) showed that the estimates of g_m using the constant J method are highly sensitive to errors in the estimation of Γ^*, such that 10% over- and underestimates in Γ^* led to errors of +92% and −32% in g_m, respectively. Our mean values of Γ^* (measured at 20 °C) for *P. radiata* were 49 μmol mol^{-1} and about 7 μmol mol^{-1} greater than the mean value of C_i^*. Piel et al. (2002) found Γ^* to be 51 μmol mol^{-1} and about 3 μmol mol^{-1} higher than those of C_i^* in *Juglans regia* L., while Warren (2006) found Γ^* to be 67 μmol mol^{-1} and about 15–24 μmol mol^{-1} higher than those of C_i^* in *P. pinaster*, but the measurements in both these studies were done at 25 °C. The response of g_m to temperature is sensitive to the value of Γ^* used in the calculations (Warren 2008). Overall, the value of Γ^* is similar across C_3 species (Evans and Loreto 2000), but the sensitivity of g_m to errors in Γ^* increase with increasing g_m and independent estimates of Γ^* under the same experimental growing conditions help to reduce errors (Pons et al. 2009). To test this sensitivity, we refitted the values of g_m using the values of C_i^* rather than those of Γ^*, and we found that the trends were similar to those obtained using Γ^* (Table 4), with only treatment effects being significant ($F_{3,40} = 5.23$, $P = 0.004$); but the mean values of g_m calculated using C_i^* were 9% lower than the values of g_m calculated using Γ^*. We are also aware that R_d could have been overestimated due to diffusion of CO_2 from the gasket of the leaf chamber (Pons and Welschen 2002), but this effect would have been minimized by the adjustment of flow rates to maintain a reasonable difference in CO_2 concentration across the chamber (Pons et al. 2009).

Most values of V_{cmax} and J_{max} reported in the literature are calculated from A/C_i response curves, rather than A/C_c curves, with the implicit assumption that mesophyll conductance is infinitely large. When this assumption is invalid, values of V_{cmax} and J_{max} are underestimated (Harley et al. 1992, Loreto et al. 1992, von Caemmerer 2000, Long and Bernacchi 2003, Ethier and Livingston 2004, Manter and Kerrigan 2004, Ethier et al. 2006). In our study, the difference in CO_2 concentration of 48 μmol mol^{-1} between C_i and C_c resulted in increased values of V_{cmax} and J_{max} of 15.4% and 3.1%, respectively, less than the increase of up to 47% for V_{cmax} reported for *P. menziesii* by Warren et al. (2003). Further, this decreased the slope of the J_{max}/V_{cmax} relationship (C_c basis: $J_{max} = 2.11V_{cmax}$, $r^2 = 0.88$, $P < 0.001$; C_i basis: $J_{max} = 2.43V_{cmax}$, $r^2 = 0.86$, $P < 0.001$), which is consistent with the findings of Singsaas et al. (2003).

In conclusion, our investigation of the effects of N and P supply on mesophyll conductance in *P. radiata* showed that g_m increased with N and P supply but, because of concomitant increases in the rates of photosynthesis, the degree of limitation attributable to mesophyll conductance did not change between treatments. Loustau et al. (1999) showed that P deficiency resulted in reductions in V_{cmax} and J_{max} in *P. pinaster*, but the effect could be partly attributable to changes in mesophyll conductance. Our work is the first attempt to measure the effects of P supply on mesophyll conductance, and our results add to the understanding of the response of g_m to the supply of nutrients. Further, there were no differences in g_m or mesophyll limitation of photosynthesis between two clones of *P. radiata*, suggesting that differences in growth rate are independent of differences in limitations to photosynthesis from mesophyll conductance. The CO_2 gradient posed by g_m was constant at 48 μmol mol^{-1}, which led to a serious underestimation of 15.4% and 3.1% in V_{cmax} and J_{max} values when expressed on a C_i rather than on a C_c basis. While estimates of V_{cmax} and J_{max} on a C_i basis are widely used in models that scale estimates of photosynthesis from leaves to canopies, the use of corrected estimates on a C_c basis enables future models to incorporate limitations to photosynthesis that are not derived solely from carboxylation and electron transport processes.

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