

The relative limitation of photosynthesis by mesophyll conductance in co-occurring species in a temperate rainforest dominated by the conifer *Dacrydium cupressinum*

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Abstract. The capacity to conduct CO₂ from the intercellular spaces in leaves to the site of fixation (mesophyll conductance, g_m) may pose a significant limitation to photosynthesis. *Dacrydium cupressinum* Sol. ex Lamb. (rimu), a native conifer of New Zealand, and other members of the Podocarpaceae evolved during the Jurassic when the partial pressure of CO₂ exceeded 200 Pa. This species has low rates of photosynthesis and high levels of leaf nitrogen, which have led to the hypothesis that low g_m restricts photosynthesis. Mesophyll conductance was estimated from gas-exchange and fluorescence measurements for this and other co-occurring tree species [*Prumnopitys ferruginea* D. Don (miro), *Weinmannia racemosa* L.f. (kāmahi), *Meterosideros umbellata* Cav. (rata)]. *Pinus radiata* D. Don (radiata pine) and *Phaseolus vulgaris* L. (bean) were included to provide comparisons with a rapidly growing tree and herbaceous plant with relatively high photosynthetic rates. Mesophyll conductance was not statistically different among indigenous tree species but was lowest for *D. cupressinum*. This species also had the lowest ratio of mesophyll to stomatal conductance, g_m/g_{st} and was the only species where the decline in partial pressure of CO₂ was greater from the intercellular air space to the site of fixation (16.3 Pa) than between the bulk air and the intercellular spaces (8.8 Pa), providing support for the hypotheses that low g_m limits photosynthesis in this species. As a group, conifers had marginally lower g_m and g_m/g_{st} ratio than angiosperms, but this difference was strongly influenced by the high values for *Phaseolus vulgaris*. That co-occurring members of the Podocarpaceae operated differently suggests that low g_m may reflect a response to evolutionary pressures other than high atmospheric CO₂ partial pressure.

Keywords: CO₂ compensation point, day respiration, gas exchange, intercellular CO₂.

Introduction

Rates of photosynthesis in leaves are co-limited by several biochemical, physiological and morphological characteristics. In conditions of saturating irradiance and moderate temperature, the catalytic capacity of ribulose-1,5-bisphosphate (rubisco) ultimately limits carbon uptake in C₃ plants (Sage 2002). However, several diffusive conductances impede the transfer of carbon dioxide along the gradient in partial pressure from the bulk atmosphere around the leaf to the site of fixation by rubisco (Nobel 1999; Evans and Loreto 2000). After transfer across the boundary layer surrounding the leaf, the diffusion of CO₂ becomes regulated by the stomata and the conductive properties of the mesophyll. The importance of g_m to CO₂ transfer in regulating the rate of photosynthesis is not well understood,

in part, because it is difficult to measure. Substantial variation in the relationship between net photosynthesis and leaf nitrogen concentration (Field and Mooney 1983) and in the ratio of CO₂ intercellular partial pressure to the partial pressure in the bulk atmosphere (Farquhar *et al.* 1982; Vitousek *et al.* 1990) suggest that at least for some species, g_m may impose a significant limitation on photosynthesis.

Epron *et al.* (1995) hypothesised that the low rates of photosynthesis in some tree species may be attributed to low g_m , and a recent study by Warren *et al.* (2003) indicates that g_m limits photosynthesis by approximately the same magnitude as stomatal conductance for douglas-fir trees (*Pseudotsuga menziesii*). The suggestion that g_m disproportionately limits photosynthesis in trees has, however, been challenged on the grounds that difference in CO₂ partial

Abbreviations used: c_a , CO₂ partial pressure in atmosphere; c_c , CO₂ partial pressure in chloroplast; c_i , CO₂ partial pressure in leaf internal airspace; g_m , mesophyll conductance; g_{st} , stomatal conductance; PFD, photon flux density.

pressure between the internal air spaces in leaves (c_i) and at the site of fixation in the chloroplasts (c_c) is not uniformly larger for tree species than other plants, and indeed, other life forms (Evans and Loreto 2000), although measurements on woody species are few. Support for the suggestion that that g_m may limit photosynthesis in the 'primitive' evergreen conifer *Dacrydium cupressinum* (rimu) that dominates forests in South Westland, New Zealand (Norton *et al.* 1988; Whitehead *et al.* 2002) results from the observation that it has low rates of photosynthesis at relatively high leaf nitrogen concentration and presumably rubisco content (Whitehead *et al.* 2003; F. Carswell personal communication).

The light-saturated rate of net photosynthesis for *D. cupressinum* in field conditions is quite low (approximately $2.7 \mu\text{mol m}^{-2} \text{s}^{-1}$) compared with co-dominant species in the temperate rainforests of South Westland, New Zealand ($2.8\text{--}13 \mu\text{mol m}^{-2} \text{s}^{-1}$; D. Tissue personal communication). A member of the family Podocarpaceae, *D. cupressinum* diverged from the Glossopterids during the Jurassic period, 190–144 million years ago, when conditions were uniformly warm and moist (Frakes 1979; Mildenhall 1980). A reconstruction of the paleo-atmosphere indicates that the partial pressure of carbon dioxide in the atmosphere during this period exceeded 200 Pa (Retallack 2001). Such high values may have minimized the importance of CO_2 as a selective agent for efficient transfer conductance from the atmosphere to the binding sites on rubisco. Geographic isolation and perhaps reduced competitive pressure on *D. cupressinum* and other Southern Hemisphere conifers from Northern Hemisphere conifers for the past 190 million years (Ogden and Stewart 1995) may have facilitated their survival as 'photosynthetic relics,' preserving the functional properties adapted to a high- CO_2 environment.

Using a comparative approach, our objective was to test the hypothesis that low g_m contributes to low observed rates of photosynthesis for *D. cupressinum*. Mesophyll conductance was estimated from gas exchange and fluorescence measurements for this species and three other co-occurring tree species consisting of the sub-dominant conifer *Prumnopitys ferruginea* (miro), and two broadleaved angiosperms: *Weinmannia racemosa* (kāmahi) and *Meterosideros umbellata* (rata). We also included *Pinus radiata* (radiata pine) and *Phaseolus vulgaris* (bean) to provide comparisons with a rapidly growing tree and herbaceous plant with relatively high photosynthetic rates. *D. cupressinum*, *Prumnopitys ferruginea* and *Pinus radiata* are members of the class Coniferales (Allan 1961). Both *W. racemosa* and *M. umbellata* co-occur ecologically with *D. cupressinum* but they are removed phylogenetically and have very different leaf morphologies. *M. umbellata* is in the family Myrtaceae while *W. racemosa* is in the family Cunoniaceae (Allan 1961).

Materials and methods

Seedlings (0.25–0.5 m in height) of *Dacrydium cupressinum* Sol. ex Lamb. and *Prumnopitys ferruginea* D. Don, were collected from the understory of a mixed conifer-angiosperm forest near Okarito, New Zealand (latitude 43.2°S , longitude 170.3°E) and grown in forest soil in a shade house (50% shade) before the experiment. Small plants for the other tree species were purchased from a nursery. Three months before the experiment, all plants were transplanted into potting mix in 10-L containers and grown in a shade house. *Phaseolus vulgaris* L. plants were grown from seed in 5-L containers for a month before the experiment in an adjacent glasshouse. All plants were well supplied with a full complement of nutrients at monthly intervals and were well watered.

This suite of species has strongly contrasting leaf morphologies that potentially influence g_m . *D. cupressinum* has small, keeled, scale-like imbricate leaves 0.5–1 mm wide surrounding the stem (Allan 1961). The foliage of *Prumnopitys ferruginea* consists of flat, distichous leaves up to 30 mm in length (Allan 1961), and *Pinus radiata* D. Don consists of three needles forming a cylindrical fascicle. Leaves of *Weinmannia racemosa* L.f. and *Meterosideros umbellata* Cav. are broad, sclerophyllous and bifacial up to 20 mm wide and 40 mm long. *Phaseolus vulgaris* has a mesophytic leaf typical of C_3 agronomic species.

For comparative purposes all foliage areas, rates of gas exchange and conductances in this paper are expressed on the basis of half the total surface area (hemi-surface area). This was obtained from measurements of projected foliage areas for *D. cupressinum* using digital photographs analysed with digitizing software (Scion Image Beta 3b Win, Scion Co., Frederick, MD). These estimates were converted to hemi-surface area using an allometric multiplier of 1.66 determined by F. Carswell (personal communication). Total surface areas for *Pinus radiata* needles were obtained from measurements of average fascicle diameter and length using geometric calculations and these were multiplied by 0.5 to give hemi-surface area. Foliage for the other species was flat and measurements of hemi-surface area were made directly from digital photographs analysed with digitizing software.

Plants were moved into a controlled environment cabinet [irradiance $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (PPFD 400–700 nm), day and night temperatures of 20 and 15°C , respectively, day length 14 h] 24 h before gas-exchange measurements.

Rates of gas exchange were measured on fully expanded, attached leaves with an open-path portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE) equipped with an integrated fluorescence detector (LI-6400-40 leaf chamber, Li-Cor Inc.). An actinic irradiance of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ (400–700 nm) was provided by blue and red photodiodes and the intensity and duration of the saturating pulses for fluorescence measurements were optimized for each species. The temperature of the leaf chamber was maintained at 20°C , resulting in leaf temperatures of approximately 21°C . Leaf temperature was measured with a fine-wire thermocouple touching the abaxial surface for the broad-leaved species and an energy balance approach was used to estimate leaf temperature from air temperature for the species with complex leaf geometry. Water vapour pressure deficit in the leaf chamber was maintained at a low level of 0.5–1.0 kPa. The angiosperm species were primarily hypostomatal (percentage of stomata on the abaxial surface *W. racemosa*, 100%; *M. umbellata*, 100%; *Phaseolus vulgaris*, 80%); with the exception of *Prumnopitys ferruginea* (80% on the adaxial surface), the conifers were amphistomatal (J. Hunt personal communication; Townrow 1965).

Mesophyll conductance was calculated by the 'constant J ' method described by Harley *et al.* (1992), Loreto *et al.* (1992), Singaas *et al.* (2003) and Long and Bernacchi (2003). This method is based on the premise that once the rate of photosynthetic electron transport

becomes constant, further increases in photosynthesis with increasing intercellular CO₂ partial pressure represent the rate of carboxylation supplanting the rate of oxygenation. Given that the properties of rubisco are relatively constant, g_m is estimated empirically as the value that gives the best description (minimum variance) of the changes in photosynthesis with changes in CO₂.

Gas exchange and chlorophyll fluorescence were measured simultaneously over a range of CO₂ partial pressures (approximately 5–140 Pa), and the rate of net photosynthesis, A , was plotted as a function of CO₂ partial pressure in the sub-stomatal air space (c_i). The photochemical efficiency of photosystem II (Φ_{PSII}) calculated from the fluorescence measurements is directly related to the rate of electron transport through photosystem II (Genty *et al.* 1989) and was used to identify the portion of the A/c_i curve where the rate of electron transport was constant.

Mesophyll conductance was estimated with the following equation from Harley *et al.* (1992):

$$J = (A + R_d) \times \frac{4 \left[\left(c_i - \frac{A}{g_m} \right) + 2\Gamma^* \right]}{\left(c_i - \frac{A}{g_m} \right) + \Gamma^*}, \quad (1)$$

where J is the rate of electron transport, A is net photosynthesis, R_d is the rate of mitochondrial respiration in the light and Γ^* is the CO₂ compensation point in the absence of day respiration (R_d). The rate of electron transport was calculated for three or more photosynthetic rates measured above the values of c_i yielding constant rates of electron transport. These calculations were repeated with different values of g_m to find the value that minimized the variance:

$$\sum_{i=1,n} \frac{(J_a - J_i)^2}{(n-1)} \quad (2)$$

where J_a is the average value of each of the measured values of J_i .

The dissolution of gas in the liquid phase is a function of its partial pressure in the adjoining air, and because diffusion in the liquid phase contributes substantially to g_m , the driving force is most appropriately expressed using partial pressure, leading to units for g_m of 'mol m⁻² s⁻¹ bar⁻¹' (Harley *et al.* 1992). We have used these units to provide consistency with the literature (von Caemmerer and Evans 1991; Evans and Loreto 2000).

Particularly when g_m is low, estimated values are sensitive to variation in Γ^* and R_d (Harley *et al.* 1992). These parameters were

therefore estimated for each species by the method of Laik and Oja (1998). The A/c_i response was measured at three different irradiances (in most cases: 30, 120 and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and extrapolation of the linear relationships between A and c_i to give the point of intersection on the A axis is the CO₂ and light-independent rate of photosynthesis and represents the rate of 'day' respiration (R_d). Extrapolation of the lines to the c_i axis was taken as the CO₂ compensation point in the absence of day respiration (Γ^*). Care was taken to use only those points on the linear portion of the curves, typically at c_i values below approximately 15 Pa.

Following the gas-exchange measurements, leaf samples were harvested and dried at 70°C to constant mass. Specific leaf area (S) was calculated as the quotient of leaf area and mass, and leaf nitrogen concentration of ground leaf tissue was measured with an elemental analyzer (NA1500, Carlo Erba, Milan, Italy).

Average values of gas-exchange parameters and leaf chemical and morphological properties were compared across species with an ANOVA (SYSTAT ver. 7.0 for Windows; SPSS, Inc.) and a Bonferroni *post hoc* test with a significance limit of $P \leq 0.05$. Comparisons of photosynthetic parameters and leaf properties between angiosperms and conifers were made using a *t*-test. Where appropriate, data were transformed to meet the assumptions of normality.

Results

Photosynthetic rates varied among species. Though not significantly different from *Prumnopitys ferruginea* or *M. umbellata*, the lowest rate of photosynthesis was for *D. cupressinum*. The highest rate was for *Phaseolus vulgaris* (Table 1). The average rate of photosynthesis, measured at 36 Pa CO₂ and an irradiance of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PFD, was higher for the angiosperms as a group than the conifers (conifers: 7.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$; angiosperms 10.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $P < 0.05$). Angiosperms had significantly higher photosynthetic nitrogen-use efficiency, ϵ , nitrogen concentration on a mass basis, N , and specific leaf area, S , but lower nitrogen concentration per unit leaf area, N_{area} , than the conifers, but the mean values of S and N were inflated by the very high values for *Phaseolus vulgaris*. The relatively low rates of photosynthesis for the two conifers, *D. cupressinum* and

Table 1. Specific leaf area (S , m² kg⁻¹), nitrogen concentration (N , mmol g⁻¹), nitrogen content (N_{area} , mmol m⁻²), net rate of photosynthesis (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and photosynthetic nitrogen use efficiency (ϵ , $\mu\text{mol N}^{-1} \text{s}^{-1}$) for the three conifers (*D. cupressinum*, *Prumnopitys ferruginea* and *Pinus radiata*) and three angiosperms (*W. racemosa*, *M. umbellata* and *Phaseolus vulgaris*)

Photosynthesis was measured at a leaf temperature of approximately 20°C and at an irradiance of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (PFD). The partial pressures of CO₂ and water vapour were 36 Pa and 0.5–1.0 kPa, respectively. The values are averages of 4–8 independent measurements ± 1 standard deviation. Values within column followed by the same superscript are not significantly different ($P > 0.05$). * indicates native forest species

Species	S	N	N_{area}	A	ϵ
Conifers					
<i>D. cupressinum</i> *	7.2 \pm 1.6 ^{bc}	1.0 \pm 0.1 ^b	149.2 \pm 49.9 ^{bc}	5.2 \pm 1.0 ^b	39.1 \pm 19.6 ^c
<i>P. ferruginea</i> *	9.7 \pm 1.6 ^{bc}	1.4 \pm 0.2 ^d	150.0 \pm 37.3 ^c	6.8 \pm 1.0 ^b	48.3 \pm 17.6 ^c
<i>P. radiata</i>	11.6 \pm 1.0 ^{bc}	1.4 \pm 0.1 ^{bd}	145.7 \pm 22.6 ^{ac}	11.3 \pm 0.5 ^{ac}	78.6 \pm 9.7 ^{bc}
Angiosperms					
<i>W. racemosa</i> *	11.6 \pm 2.0 ^b	0.9 \pm 0.3 ^b	94.1 \pm 9.2 ^{abc}	9.9 \pm 1.1 ^{ad}	105.9 \pm 18.3 ^{ab}
<i>M. umbellata</i> *	5.0 \pm 0.4 ^c	0.5 \pm 0.1 ^c	99.6 \pm 13.1 ^{acd}	6.7 \pm 1.0 ^{bcd}	67.5 \pm 7.2 ^{bc}
<i>P. vulgaris</i>	45.1 \pm 3.7 ^a	4.2 \pm 0.1 ^a	93.5 \pm 8.3 ^{ad}	12.2 \pm 2.8 ^a	132.4 \pm 35.5 ^a

Prumnopitys ferruginea, were associated with high values of N_{area} , resulting in the lowest values of ϵ for all the species.

In most, but not all, cases the A/c_i curves measured at different irradiances converged at a single point (Fig. 1). Those cases where a clear point of convergence was not attained typically occurred in leaves where stomatal conductance decreased at low irradiances. In these cases, new leaves were selected until a point of convergence could be defined. There was statistically significant variation in Γ^* and R_d among species, but at least for Γ^* the variation was small (Table 2). *D. cupressinum* and *Prumnopitys ferruginea*, the two podocarps, had the lowest and highest values of Γ^* , respectively, but the values deviated only approximately 0.5–0.6 Pa from the average for all species (3.7 Pa). There was more than 3-fold variation in the rates of day respiration with the lowest and highest rates for *Pinus radiata* and *Phaseolus vulgaris*, respectively. The rates for *D. cupressinum* and *Prumnopitys ferruginea* were intermediate.

For a given leaf the estimate of g_m depended on identifying the values of c_i where photosynthesis continued to increase but where electron transport, estimated from measurements of Φ_{PSII} , remained constant. In this region, the rate of ribulose biphosphate (RuBP) regeneration is constant and further increases in photosynthesis resulted from the suppression of photorespiration, as mesophyll conductance was reduced by an increasing gradient in CO_2 partial pressure from the intercellular airspaces in the leaf to the sites of fixation by rubisco. This region varied among species but typically occurred at c_i values of approximately

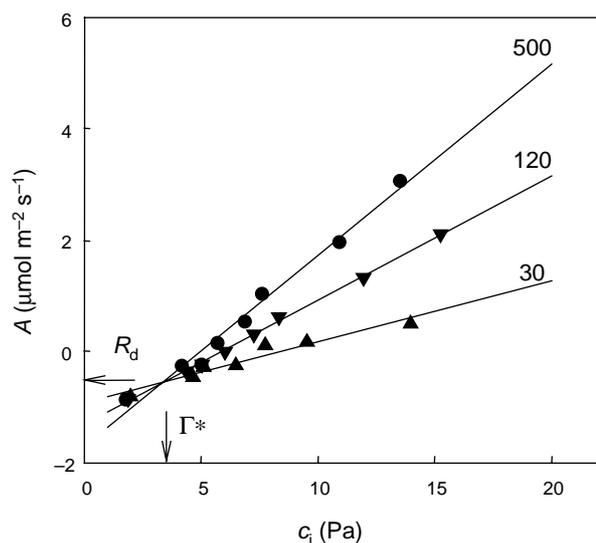


Fig. 1. The response of net photosynthesis (A) to variation in intercellular CO_2 partial pressure, c_i , at three different levels of irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$; 400–700 nm, shown to the right of each line) for representative *D. cupressinum* foliage. The CO_2 compensation partial pressure in the absence of day respiration (Γ^*) was estimated by extrapolating the point of intersection to the c_i axis; day respiration (R_d) was estimated by extrapolating the point of intersection to the A axis.

90 Pa and above (Fig. 2A). For the *D. cupressinum* foliage depicted in Fig. 2, a value of g_m of $0.026 \text{ mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$ resulted in a minimum variance for values of J (ranging from 9.2–59.5 $\mu\text{mol m}^{-2} \text{ s}^{-1}$; Fig. 2B) and was therefore deemed the best estimate of g_m .

Conductance values varied significantly among species and as for photosynthesis, higher values were found in *Pinus radiata* and *Phaseolus vulgaris* than in the indigenous forest species (Table 3). Though not statistically different from the values for *W. racemosa* and *M. umbellata*, g_m for *D. cupressinum* and *Prumnopitys ferruginea* were the lowest, being about one third or less of the values for *Phaseolus vulgaris*. The marginal difference in the group mean value for g_m ($P < 0.10$), when combined with the absence of a difference for g_{st} , caused a higher value for the ratio g_m/g_{st} for the angiosperms than for the conifers (angiosperms 2.1, conifers 0.9), but this difference was heavily influenced by the high value for *Phaseolus vulgaris*. There was 4-fold variation in g_m/g_{st} among species with the lowest and highest values for *D. cupressinum* and *Phaseolus vulgaris*, respectively.

Net photosynthesis was positively correlated with g_m , but high values of g_m relative to photosynthesis for *Phaseolus vulgaris* resulted in the overall relationship appearing to be non-linear (Fig. 3). With the exception of *Phaseolus vulgaris*, the relationship between photosynthesis and g_m in this study was consistent with data representing eight species (solid line, Fig. 3) presented by Evans and Loreto (2000). That some of the bean leaves used in this experiment were not fully mature may have contributed to the disproportionately low rates of photosynthesis for a given g_m .

Table 2. The CO_2 compensation partial pressure in the absence of mitochondrial respiration (Γ^* , Pa) and the rate of mitochondrial respiration in the light (R_d , $\mu\text{mol m}^{-2} \text{ s}^{-1}$) for the three conifers (*D. cupressinum*, *Prumnopitys ferruginea* and *Pinus radiata*) and three angiosperms (*W. racemosa*, *M. umbellata* and *Phaseolus vulgaris*)

Measurements were made at a leaf temperature of approximately 20°C ; the maximum variation in leaf temperature during the sequence of measurements on each leaf was 19.6 – 20.8°C . The water vapour pressure deficit in the cuvette was 0.5 – 1.0 kPa . The values are averages of 4–8 independent measurements ± 1 standard deviation. Different subscripts indicate means are significantly different ($P > 0.05$).

* indicates native forest species

Species	Γ^*	R_d
Conifers		
<i>D. cupressinum</i> *	3.2 ± 0.5^a	$-1.0 \pm 0.3^{\text{bd}}$
<i>P. ferruginea</i> *	4.3 ± 0.6^b	-1.2 ± 0.2^d
<i>P. radiata</i>	$3.5 \pm 0.1^{\text{ab}}$	-0.6 ± 0.1^b
Angiosperms		
<i>W. racemosa</i> *	$3.9 \pm 0.4^{\text{ab}}$	$-0.8 \pm 0.3^{\text{bc}}$
<i>M. umbellata</i> *	$3.7 \pm 0.4^{\text{ab}}$	$-1.0 \pm 0.1^{\text{bd}}$
<i>P. vulgaris</i>	$3.6 \pm 0.1^{\text{ab}}$	-1.7 ± 0.1^a

Discussion

The lowest photosynthetic rate for the species investigated was for *D. cupressinum*, and this low rate was associated with a relatively large investment in leaf nitrogen on an area basis, N_{area} (Table 1). Insofar as leaf nitrogen concentration reflects the amount of rubisco, this pattern of low photosynthesis and high N_{area} led us to hypothesise that mesophyll conductance may be the major limitation of photosynthesis in this species. In addition to low g_{st} , there is some indication that low g_{m} contributed to the reduced photosynthetic rates

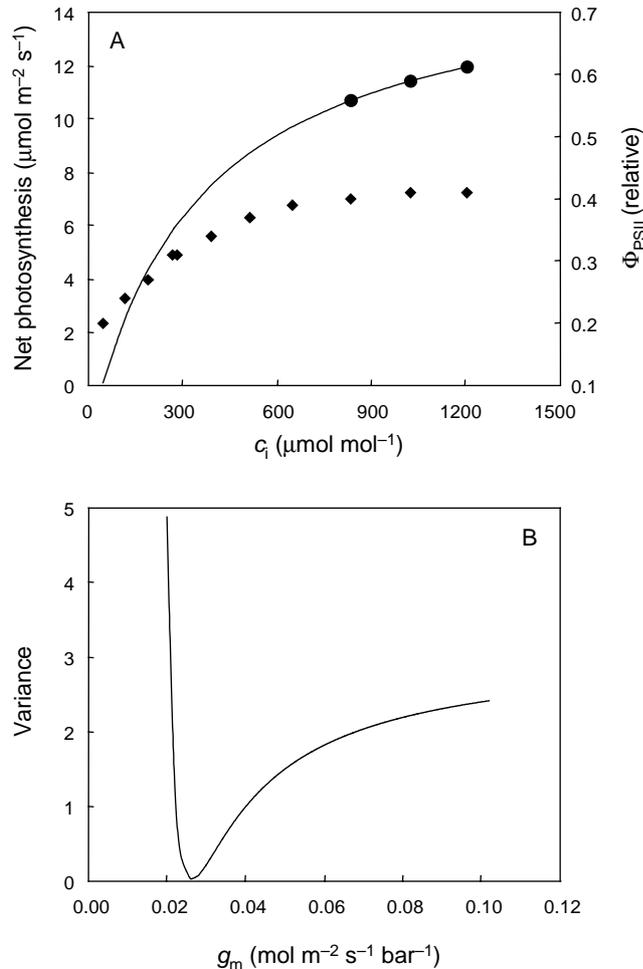


Fig. 2. (A) The response of net photosynthesis (A, line) and the photochemical efficiency of photosystem II (Φ_{PSII} ; \blacklozenge) as a function of the sub-stomatal CO₂ partial pressure (c_i) for representative *D. cupressinum* foliage. The solid line represents a least-squares fit to the A/c_i response. The three solid circles are measured values and indicate the values of photosynthesis used to estimate mesophyll conductance; these values occurred in a range of c_i where Φ_{PSII} indicated electron transport was constant. The diamonds represent relative values of Φ_{PSII} . (B) The variance among estimates of J for each of the three values of A indicated by the circles in Fig. 2A for different values of g_m . The minimum variance occurred at a g_m of $0.026 \text{ mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$.

for *D. cupressinum* since the values of g_m and the ratio g_m/g_{st} were the lowest for all the species investigated.

The Ohm's law analogy revealed differentials of 8.8 and 16.3 Pa, respectively, for *D. cupressinum* in the decrease in CO₂ partial pressure from the bulk atmosphere to the intercellular air space ($c_a - c_i$) and from the intercellular air space to the site of fixation by rubisco ($c_i - c_c$). This was the only species with a larger gradient in CO₂ partial pressure from c_i to c_c than from c_a to c_i . *Pinus radiata* had considerably higher rates of net photosynthesis but approximately the same N_{area} as *D. cupressinum*; the decrease in CO₂ partial pressure from c_i to c_c (8.1 Pa) was roughly the same as the decrease from $c_i - c_c$ (7.4 Pa) for this species. For *Prumnopitys ferruginea*, the other native conifer in this study, the gradients between $c_a - c_i$ and $c_i - c_c$ also were similar (13.3 and 13.1 Pa, respectively). The gradients in CO₂ partial pressure were reversed for *Phaseolus vulgaris*, the species with the highest value of g_m ; there was a greater decrease in CO₂ partial pressure from the bulk air to the intercellular air space (6.6 Pa) than from the intercellular air spaces to the chloroplasts (3.1 Pa). Though tempered by the low replication and the lack of strong statistical differences among species, the low values of the g_m/g_{st} ratio for *D. cupressinum* tacitly supports our hypothesis that g_m disproportionately limits photosynthesis in this species. The value of g_m for the other native conifer, *Prumnopitys ferruginea*, also was low, but because the value of g_m was similar to g_{st} it is not likely that it disproportionately limited photosynthesis (Table 3).

Mesophyll conductance regulates the rate of CO₂ diffusion from the substomatal cavity to the active sites of rubisco, and includes limitations imposed by the intercellular air spaces, cell walls and the liquid phases of the cytoplasm and stroma (Evans 1983; Nobel 1999). Photosynthetic rates across species are highly correlated with g_m

Table 3. Mesophyll (g_m , $\text{mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$) and stomatal (g_{st} , $\text{mol m}^{-2} \text{ s}^{-1}$) conductance to CO₂ transfer, and the ratio (g_m/g_{st}), for three conifers (*D. cupressinum*, *Prumnopitys ferruginea* and *Pinus radiata*) and three angiosperms (*W. racemosa*, *M. umbellata* and *Phaseolus vulgaris*)

The values of g_m are directly comparable to g_{st} when atmospheric pressure is 1 bar. Each value is the mean of 4–6 independent measurements ± 1 standard deviation. Values followed by different superscripts are different at $P < 0.05$. * indicates native forest species

Species	g_m	g_{st}	g_m/g_{st}
Conifers			
<i>D. cupressinum</i> *	0.032 ± 0.005^b	0.059 ± 0.015^b	0.6 ± 0.2^b
<i>P. ferruginea</i> *	0.052 ± 0.013^b	0.051 ± 0.016^b	1.1 ± 0.3^b
<i>P. radiata</i>	0.153 ± 0.002^a	0.139 ± 0.018^a	1.1 ± 0.1^b
Angiosperms			
<i>W. racemosa</i> *	0.085 ± 0.008^b	0.085 ± 0.010^b	1.0 ± 0.2^b
<i>M. umbellata</i> *	0.060 ± 0.011^b	0.068 ± 0.024^b	1.1 ± 0.5^b
<i>P. vulgaris</i>	0.399 ± 0.304^a	0.175 ± 0.098^a	4.2 ± 2.2^a

(Fig. 3, Evans and Loreto 2000) and, like photosynthesis, g_m varies with environment, leaf morphology and developmental stage. Leaf temperature (Bernacchi *et al.* 2002), water stress (Flexas and Medrano 2002; Flexas *et al.* 2002), and growth irradiance (Hanba *et al.* 2002) are the environmental factors that strongly influence g_m .

Earlier studies suggested that morphological features such as leaf thickness and the corresponding path length through the internal air spaces were primarily responsible for determining g_m (Evans and Loreto 2000). It is, however, unlikely that these features could explain the rapid responses of g_m to changing environmental conditions and it now appears that enzymatic processes may be primarily responsible for rapid regulation of g_m . Bernacchi *et al.* (2002) observed that the temperature dependence of g_m in tobacco was much steeper than expected if values were regulated solely by diffusion of CO_2 in water. The rate of change more closely resembled the temperature responsiveness typical of enzymatic processes. Recent evidence suggests that two enzymes, carbonic anhydrase (Gillon and Yakir 2000) and chloroplast aquaporins (Terashima and Ono 2002), may play an important role in regulating g_m .

By facilitating the inter-conversion of CO_2 and HCO_3^- , carbonic anhydrase maintains a steep diffusion gradient through the cytosol and stroma in the chloroplasts (Cowan 1986). Using a combination of isotopic and gas-exchange methods, Gillon and Yakir (2000) found that of the two major components of g_m , cell wall conductance was

consistently greater than 'chloroplast' conductance, suggesting that diffusion through the liquid phase provided a disproportionately low conductance for the transfer of CO_2 . Furthermore, chloroplast conductance was correlated with carbonic anhydrase activity. Cell wall conductance was consistently lower in the sclerophyllous leaves of *Quercus robur* than for the mesophytic leaves of *Nicotiana tabacum* or *Glycine max*, but carbonic anhydrase activity was higher in *Q. robur*, thereby compensating for the low conductance imposed by the cell wall (Gillon and Yakir 2000).

As a small, uncharged molecule CO_2 readily moves through cell membranes but trans-membrane channels can greatly enhance the rate of diffusion. Aquaporins facilitate the diffusion of water and CO_2 across the cell membrane of animal cells and Terashima and Ono (2002) postulated that they play a similar role in plant cells. Using HgCl_2 as an inhibitor, Terashima and Ono (2002) found that reducing the activity of aquaporins in pea and bean reduced hydraulic conductivity into mesophyll cells by as much as 80%, and the conductance of CO_2 from the intercellular air spaces to the chloroplast stroma declined by 60–70%. Thus, mercury-sensitive aquaporins facilitate diffusion of CO_2 through the plasma membrane and may therefore contribute to short-term adjustments of g_m .

Our understanding of the relative importance of different processes regulating g_m is incomplete, but the emerging framework suggests that g_m is regulated by the interplay between structural and biochemical elements. Mesophyll conductance should be proportional to the porosity of the mesophyll and inversely proportional to mesophyll thickness. However, in all but the thickest hypostomatous leaves, gas-phase conductance through the intercellular air spaces is high (Parkhurst 1994). Because of this, there often is no clear relationship between leaf thickness and g_m (Evans *et al.* 1994; Lauteri *et al.* 1997), and in cases where there is a positive relationship, it may emerge from an autocorrelation with chloroplast surface area (Hanba *et al.* 1999). The primary 'structural' element regulating g_m appears to be the chloroplast surface area (Evans and Loreto 2000), which can be modified as environmental conditions change. It seems there is a continuum of factors from slow-responding structural attributes (mesophyll porosity and thickness, chloroplast surface area) to fast responding biochemical (carbonic anhydrase and aquaporins) processes that regulate g_m , and currently we have no explanation for the combination of these factors that contribute to the low g_m in *D. cupressinum* and, to a lesser extent, low g_m in the co-occurring species in the forest at our site. The low value of specific leaf area (S , Table 1) may indicate that low mesophyll porosity and thick mesophyll cell walls contribute to low g_m in *D. cupressinum*. Given the complex shoot structure and small, scale-like leaves in *D. cupressinum*, a closer examination of the specific factors regulating liquid-phase diffusion in this species is warranted.

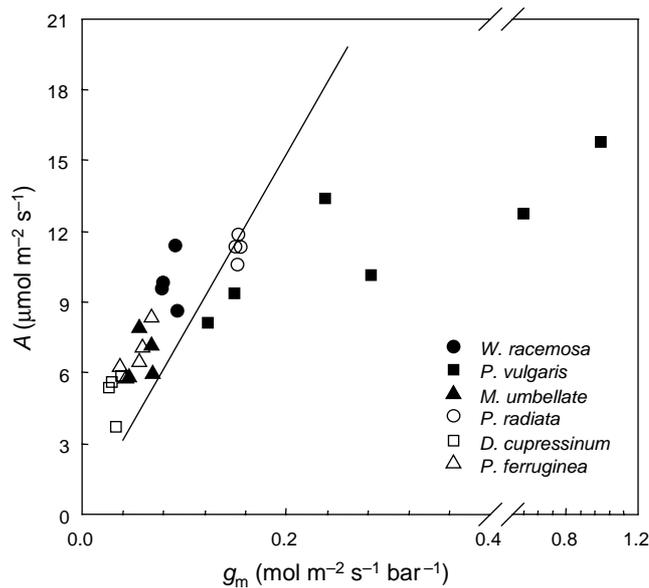


Fig. 3. Relationship between net photosynthesis (A) and mesophyll conductance (g_m) for three conifers (*D. cupressinum*, *Prumnopitys ferruginea* and *Pinus radiata*) and three angiosperms (*W. racemosa*, *M. umbellata* and *Phaseolus vulgaris*). Each value represents a single measurement and the line represents the relationship for several species given by Evans and Loreto (2000).

Estimates of g_m are very sensitive to variation in the CO_2 compensation partial pressure in the absence of day respiration (Γ^* , Harley *et al.* 1992). The values of Γ^* in this study (approximately 3.2–4.3 Pa) were consistent with those reported by Evans and Loreto (2000). For several agronomic and one tree species Γ^* was between 3.3 and 4.6 Pa, with one estimate as high as 5.5 Pa. Recently, Hovenden (2003) reported values of Γ^* for poplar (*Populus alba*) clones of 5–6 Pa. It should be noted that the values in this study are ‘apparent’ Γ^* as our data were not corrected for mitochondrial respiration in the light (von Caemmerer *et al.* 1994). The correction expressed as $\Gamma^* = \Gamma^*_{\text{apparent}} + R_d/g_m$, requires an independent estimate of g_m and the absence of this correction generates a small underestimate of Γ^* . For walnut (*Juglans nigra* \times *regia* and *J. regia*) trees, correction of Γ^* for R_d increased the values by approximately 0.3 Pa (Piel *et al.* 2002).

There was significant variation in Γ^* among species in this study (Table 2) but this variation did not appear to correlate with different phylogenetic groups. Rubisco serves as an oxygenase as well as a carboxylase, and at a given temperature Γ^* is regulated by the specificity of rubisco for CO_2 relative to O_2 ; low values of Γ^* correspond with a high specificity for CO_2 . There is a loose correlation between the specificity of rubisco and its specific reaction rate (Raven 2000), leading to the prediction that during times of high atmospheric CO_2 partial pressure, specificity may have been reduced in favour of higher specific rates of carboxylation. There was no detectable difference in Γ^* between angiosperms and conifers in this study. For the native conifer species, a group that evolved during a time of considerably higher atmospheric CO_2 partial pressure than today, the lowest value of Γ^* (3.2 Pa) was for *D. cupressinum* while the highest (4.3 Pa) was for *Prumnopitys ferruginea*. Across broad taxonomic groups, including CAM, C_3 and C_4 plants, aquatic bryophytes and freshwater algae, the affinity of rubisco for CO_2 varies by more than 5-fold and is higher in groups without active CO_2 concentrating mechanisms (Yeoh *et al.* 1981; Seemann *et al.* 1984; Kane *et al.* 1994). There is some evidence that in hot, dry climates where photorespiration is pronounced, natural selection favours a high specificity factor for rubisco and thus, low Γ^* (Delgado *et al.* 1995).

Although there was no systematic difference between conifers and angiosperms in our study, it is reasonable to argue that low g_m disproportionately limited photosynthesis in *D. cupressinum* relative to the other co-occurring species in the ecosystem. An explanation for the low g_m in this species is, however, unclear, and may be related to aspects of leaf anatomy, position and size of chloroplasts or the activity of leaf carbonic anhydrase or other biochemical attributes. The draw-down of CO_2 partial pressure from c_i to c_c was greater for *D. cupressinum* than for the other native conifer species *Prumnopitys ferruginea*. That these species

appeared to operate differently may suggest that low g_m is not necessarily a property of species that diverged during a time of extraordinary high atmospheric CO_2 partial pressure, but may reflect other evolutionary pressures that result in specific leaf anatomical and functional properties, such as adaptation to low nutrient availability.

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