

# A test of the hydraulic limitation hypothesis in fast-growing *Eucalyptus saligna*

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## ABSTRACT

The hydraulic limitation hypothesis proposes that (1) reduced growth in taller trees is caused by decreased photosynthesis resulting from a decrease in hydraulic conductance promoted by a longer root-to-leaf flow path, and (2) this mechanism reduces stand productivity after canopy closure. This hypothesis was tested by comparing the physiology of 7 m (1 year) and 26 m (5 year) *Eucalyptus saligna* plantations where above-ground productivity for the 26 m trees was approximately 69% of that for the 7 m trees, and water and nutrients were not limiting. The study compared whole tree physiology [water flux ( $Q_i$ ), average crown conductance ( $G_T$ ), crown hydraulic conductance per unit leaf area ( $K_L$ ), carbon isotope discrimination ( $\delta^{13}C$ )] and leaf physiology under light saturation (leaf water potential at the canopy top ( $\Psi_{LEAF}$ ), photosynthetic capacity ( $A_{max}$ ), and photosynthesis ( $A$ ) and stomatal conductance ( $g_s$ ).  $K_L$  was 50% lower in the taller trees, but whole tree  $Q_i$  and  $G_T$  were the same for the 7 m and 26 m trees. Photosynthetic capacity was the same for leaves at the canopy top, but  $\delta^{13}C$  was  $-1.8\%$  lower for the 26 m trees.  $A$  and  $g_s$  were either lower in the taller trees or equal, depending on sampling date. The taller trees maintained 0.8 MPa lower  $\Psi_{LEAF}$  during the day and had 2.6-times higher sapwood area per unit leaf area; these factors compensated for the effects of increased height and gravitational potential in the taller trees to maintain higher  $G_T$ . The hydraulic limitation hypothesis (as originally stated) failed to explain the sharp decline in net primary productivity after canopy closure in this study. The effects of increased height appear to be a universal hydraulic problem for trees, but compensation mitigated these effects and maintained  $Q_i$  and  $G_T$  in the present study. Compensation may induce other problems (such as lower  $\Psi_{LEAF}$  or higher respiratory costs) that could reduce carbon gain or shift carbon allocation, and future studies of hydraulic limitation should consider compensation and associated carbon costs. In this study, the combination of similar  $G_T$  and lower  $\delta^{13}C$  for the 26 m trees suggests that total crown photosynthesis was lower for the 26 m trees, perhaps a result of the lower  $\Psi_{LEAF}$ .

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## INTRODUCTION

The hydraulic limitation hypothesis proposes that increased path length (in roots, stems and branches) decreases leaf-specific hydraulic conductance as trees grow in height. If stomata close to regulate leaf water status to a constant mid-day minimum as trees grow taller, tall trees must close stomata at a lower leaf to air saturation deficit ( $D$ ) than short trees. Closure of stomata will restrict the diffusion of  $CO_2$  into the leaf and reduce net photosynthesis and tree growth and perhaps the ultimate height of the tree (Ryan & Yoder 1997).

We can examine the hydraulic limitation hypothesis using an Ohm's Law analogy for water flux through trees (Tyree & Ewers 1991):

$$G_c = K_L \Delta \Psi / D \quad (1)$$

where  $G_c$  is average stomatal conductance of the tree's foliage,  $K_L$  is average hydraulic conductance for the whole tree from soil to leaf (per unit leaf area),  $D$  is the leaf-to-air vapour pressure deficit and  $\Delta \Psi$  is the soil-to-leaf water potential difference. A reduction in  $K_L$  as trees increase in height would result in a proportional reduction in  $G_c$  if  $D$  and  $\Delta \Psi$  remain constant.  $\Delta \Psi$  will remain constant only if leaf water potential ( $\Psi_{LEAF}$ ) is regulated and tall trees have access to the same soil water as shorter trees.

To critically examine the hydraulic limitation hypothesis, we identified five necessary components: (1) stomata must close to maintain  $\Psi_{LEAF}$  above a minimum, critical threshold and this threshold must be the same for all tree heights; (2) stomata must close in response to decreased hydraulic conductance; (3) hydraulic conductance must decrease with tree height; (4) stomatal closure promoted by reduced hydraulic conductance must cause lower photosynthesis; and (5) reduction in photosynthesis in older, taller trees must be sufficient to account for reduced growth. Below, we review evidence that these components occur.

## Component 1

Much evidence shows that stomata close in order to maintain  $\Psi_{LEAF}$  above a critical threshold (for examples, see Hinckley, Lassoie & Running 1978; Tyree & Sperry 1988; Tyree & Ewers 1991; Sperry & Pockman 1993; Saliendra,

Sperry & Comstock 1995; Bond & Kavanagh 1999; Hubbard, Bond & Ryan 1999; Oren *et al.* 1999). Tyree & Sperry (1988) suggest that maintaining  $\Psi_{\text{LEAF}}$  above a minimum threshold ( $\Psi_{\text{MIN}}$ ) protects the water-conducting system from damage by embolism and, further, that reducing  $\Psi_{\text{LEAF}}$  below  $\Psi_{\text{MIN}}$  would result in a chain reaction of unreparable embolisms forming in the xylem elements, ultimately causing death of some or all living tissue from water stress. Critical thresholds differ among species and can vary among sites within a species (Hinckley *et al.* 1978; Tyree *et al.* 1991; Kolb & Davis 1994; Bond & Kavanagh 1999). In some cases, stomata apparently do not regulate  $\Psi_{\text{LEAF}}$  to remain above a critical threshold (Phillips, Bond & Ryan 2001).

Given that  $\Psi_{\text{MIN}}$  exists for most trees, the critical issue for the hydraulic limitation hypothesis is whether  $\Psi_{\text{MIN}}$  changes with tree height for trees of the same species growing at the same site. A decrease in  $\Psi_{\text{MIN}}$  with height could compensate for decreased  $K_L$  and maintain  $G_C$ , perhaps at the cost of increased risk for loss of the conducting system. For ponderosa pine (Yoder *et al.* 1994; Hubbard *et al.* 1999; Ryan *et al.* 2000), Oregon white oak (N. Phillips *et al.* unpublished data) and mountain beech (M. G. Ryan & D. Whitehead, unpublished data),  $\Psi_{\text{MIN}}$  did not vary with tree height. However,  $\Psi_{\text{MIN}}$  decreased with height for lodgepole pine (Yoder *et al.* 1994) and Douglas-fir (McDowell *et al.* 2002b).

### Component 2

Experimental manipulations of the conducting system have generally demonstrated that stomata respond directly to experimental changes in hydraulic conductance by induced cavitation (Sperry & Pockman 1993; Hubbard *et al.* 2001), notching (Sperry, Alder & Eastlack 1993), root pressurization (Saliendra *et al.* 1995), defoliation (Pataki, Oren & Phillips 1998; Hubbard *et al.* 1999), shading (Whitehead *et al.* 1996) and root pruning (Teskey, Hinckley & Grier 1983; Meinzer & Grantz 1990). Stomatal response occurred rapidly in these studies and, where  $\Psi_{\text{LEAF}}$  was measured it remained constant or nearly so (Teskey *et al.* 1983; Sperry & Pockman 1993; Hubbard *et al.* 2001). These studies suggest that stomatal conductance will change with hydraulic conductance and the response follows that expected from Eqn 1. In some cases, stomata did not respond to manipulations of the conducting system by notching (Hubbard *et al.* 1999) and defoliation (Phillips *et al.* 2001).

### Component 3

Hydraulic conductance and water flux decreased with increasing tree height and age in Scot's pine (Mencuccini & Grace 1996b), ponderosa pine (Hubbard *et al.* 1999; Ryan *et al.* 2000), European beech (Schäfer, Oren & Tenhunen 2000) and Oregon white oak (N Phillips *et al.* unpublished manuscript). A decrease was not found in Douglas-fir (Phillips *et al.* 2002). In each of these studies, an increase in the ratio of sapwood area-to-leaf area ratio ( $A_s : A_l$ ) may have partially compensated for the longer

path length and increased gravitational potential of taller trees; an increase in  $A_s : A_l$  with tree height is found in many species (McDowell *et al.* 2002b). Magnani, Mencuccini & Grace (2000) suggest that shifts in allocation from foliage to conductive tissue could reduce tree growth by lowering canopy photosynthesis and increasing the carbon costs of fine root turnover and respiration for sapwood. In their study, modelled fine root biomass increased with stand age, supporting an allocation explanation. However, direct measurements of below-ground allocation did not increase with tree height and stand age (Smith & Resh 1999; Giardina & Ryan 2002) and respiration costs for woody tissue did not increase with increasing tree size or age (Ryan & Waring 1992), so there is no direct evidence that changing  $A_s : A_l$  will lower growth.

### Component 4

This component is included for completeness, as photosynthesis is strongly regulated by stomatal conductance, which controls the diffusion of  $\text{CO}_2$  to the chloroplasts. The relationship between  $g_s$  and  $A$  varies with environmental conditions, but is approximately linear (Leuning 1995). Hubbard *et al.* (2001) demonstrate that manipulating  $K_L$  can change both stomatal conductance and photosynthesis.

### Component 5

The pattern of reduced tree growth as trees grow in size appears to be universal (Ryan, Binkley & Fownes 1997), but few studies have directly linked reductions in photosynthesis in taller trees with reductions in growth. Ryan & Waring (1992) used a model analysis and a carbon budget approach to suggest lower photosynthesis accounted for lower growth in lodgepole pine, and such a reduction was later corroborated by leaf-level photosynthesis measurements (Yoder *et al.* 1994). Furthermore, reductions in leaf-level photosynthesis (Yoder *et al.* 1994; Hubbard *et al.* 1999) and  $G_C$  (Ryan *et al.* 2000) were linked with reduced above-ground production per unit leaf area in ponderosa pine. However, McDowell *et al.* (2002a) found only minor differences in stomatal conductance and photosynthesis among Douglas-fir trees of different height during drought. Such measurements are difficult to obtain for tall trees, but will be necessary to further explore how tree physiology changes with height and age.

In general, past studies have supported conditions 1–5 as being necessary for the hydraulic limitation hypothesis. However, factors that can compensate for the effects of increased path length and gravity in taller trees (increased  $A_s : A_l$  and reduced  $\Psi_{\text{MIN}}$ ) occur in several species. Studies linking reduced photosynthesis with reduced growth are rare.

A strong test of the hydraulic limitation hypothesis would occur where growth declines have been measured, but other factors that influence growth in forest systems (nutrition, water and competition) are controlled so that differences in physiology can be directly related to changes in tree

height. Our objective was to test the hydraulic limitation hypothesis in a system with no water or nutrient limitation and no competition from other species. We measured a variety of whole tree and leaf-level physiological characteristics in fast-growing *Eucalyptus saligna* trees of different heights (1 year, 7 m and 5 year, 26 m) to evaluate the physiological components necessary for the hydraulic limitation hypothesis. Component 1 was assessed by diurnal measurements of  $\Psi_{\text{LEAF}}$ ; components 2 and 3 by estimates of average crown conductance ( $G_T$ ) (estimated from water flux per unit leaf area,  $Q_i$ ),  $K_L$ ,  $A$ ,  $g_s$  and the response of  $G_T$  to  $D$ ; and component 5 by comparing estimates of whole tree photosynthesis derived from measurements of  $G_T$  and  $\delta^{13}\text{C}$  with differences in above-ground production. If hydraulic limitation operated for these trees, then the longer path length from soil to leaf in taller trees would result in lower  $K_L$ , lower  $Q_i$  and lower whole tree stomatal conductance ( $G_C$ ),  $g_s$  and  $A$  in tall trees compared to short trees.

## MATERIALS AND METHODS

### Site description

The study site is an 4.5 ha experimental forest of *Eucalyptus saligna* located 13 km NNE of Hilo, HI (19°50'28.1" N, 155°7'28.3" W) on the Hamakua coast of the Island of Hawaii. The site is located at an elevation of 350 m with soils derived from deep volcanic ash that belong to the Akaka series (Binkley & Resh 1999). The site receives an average precipitation of approximately 4000 mm year<sup>-1</sup> distributed evenly throughout the year, and has a mean annual temperature of 21 °C with little seasonal variation (Binkley *et al.* 1992). The site was intensively managed for the production of sugarcane for 80 years and was fallow for less than 1 year prior to the establishment of the *Eucalyptus* forest.

The forest contained stands of two age classes: 1 year and 5 year. The 5 year stand was planted in May 1994 using seedlings grown from seed in a greenhouse for 6 months, and consisted of 18 plots (30 m × 30 m) with different tree density (1111 ha<sup>-1</sup> and 10 000 ha<sup>-1</sup>) and fertility treatments [control = fertilized at planting and at 7 months, high fertility = quarterly fertilizations, restore fertility = quarterly additions when trees were 43 months old (Giardina & Ryan 2002)]. The 1 year stand was planted in August 1998 (also from 6-month-old, greenhouse-grown seedlings), and consisted of six 30 m × 30 m plots with the same tree density treatments as above and only the high fertility treatment.

### Experimental design

We selected one plot from each of the 1 year and 5 year stands for this study. These plots were both planted at a spacing of 3 m × 3 m (1111 ha<sup>-1</sup>) in the high fertility treatment. The fertilization regime was formulated to minimize any nutrient limitation on the stands and consisted of quarterly applications of 56 kg N ha<sup>-1</sup>, 24 kg P ha<sup>-1</sup>, 46 kg K ha<sup>-1</sup>, 20 kg Ca ha<sup>-1</sup>, and yearly applications of 100 kg ha<sup>-1</sup> of

Granusol (API Technologies, Inc., King of Prussia, PA, USA) 2GB5 micronutrient fertilizer (5% Mn, 5% Zn, 5% Mg, 5% Fe, 1.5% Cu, and 0.5% B). Periodic applications of glyphosate (Roundup®; Monsanto Technology, St Louis, MO, USA) controlled understorey vegetation so that virtually none was present during the study. At the end of this study, mean height of the 1 year plot was 6.9 m and the 5 year plot had a mean height of 25.6 m (Table 1).

### Weather

Meteorological variables were measured from a weather station located about 300 m upwind of the plantation where light and wind were unobstructed. We measured photosynthetically active radiation ( $I_p$ ) with a LI-190SB quantum sensor (Li-Cor, Inc., Lincoln, NE, USA), air temperature ( $T_a$ ) and relative humidity ( $RH$ ) with a CS-500 sensor (Campbell Scientific Instruments, Logan, UT, USA), and precipitation with a CS-700 tipping bucket rain gauge (Campbell Scientific). All measurements were recorded at 10 s intervals and stored as 15 min averages ( $I_p$ ,  $T_a$ ,  $RH$ ) or hourly totals (precipitation) with a CR-10 data logger (Campbell Scientific). To compare  $D$  in the canopy between the two plots, we measured  $T_a$  and  $RH$  at the mid-point of each canopy for 14–21 d with a CS-500 sensor in the 5 year plot and a HMP45C sensor in the 1 year plot (Campbell Scientific). To ensure all three  $T_a$  and  $RH$  sensors gave the same reading under identical conditions, the sensors from both plots were moved to the main weather station where all three sensors recorded measurements simultaneously for 14 d. After the concurrent readings, we calibrated one of the CS-500 sensors with a Li-Cor LI-610 dew point generator. Values of  $T_a$  and  $RH$  recorded *in situ* were adjusted to match true values using a calibration curve for each sensor.

### Tree-level measurements of water flux

We used Granier-type sensors (Granier 1987) to measure water flux ( $Q$ ) for 12 trees in the 5 year plot and 13 trees in the 1 year plot for days 165–234 in 1999. The measured trees represented the range of tree diameters in each plot. For the 5 year plot, average height of the measured trees (25.6 m; SE, 1.1) was larger than the stand average tree height (21.6 m; SE, 1.0), because larger trees represented most of the growth on the plot, and because we wished to test the hypothesis using the greatest height difference. Sensor probes were constructed of pairs of 20-mm-long, 1.65-mm-diameter stainless-steel needles (sapwood thickness as 20–25 mm). Sensors were inserted at a height of 2 m in the 5 year trees and the base of the live crown in the 1 year trees (approximately 0.25 m in height). To reduce the effect that natural temperature gradients in the stem have on the difference in temperature between probes, sensors were inserted at the same height above the ground and 8–15 cm apart. Temperature differences between unheated probes within an individual tree averaged <0.1 °C for both age classes. In three trees per plot, two sensors were placed opposite of each other at the same height. The estimated  $Q$

**Table 1.** Characteristics of sample trees from the 1 and 5 year plots

Characteristic	1 year	<i>n</i>	5 year	<i>n</i>
Height (m)	6.94 ± 0.21	13	25.6 ± 1.1	12
Diameter (m)	0.07 ± 0.01	13	0.21 ± 0.02	12
Leaf area (m <sup>2</sup> )	47.3 ± 4.9	13	97.1 ± 14.5	12
Sapwood area (m <sup>2</sup> )	0.003 ± 0.001	13	0.015 ± 0.001	12
Sapwood area : leaf area (10 <sup>-6</sup> m <sup>2</sup> : m <sup>2</sup> )	67.6 ± 5.5	13	179 ± 22	12
Leaf area : dry weight (m <sup>2</sup> : kg)	10.8 ± 0.17	13	10.4 ± 0.5	12
N concentration by canopy thirds (%)				
Top	1.87 ± 0.08	13	1.94 ± 0.07	12
Middle	1.70 ± 0.07	13	1.90 ± 0.06	12
Bottom	1.43 ± 0.02	13	1.93 ± 0.07	12
δ <sup>13</sup> C by canopy thirds (‰)				
Top	-28.7 ± 0.11	13	-30.7 ± 0.24	21
Middle	-29.1 ± 0.23	5	-30.5 ± 0.19	5
Bottom	-29.7 ± 0.22	5	-31.9 ± 0.4	5
A <sub>max</sub> by leaf position (μmol m <sup>-2</sup> s <sup>-1</sup> )				
1	17.8 ± 2.4	3	16.3 ± 2.1	4
2	25.5 ± 0.8	4	23.1 ± 0.9	4
3	15.7 ± 1.4	3	14.5 ± 0.5	7
4	14.5 ± 1.0	3	8.5 ± 0.5	12
5	6.3 ± 1.0	3	9.7 ± 0.2	3
V <sub>c,max</sub> (position 2 leaves, μmol <sup>-2</sup> s <sup>-1</sup> )	76.0 ± 6.2	9	85.4 ± 5.1	12
A (position 2 leaves, μmol m <sup>-2</sup> s <sup>-1</sup> ), days 179–184	18.2 ± 0.4	80	19.2 ± 0.2	248
A (position 2 leaves, μmol m <sup>-2</sup> s <sup>-1</sup> ), day 211	25.1 ± 0.3	91	21.6 ± 0.3	63
g <sub>s</sub> (position 2 leaves, mmol m <sup>-2</sup> s <sup>-1</sup> ), days 179–184	340 ± 16	80	355 ± 10	248
g <sub>s</sub> (position 2 leaves, mmol m <sup>-2</sup> s <sup>-1</sup> ), day 211	518 ± 10	91	248 ± 10	63

Values are mean ± 1 SE. A<sub>max</sub> measured with  $D < 0.6$  kPa,  $I_p > 1300$  μmol m<sup>-2</sup> s<sup>-1</sup>,  $C_a = 360$  μmol mol<sup>-1</sup>. A measured at ambient  $D$ ,  $I_p > 800$  μmol m<sup>-2</sup> s<sup>-1</sup>,  $C_a = 360$  μmol mol<sup>-1</sup>.

differed between the two sensors in an individual tree by an average of 5% in the 1 year trees and by 11% in the 5 year trees. We insulated and shielded the sensors from incident radiation by wrapping the stems with reflective insulation from 0.5 m above the insertion point to 0.5 m below the insertion point (or to the base of the tree in the 1 year trees). Sensors were protected from rain and stem-flow by wrapping the stem and reflective insulation with plastic sheeting, and sealing the plastic sheeting at the top with pruning wax and vinyl tape. Temperature differences of all sensors were recorded every 15 s and stored as 15 min means with two CR-21x data loggers (Campbell Scientific).

### Leaf area, sapwood area, foliar N, and <sup>13</sup>C : <sup>12</sup>C for trees measured for water flux

We harvested each tree that had water flux measurements at the end of the study period and determined total leaf area, and average foliar N and <sup>13</sup>C : <sup>12</sup>C for all foliage in each crown third. After felling a tree, each crown was divided into thirds by length. In each third, every leaf was removed and the total wet weight of leaves was recorded. Leaves were mixed and a representative subsample (approximately 150 g) was taken and measured for wet weight, dry weight and total leaf area using a LI-3100 area meter (Li-Cor, Inc.). Total leaf area was calculated using the ratio of leaf area to leaf wet weight for each specific subsample. Subsamples were dried, ground and analyzed

for nitrogen (details below under 'Leaf measurements') and for <sup>13</sup>C : <sup>12</sup>C in a Europa Scientific Integra isotope ratio mass spectrometer (PDZ Europa, Northwich, Cheshire, UK).

Conducting sapwood was determined by either examining the translucency of cross-sections when held up to a bright light or by the colour change between heartwood and sapwood (both methods gave the same results). To calculate sapwood cross-sectional area at the sapflow sensor insertion point and at 1.4 m, we measured the total length inside the bark of two opposing radii (long axis and perpendicular to the long axis) and the width of the heartwood along each radius to the nearest 2 mm. Sapwood area was calculated as the difference between total cross-sectional area and heartwood cross-sectional area, where each cross-section was treated as an ellipse.

### Leaf measurements

We selected three to four trees for leaf gas exchange measurements and defined five diagnostic leaf positions in the tree crown: (1) expanding foliage in the upper third of the crown; (2) fully expanded foliage in the outer upper third of the crown; (3) fully expanded foliage in the outer middle third of the crown; (4) fully expanded foliage in the outer lower third of the crown; and (5) fully expanded foliage in the inner lower third of the crown. Scaffolding towers were used to access foliage.

We assessed photosynthetic capacity by measuring maximum assimilation ( $A_{\max}$ ) rates at the five crown positions and estimating maximum carboxylation velocity ( $V_{c,\max}$ ) by measuring the response of  $A$  to intercellular  $\text{CO}_2$  concentration ( $C_i$ ) on position 2 leaves ( $C_i$  from 0 to  $400 \mu\text{mol mol}^{-1}$ ). Preliminary measurements showed that photosynthesis saturated at  $I_p > 800 \mu\text{mol m}^{-2} \text{s}^{-1}$ .  $A_{\max}$  and  $V_{c,\max}$  were measured on overcast days with  $D < 0.6 \text{ kPa}$ ,  $I_p > 1300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and  $C_a$  of  $360 \mu\text{mol mol}^{-1}$ , using a CIRAS-1 infrared gas analyzer (PP Systems, Haverhill, MA, USA) and an artificial light source.  $V_{c,\max}$  was estimated by a non-linear regression fit to the Farquhar photosynthesis equation (Farquhar, von Caemmerer & Berry 1980). Ambient measurements of photosynthesis ( $A$ ) and stomatal conductance ( $g_s$ ) were taken under  $I_p > 800 \mu\text{mol m}^{-2} \text{s}^{-1}$  and ambient  $D$  on days 179–184 and day 211. Measured leaves were collected, dried at  $70^\circ\text{C}$  for  $> 72 \text{ h}$ , ground with a Wiley mill and a roller mill, and analysed for nitrogen concentration with a NA1500 C/H/N Analyzer (Carlo Erba Strumentazione, Milan, Italy).

Hourly leaf water potential measurements were taken on sunny, clear days from 0900 to 1500 h on days 179, 181, and 211 using a Scholander style pressure chamber (PMS Instrument Company, Corvallis, OR, USA). Leaves were dropped from the top of the trees in sealed bags with moist tissue paper and measurements were taken within 2 min of sampling. Predawn water potential measurements were taken on leaves located in position 2 of the canopies every 2 weeks beginning on day 172. Predawn measurements were corrected for gravitational potential.

## Data analysis

We selected days 203–233 for analysis of water flux data because they were within 40 d of harvest for leaf area measurement. The 1 year trees were growing so rapidly during measurements that leaf area at harvest would not apply to measurements at the beginning of summer. Under similar environmental conditions, xylem water flux per unit sapwood area ( $Q_s$ ) during days 170–200 was similar to that during days 203–233 for both the 1 year or 5 year trees ( $P > 0.24$ ).

Whole tree conductance to water vapour was calculated as:

$$G_T = Q_l/D \quad (2)$$

where  $Q_l$  is xylem water flux per unit leaf area ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) and  $D$  is air saturation deficit in partial pressure units ( $\text{kPa kPa}^{-1}$ ) (Whitehead *et al.* 1996). We estimated  $G_T$  only for  $D \geq 0.6 \text{ kPa}$ , because of the large error associated with low  $D$  estimates (Ewers & Oren 2000). To estimate  $G_T$  from  $Q$ , water capacitance within the conducting system is assumed negligible. To evaluate this assumption, capacitance was analysed using an approach that examines whether the correlation of  $Q$  with  $D$  is stronger for current  $D$ , or  $D$  for some prior time period (Phillips *et al.* 1997; Ryan *et al.* 2000). If capacitance affects transpiration, the correlation of  $Q$  and  $D$  will be greater for some earlier time than for current  $D$ .

Whole tree conductance to water vapour represents the conductance of the average leaf in the tree crown and consists of aerodynamic ( $G_A$ ) and canopy ( $G_C$ ) components (Thom 1972). Testing the hydraulic limitation hypothesis requires knowledge of  $G_C$ , because that is the component which is potentially influenced by hydraulic conductance. To estimate the relative importance of  $G_C$  in  $G_T$ , we examined the magnitude of the influence of wind speed ( $u$ ) on the relationship between  $Q_l$  and  $D$  at high light ( $I_p > 800 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). We compared  $Q_l$  between high wind (average  $u \geq 2.5 \text{ m s}^{-1}$  for 15 min) and low wind (average  $u < 2.5 \text{ m s}^{-1}$  for 15 min) conditions. We reasoned that if  $G_A \gg G_C$ , the difference between  $Q_l$  at low and high  $u$  would be small.

Whole tree leaf-specific hydraulic conductance was estimated as:

$$K_L = Q_l/\Psi_{\text{SOIL}} - \Psi_{\text{LEAF}} \quad (3)$$

The sampling unit was the individual tree for  $Q_l$ ,  $Q_s$ ,  $G_T$ ,  $K_L$ ,  $A_s$ :  $A_l$ ,  $\delta^{13}\text{C}$ , and nitrogen concentration. Differences in means between the two age classes were determined by independent samples  $t$ -tests. The sampling unit was the leaf for  $\Psi_{\text{LEAF}}$ ,  $A_{\max}$ ,  $V_{c,\max}$ ,  $A$  and  $g_s$ , and differences between means for the two age classes were assessed by independent samples  $t$ -tests. We tested for whether the response of  $\Psi_{\text{LEAF}}$  to  $D$  differed between the two age classes with analysis of covariance with  $D$  as a covariate. Differences in ambient  $A$  and  $g_s$  were assessed using analysis of covariance with leaf-to-air vapour pressure deficit (calculated from leaf temperature air temperature and relative humidity) as a covariate. Differences for all statistical tests were evaluated at  $\alpha = 0.05$ .

## RESULTS

### Weather

Temperature and vapour pressure deficit within the canopy differed little between two plots, although the 5 year plot tended to be slightly warmer and drier.  $T_a$ , and  $D$  differed by  $< 0.2^\circ\text{C}$ , and  $< 0.05 \text{ kPa}$ , respectively, between the plots. Precipitation for days 203–233 totalled 116 mm.

### Water flux and crown conductance

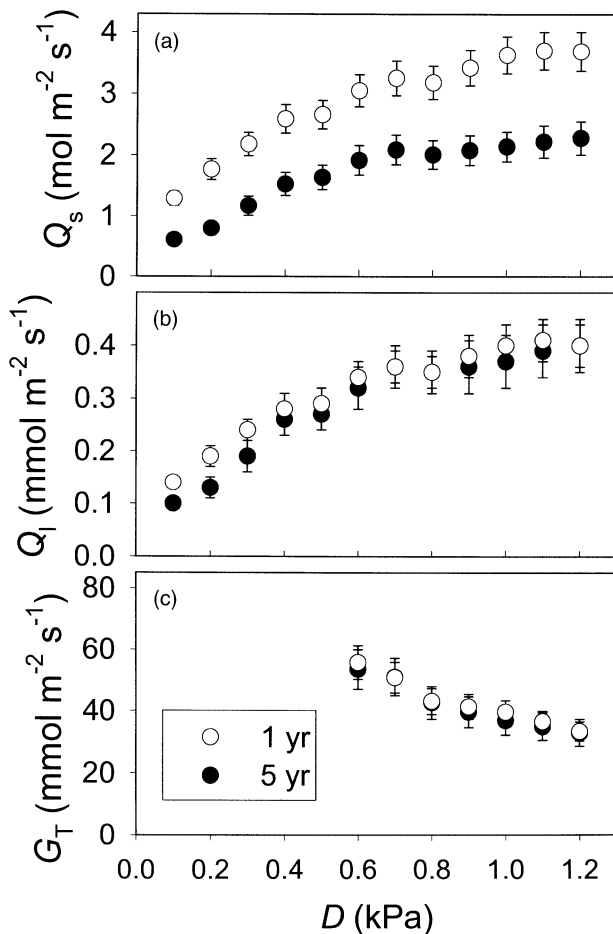
Water flux per unit sapwood area was much larger for the 1 year than for the 5 year trees under high light. For  $I_p > 800 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $Q_s$  for the 1 year trees was  $3.21 \text{ mol m}^{-2} \text{s}^{-1}$  (SE = 0.27) compared with  $1.94 \text{ mol m}^{-2} \text{s}^{-1}$  (SE = 0.23) for the 5 year trees ( $P < 0.01$ , Fig. 1a). Mean  $Q_s$  over 24 h was  $0.85 \text{ mol m}^{-2} \text{s}^{-1}$  (SE = 0.07) compared with  $0.65 \text{ mol m}^{-2} \text{s}^{-1}$  (SE = 0.08) for the 5 year trees ( $P = 0.07$ ). The value of  $Q_s$  steadily increased with increasing  $D$  between 0.0 and 0.6 kPa, and saturated at  $D > 0.6 \text{ kPa}$  (Fig. 1a).

In contrast to  $Q_s$ , water flux per unit leaf area was the same for the 1 year and 5 year trees under all conditions. Under high light,  $Q_l$  was  $0.35 \text{ mmol m}^{-2} \text{s}^{-1}$  (SE = 0.03) for

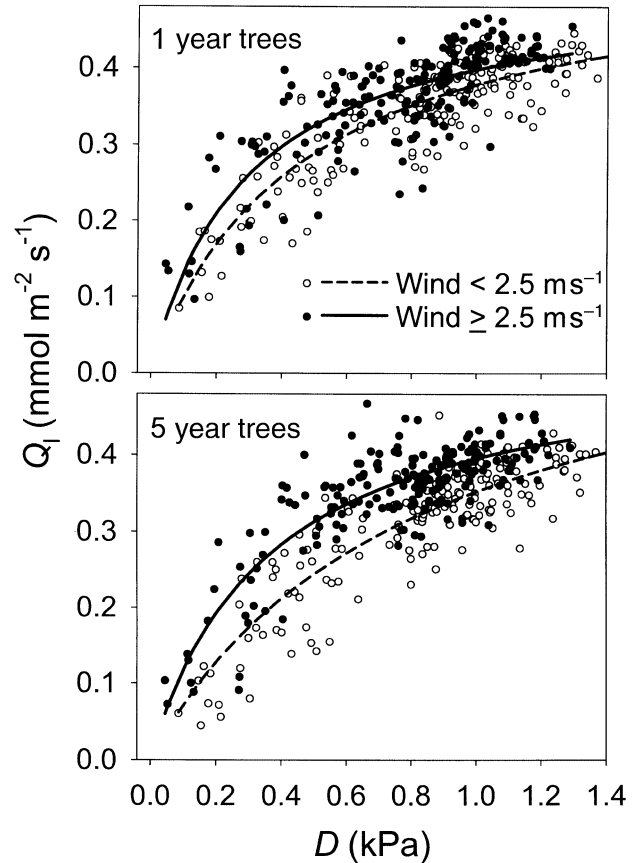
1 year trees compared with  $0.33 \text{ mmol m}^{-2} \text{ s}^{-1}$  (SE = 0.04) for the 5 year ( $P = 0.74$ , Fig. 1b). Average  $Q_1$  over 24 h also did not differ between 1 year trees ( $0.09 \text{ mmol m}^{-2} \text{ s}^{-1}$ , SE = 0.01) and 5 year trees ( $0.11 \text{ mmol m}^{-2} \text{ s}^{-1}$ , SE = 0.01,  $P = 0.36$ ). Average  $G_T$  at high light did not differ between the two age classes [1 year:  $40.0 \text{ mmol m}^{-2} \text{ s}^{-1}$  (SE = 3.8); 5 year:  $38.7 \text{ mmol m}^{-2} \text{ s}^{-1}$  (SE = 4.9),  $P = 0.83$ , Fig. 1c];  $G_T$  declined with increasing  $D$ . The 5 year trees had a greater ratio ( $2.7 \times$ ) of conducting to transpiring area ratio ( $A_s : A_i$ ; Table 1,  $P < 0.01$ ).

Capacitance did not strongly influence  $Q_s$  in the analysis. For all trees, the correlation of  $Q_s$  with current  $D$  was greater than the correlation of  $Q_s$  and  $D$  from 15 to 180 min prior to measurement of  $Q_s$ . Additionally, the average daily proportion of total flux that occurred at night (another estimate of capacitance) was 4% for the 1 year trees and 9% for the 5 year trees.

Analysis of the effect of wind speed on  $Q_1$  showed that the foliage of the sample trees was well-coupled to the atmosphere ( $G_A \gg G_C$ ), so that stomatal conductance of



**Figure 1.** Water flux per unit sapwood area ( $Q_s$ ) is greater for 1 year trees, but not water flux per unit leaf area ( $Q_1$ ) or whole tree conductance ( $G_T$ ).  $Q_s$ ,  $Q_1$  and  $G_T$  were calculated from sapflow measurements for  $I_p > 800 \mu\text{mol m}^{-2} \text{ s}^{-1}$  by  $0.1 \text{ kPa } D$  classes. (a)  $Q_s$ ; (b)  $Q_1$ ; and (c)  $G_T$ . Error bars are standard errors of the variation among trees ( $n = 13$  for 1 year and 12 for 5 year).



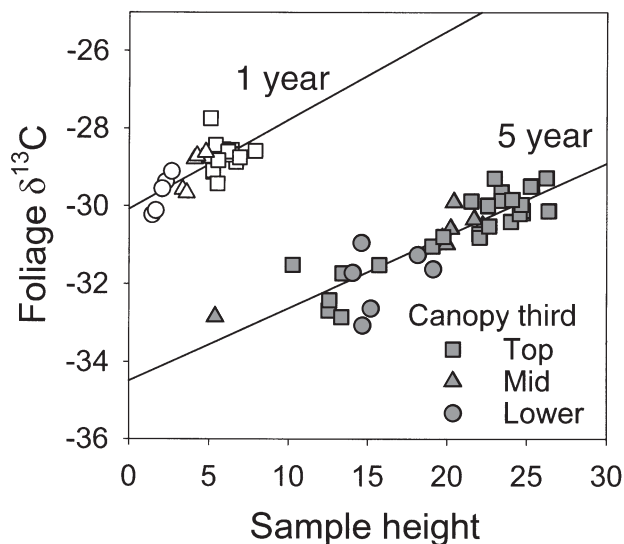
**Figure 2.** Wind speed has little effect on water flux per unit leaf area ( $Q_1$ ), indicating that canopy stomatal conductance ( $G_C$ )  $\approx$  whole tree conductance ( $G_T$ ). Points are the 15 min plot averages for  $I_p > 800 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Lines are fits to equation  $Q_1 = a \times D / (b + D)$ , where  $a$  and  $b$  are coefficients determined using non-linear regression. Solid line is model fit with 15 min average wind  $\geq 2.5 \text{ m s}^{-1}$ ; dashed line is model fit with 15 min average wind  $< 2.5 \text{ m s}^{-1}$ .

the tree crowns largely regulated  $G_T$ . Under high light,  $Q_1$  showed only small decreases under low wind compared to high wind conditions (Fig. 2, average decrease was 11% for the 1 year trees and 20% for the 5 year trees,  $P < 0.01$  for the difference in response between the two stands). Other studies of *Eucalyptus* have found values of a dimensionless decoupling coefficient (0 = perfectly coupled) to range from 0.12 to 0.63 (Mielke *et al.* 1999; White, Beadle & Worledge 2000).

Average whole tree leaf-specific hydraulic conductance ( $K_L$ ) was lower in the 5 year trees than in the 1 year trees, because of similar  $Q_1$  and lower  $\Psi_{\text{LEAF}}$ .  $K_L$  averaged  $1.52 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$  (SE = 0.18) in the 5 year trees and  $4.50 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$  (SE = 0.38) in the 1 year trees ( $P < 0.01$ ).

### Crown $\delta^{13}\text{C}$ and foliar N

Foliage on the 5 year trees had  $\delta^{13}\text{C}$  that averaged  $1.8\text{‰}$  lower than foliage on the 1 year trees, and  $2.0\text{‰}$  lower in



**Figure 3.** Foliar  $\delta^{13}\text{C}$  is 1.8‰ more negative for 5 year trees and varies with sample height (mid-point of canopy third). Lines are linear regressions of  $\delta^{13}\text{C}$  and sample height (1 year:  $y = -30.08 + 0.229x$ ,  $R^2 = 0.52$ ; 5 year:  $y = -34.45 + 0.183x$ ,  $R^2 = 0.77$ ).

the upper third ( $P < 0.01$ , Table 1, Fig. 3). Carbon isotope discrimination for these trees probably represents stomatal and photosynthetic characteristics, because respired  $\text{CO}_2$  has little effect on the isotopic composition of source air for canopies  $>1$  m (Buchmann, Brooks & Ehleringer 2002), and differences in the isotopic composition of source air for stands of different heights are likely to be 0.01–0.04‰ (Buchmann *et al.* 2002). If  $G_c$  is similar for the 1 and 5 year trees (as the analysis in ‘Water flux and crown conductance’ suggests), and photosynthetic capacity is similar for 1 and 5 year trees (see ‘Leaf measurements’, below), then the relative difference in whole tree photosynthesis per unit leaf area can be derived from  $C_i$  calculated from  $\delta^{13}\text{C}$ :

$$A = G_c (C_a - C_i) \quad (4)$$

as:

$$A_{5\text{ year}}/A_{1\text{ year}} = (C_a - C_i)_{5\text{ year}}/(C_a - C_i)_{1\text{ year}} \quad (5)$$

Calculation shows  $A_{5\text{ year}}/A_{1\text{ year}}$  was 0.71 per unit leaf area. Leaf area index at the time of harvest was 4.9 for the 1 year trees and 5.1 for the 5 year trees, so the ratio of whole tree photosynthesis for the two plots was 0.74. Above-ground wood production for the 1 year study plot from May to August 1999 was  $1.31 \text{ kg C m}^{-2} \text{ year}^{-1}$ , compared with  $0.90 \text{ kg C m}^{-2} \text{ year}^{-1}$  for the 5 year plot. The ratio of above-ground wood production for the study plots was 0.69. Growth for the 1 year plots was similar to the 5 year plots at the same age (M.G. Ryan, D. Binkley, J.H. Fownes, unpublished data).

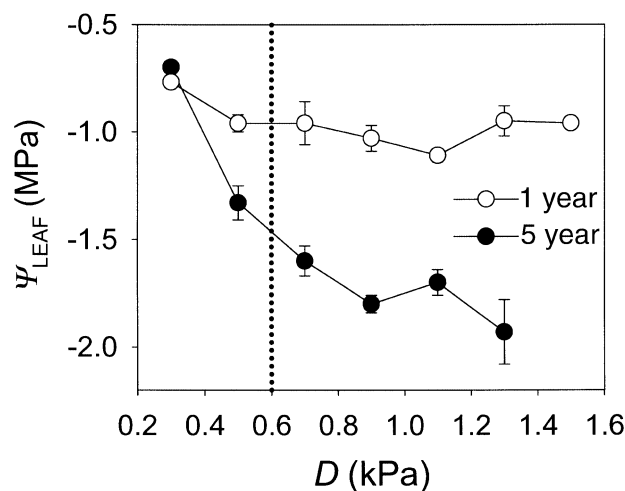
Foliar N declined from 1.87% at the canopy top to 1.43% in the bottom third in the 1 year trees, whereas in the 5 year trees foliar N was similar for the upper, middle, and lower canopy thirds (1.90–1.94%, Table 1). Nitrogen concentra-

tion was similar for the 1 and 5 year trees at the canopy top ( $P = 0.55$ ), but not for the lower two-thirds ( $P < 0.04$ ).

### Leaf measurements

Photosynthetic capacity, as estimated by  $A_{\text{max}}$  (Table 1), was similar for 1 and 5 year trees for foliage located at positions 1, 2, or 3 ( $P = 0.66, 0.09$  and  $0.33$ , respectively). For position 4 leaves, the 1 year trees had higher rates than 5 year trees ( $P < 0.01$ ) whereas for position 5 leaves, the 5 year trees had higher  $A_{\text{max}}$  ( $P < 0.01$ ).  $V_{c,\text{max}}$  did not differ between 1 and 5 year leaves ( $P = 0.25$ , Table 1). Ambient  $A$  and  $g_s$ , adjusted for any differences in leaf-to-air vapour pressure deficit, were higher for 1 year trees on day 211 ( $P < 0.04$ ), but statistically identical on days 179–184 ( $P > 0.24$ , Table 1). Leaf nitrogen concentration did not differ between the two plots for leaves measured for  $A_{\text{max}}$  for positions 2 ( $P = 0.70$ ) and 4 ( $P = 0.08$ ). Percentage N was consistently lower in 1 year trees for positions 3 and 5 ( $P < 0.01$ ), but was higher in 1 year trees for position 1 ( $P = 0.05$ ).

The value of  $\Psi_{\text{LEAF}}$  was regulated for  $D > 0.6$  kPa in both 1 year and 5 year trees, and  $\Psi_{\text{MIN}}$  was lower in the 5 year trees (Fig. 4). For both age classes,  $\Psi_{\text{LEAF}}$  declined with increasing  $D$  for  $D < 0.6$  kPa ( $P < 0.05$ ). For  $D > 0.6$  kPa,  $\Psi_{\text{LEAF}}$  was unrelated to  $D$  ( $P > 0.19$ ). Mean predawn  $\Psi_{\text{LEAF}}$  (corrected for gravitational potential) ranged from  $-0.07$  to  $-0.27$  MPa throughout the summer and did not differ between the two age classes ( $P = 0.16$ ). For  $D > 0.6$  kPa,  $\Psi_{\text{LEAF}}$  for the 5 year trees ( $-1.7$  MPa, SE = 0.03) was substantially lower than the 1 year trees ( $-1.0$  MPa, SE = 0.03,  $P < 0.01$ , Fig. 4).



**Figure 4.** Mid-day leaf water potential ( $\Psi_{\text{LEAF}}$ ) is lower for 5 year trees. Points are means for days 179, 181, and 211 by 0.2 kPa  $D$  classes. Error bars are the standard errors for the variability between foliar samples ( $n = 171$  for 1 year trees and  $n = 205$  for 5 year trees).  $\Psi_{\text{LEAF}}$  was not related to  $D$  for  $D > 0.6$  kPa (dotted line).

## DISCUSSION

The hydraulic limitation hypothesis, as originally stated by Yoder *et al.* (1994) and Ryan & Yoder (1997), failed to account for the decline in growth between trees of different heights. The value of  $\Psi_{\text{LEAF}}$  was regulated to restrict  $G_C$  at high  $D$ , but  $\Psi_{\text{MIN}}$  of the taller trees was substantially lower than that of the shorter trees. Hydraulic conductance did decrease with increased path length, but the lower hydraulic conductance in the taller trees did not result in lower water flux or  $G_T$ . An increase in sapwood to leaf area ratio and a decrease in  $\Psi_{\text{MIN}}$  compensated for increased path length and gravitational potential in the taller trees, and maintained  $G_T$ . Future tests of the hydraulic limitation hypothesis should consider compensatory changes with tree height and the carbon cost of these changes.

Evidence suggests that a decline in canopy photosynthesis was responsible for the difference in growth for the trees of different heights. Photosynthetic capacity, as estimated by  $A_{\text{max}}$  and  $V_{\text{c,max}}$ , were similar for the trees with different heights, but  $\delta^{13}\text{C}$  indicated that average whole tree  $C_i$  was higher in the tall trees. Calculations using  $\delta^{13}\text{C}$  show that with similar  $G_C$ , whole tree photosynthesis for the taller trees was 0.74 of the shorter trees, similar to the ratio of growth for the 5 year plot to growth for the 1 year plot (0.69).

### Compensation in $A_s : A_l$ and $\Psi_{\text{MIN}}$ maintained $G_T$

Our estimates of  $Q_l$  did not differ between the 1 year and 5 year trees despite large differences in  $Q_s$  and tree height, primarily because of the rapid shift in  $A_s : A_l$  and  $\Psi_{\text{MIN}}$  as the trees grew. The 5 year trees had nearly twice as much sapwood area to supply water to a unit of leaf area as the 1 year trees, and 0.8 MPa lower  $\Psi_{\text{MIN}}$ . An increase in  $A_s : A_l$  with tree height and age appears to be common, but not universal (McDowell *et al.* 2002b). In other studies, an increase in  $A_s : A_l$  did not completely compensate for the increased path length and gravitational potential in tall trees (Mencuccini & Grace 1996b; Ryan *et al.* 2000; Schäfer *et al.* 2000). For example, Schäfer *et al.* (2000) found that although  $A_s : A_l$  doubled with a 30 m increase in tree height in *Fagus sylvatica*,  $G_T$  decreased by about 60%.

A simple hydraulic model predicts how  $G_C$  at the same value of  $D$  should vary with tree height ( $h_m$ , tree height at crown mid point),  $A_s : A_l$  and  $\Delta\Psi_{\text{SOIL-LEAF}}$ , assuming that hydraulic conductivity is constant with tree size (Whitehead, Edwards & Jarvis 1984; Schäfer *et al.* 2000):

$$G_C \propto (1/h_m) (A_s : A_l) (\Delta\Psi_{\text{SOIL-LEAF}} - 0.01 h_m) \quad (6)$$

The ratio of  $G_C$  for two trees with heights  $h_1$  and  $h_2$  ( $G_{C-h2} : G_{C-h1}$ ) will then be proportional to the ratios of  $A_s : A_l$  and  $\Delta\Psi_{\text{SOIL-LEAF}}$  and inversely proportional to the ratio of tree heights. Equation 6 can be used to assess the effect of changes in  $A_s : A_l$  and  $\Delta\Psi_{\text{MIN}}$  in compensating for increased height. With no compensation for  $A_s : A_l$  or  $\Psi_{\text{MIN}}$ ,

Eqn 6 shows that adding 18.7 m in height should reduce  $G_C$  to 0.22 of the 1 year trees. Using actual  $A_s : A_l$  reduced  $G_C$  to 0.46 of the 1 year trees, and using both actual  $A_s : A_l$  and  $\Psi_{\text{MIN}}$  reduced  $G_C$  to only 0.94 of the 1 year trees. This analysis agrees with measurements of  $G_T$  derived from sapflow, and shows that both increased  $A_s : A_l$  and decreased  $\Psi_{\text{MIN}}$  nearly completely compensated for the increased height.

Decreased  $K_L$  appears to be a common, if not universal, consequence of increased tree height (Mencuccini & Grace 1996a; Hubbard *et al.* 1999; Ryan *et al.* 2000; Schäfer *et al.* 2000; Phillips *et al.* 2001; McDowell *et al.* 2002a; Phillips *et al.* 2002). However, adjustments in  $A_s : A_l$  and perhaps hydraulic conductivity (Pothier, Margolis & Waring 1989) keep  $K_L$  from further reductions, and reduced  $\Psi_{\text{MIN}}$  and increased capacitance partially or entirely compensate for decreased  $K_L$  to mitigate a decline in  $G_C$  (McDowell *et al.* 2002a). The presence of compensatory mechanisms and their effect can be variable, even within a genus. For example, in contrast to the results in this study, Olbrich (1994) found that 16 year (56 m) *Eucalyptus grandis* had transpiration per unit leaf area (proportional to  $Q_l$  and  $G_C$ ) that was only 60% of 3 year (18 m) trees. Roberts, Vertessy & Grayson (2001) examined height/age-related differences in *E. sieberi* and found  $Q_l$  in 45 year trees reduced to 50% of that measured in 14 year trees; surprisingly,  $A_s : A_l$  was also reduced 50% in the 45 year trees.

Greater capacitance in the 5 year trees could also increase  $G_C$  over that predicted by Eqn 6 (Waring & Running 1978; Waring, Whitehead & Jarvis 1979). Capacitance did not influence the response of  $Q$  to  $D$  in this study, but may have supplemented total flux. The 9% of the daily  $Q$  occurred at night in the 5 year trees can be interpreted as a measure of capacitance within the tree (Ryan *et al.* 2000) and may have reduced demand on the root systems during the day (Tyree & Ewers 1991) and perhaps contributed to a reduction in differences in  $Q_l$  between the two age classes.

Inferring  $G_C$  from  $Q_l$  measurements requires that foliage stomatal conductance be well coupled with atmospheric conditions and that leaf temperature  $\approx$  air temperature. Although we did not directly estimate foliar-atmospheric coupling, the slight response of  $Q_l$  to wind speed indicates that  $G_C$  largely controls water loss and that  $G_C \approx G_T$ . Good coupling between the foliage and the atmosphere also suggests that leaf temperature  $\approx$  air temperature. The wind speed analysis does suggest that  $G_A$  is slightly lower in the 5 year canopies and that they are slightly less coupled to the atmosphere. If so,  $G_C$  for the 5 year trees would be slightly greater than that for the 1 year canopies, providing further evidence against a hydraulic limitation to  $G_T$ .

### What is the cost of compensation?

As originally described, the hydraulic limitation hypothesis (Yoder *et al.* 1994; Ryan & Yoder 1997) did not consider adjustments to compensate for increased tree height, and any potential effects of compensatory mechanisms on the

carbon balance of a tree. However, compensation appears to be an important feature that accompanies height growth, and adjustments could alter both photosynthesis and allocation. Below, we consider two adjustments, increased  $A_s : A_l$  and reduced  $\Psi_{\text{MIN}}$ .

Increased  $A_s : A_l$  with height implies a greater redundancy in the amount of area conducting water, but also a potentially greater respiration cost to support the ray parenchyma in the xylem (Sprugel 1990). However, the available evidence suggests that woody respiration costs are not greater in larger trees (Ryan & Waring 1992; Lavigne & Ryan 1997). Additionally, measurements made at our study site show a marked decline in respiration per unit sapwood with tree age and height and a constant ratio (~10%) of woody respiration to net photosynthesis from age 1 to age 6 (M.G. Ryan, D. Binkley, J.H. Fownes, C.P. Giardina, and R. Senock, unpublished manuscript). These measurements suggest that neither increased  $A_s : A_l$  nor increased sapwood volume with tree height incur a greater respiration cost as trees grow. Increased below-ground allocation could also compensate for increased height (Magnani *et al.* 2000), but there is no evidence of it doing so (Smith & Resh 1999; Giardina & Ryan 2002; Litton 2002).

The carbon cost of compensation by maintaining lower  $\Psi_{\text{MIN}}$  is more difficult to assess. Lower  $\Psi_{\text{MIN}}$  exposes the conducting system to greater risk of cavitation and a reduction or loss in the ability to carry water (Tyree & Sperry 1988), with the potential for lower stomatal opening and photosynthesis. However, water freed by cavitation may be available for use during the day (capacitance), which may increase stomatal opening. If embolized vessels or tracheids are refilled at night (Holbrook *et al.* 2001), then redundancy in sapwood provides a real benefit to a larger tree, at a low cost. Finally, lower  $\Psi_{\text{MIN}}$  may affect translocation (Hsiao 2000; Hsiao & Xu 2000) and indirectly lower photosynthesis.

We recommend that future evaluations of the hydraulic limitation hypothesis consider the carbon costs of compensation. Such costs are easier to assess in even-aged stands of trees than for isolated trees because below-ground allocation cannot yet be measured for individuals and because growth comparisons are difficult if individuals differ in leaf area.

### A potential mechanism

Crown level measurements of  $\delta^{13}\text{C}$  and  $G_{\text{T}}$  indicate lower canopy photosynthesis for the 5 year trees. One possible mechanism consistent with these data is a reduced triose phosphate utilization (Harley & Sharkey 1991; Harley *et al.* 1992) in the 5 year trees, perhaps caused by the effect of a 0.8 MPa lower  $\Psi_{\text{LEAF}}$  on translocation and growth (Janoudi, Widders & Flore 1993; Hsiao 2000; Hsiao & Xu 2000). Differences in triose phosphate utilization could cause differences in diurnal photosynthesis in the 1 and 5 year trees, even though our assessments of photosynthetic capacity ( $A_{\text{max}}$ ,  $V_{\text{c,max}}$ , and foliar N) were similar. Lower  $\Psi_{\text{LEAF}}$  would

be a chronic problem for tall trees; even at night,  $\Psi_{\text{LEAF}}$  would reflect differences in gravitational potential.

Estimates of net photosynthesis at our study site derived from summing carbon used for above-ground respiration, above-ground production, and total below-ground carbon allocation (M.G. Ryan, D. Binkley, J.H. Fownes, C.P. Giardina, and R. Senock, unpublished manuscript) support the contention that a decline in canopy photosynthesis is responsible for the decline in wood production. Our estimate of lower canopy photosynthesis for the 5 year trees agrees with diurnal measurements of photosynthesis on position 2 leaves on day 211, but not with photosynthesis measurements made on days 179–184. We do not have an explanation for this discrepancy. Estimates of  $C_i$  from  $\delta^{13}\text{C}$ , and canopy  $A$  from  $C_i$  and  $G_{\text{T}}$  are measurements at the canopy level integrated over a much longer period of time than the leaf-level measurements, and these likely reflect a better long-term average condition than the instantaneous leaf-level measurements.

### CONCLUSION

Our findings refute the hypothesis that hydraulic limitation to liquid phase water conductance, acting through a lowering of  $G_{\text{C}}$ , is responsible for reduced growth after canopy closure in this moist tropical ecosystem. The water-conducting system changed rapidly with tree height, and these changes (reduced  $\Psi_{\text{MIN}}$  and increased  $A_s : A_l$ ) compensated for the effects of increased path length and gravitational potential and maintained canopy conductance while growth declined. The effect of these compensatory changes on the tree's carbon budget and the vulnerability to loss of conducting system remain to be explored. The hydraulic limitation hypothesis should be broadened to include the effects of compensation, and future tests of the hypothesis should consider the entire carbon budget of the tree, in addition to changes in canopy conductance and photosynthesis. The  $^{13}\text{C}$  data suggest that a mechanism not observed in this study may also play a role in changing  $C_i$  with tree height or age.

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## REFERENCES

- Binkley D. & Resh S.C. (1999) Rapid changes in soils following *Eucalyptus* afforestation in Hawaii. *Soil Science Society of America Journal* **63**, 222–225.
- Binkley D., Dunkin K.A., Debell D. & Ryan M.G. (1992) Production and nutrient cycling in mixed plantations of *Eucalyptus* and *Albizia* in Hawaii. *Forest Science* **38**, 393–408.
- Bond B.J. & Kavanagh K.L. (1999) Stomatal behavior of four woody species in relation to leaf-specific hydraulic conductance and threshold water potential. *Tree Physiology* **19**, 503–510.
- Buchmann N., Brooks J.R. & Ehleringer J.R. (2002) Predicting daytime carbon isotope ratios of atmospheric CO<sub>2</sub> within forest canopies. *Functional Ecology* **16**, 49–57.
- Ewers B.E. & Oren R. (2000) Analyses of assumptions and errors in the calculation of stomatal conductance from sap flux measurements. *Tree Physiology* **20**, 579–589.
- Farquhar G.D., von Caemmerer S. & Berry J.A. (1980) A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C<sub>3</sub> species. *Planta* **149**, 78–90.
- Giardina C.P. & Ryan M.G. (2002) Total belowground carbon allocation in a fast growing *Eucalyptus* plantation estimated using a carbon balance approach. *Ecosystems* **5**, 487–499.
- Granier A. (1987) Sap flow measurements in Douglas-fir tree trunks by means of a new thermal method. *Annales Des Sciences Forestieres* **44**, 1–14.
- Harley P.C. & Sharkey T.D. (1991) An improved model of C<sub>3</sub> photosynthesis at high CO<sub>2</sub>: Reversed O<sub>2</sub> sensitivity explained by lack of glycerate reentry into the chloroplast. *Photosynthesis Research* **27**, 169–178.
- Harley P.C., Thomas R.B., Reynolds J.F. & Strain B.R. (1992) Modeling photosynthesis of cotton grown in elevated CO<sub>2</sub>. *Plant, Cell and Environment* **15**, 271–282.
- Hinckley T.M., Lassoie J.P. & Running S.W. (1978) Temporal and spatial variations in water status of forest trees. *Forest Science* **24**, 1–72.
- Holbrook N.M., Ahrens E.T., Burns M.J. & Zwieniecki M.A. (2001) In vivo observation of cavitation and embolism repair using magnetic resonance imaging. *Plant Physiology* **126**, 27–31.
- Hsiao T.C. (2000) Leaf and root growth in relation to water status. *Hortscience* **35**, 1051–1058.
- Hsiao T.C. & Xu L.K. (2000) Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *Journal of Experimental Botany* **51**, 1595–1616.
- Hubbard R.M., Bond B.J. & Ryan M.G. (1999) Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. *Tree Physiology* **19**, 165–172.
- Hubbard R.M., Ryan M.G., Stiller V. & Sperry J.S. (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell and Environment* **24**, 113–121.
- Janoudi A.K., Widders I.E. & Flore J.A. (1993) Water deficits and environmental factors affect photosynthesis in leaves of cucumber (*Cucumis sativus*). *Journal of the American Society for Horticultural Science* **118**, 366–370.
- Kolb K.J. & Davis S.D. (1994) Drought tolerance and xylem embolism in co-occurring species of coastal sage and chaparral. *Ecology* **75**, 648–659.
- Lavigne M.B. & Ryan M.G. (1997) Growth and maintenance respiration rates of aspen, black spruce and jack pine stems at northern and southern BOREAS sites. *Tree Physiology* **17**, 543–551.
- Leuning R. (1995) A critical appraisal of a combined stomatal-photosynthesis model for C<sub>3</sub> plants. *Plant, Cell and Environment* **18**, 339–355.
- Litton C.M. (2002) *Above- and Belowground Carbon Allocation in Post-fire Lodgepole Pine Forests: Effects of Tree Density and Standage*. Ph.D. Dissertation. University of Wyoming, Laramie, WY, USA.
- Magnani F., Mencuccini M. & Grace J. (2000) Age-related decline in stand productivity: the role of structural acclimation under hydraulic constraints. *Plant, Cell and Environment* **23**, 251–263.
- McDowell N.G., Phillips N., Lunch C., Bond B.J. & Ryan M.G. (2002a) An investigation of hydraulic limitation and compensation in large, old Douglas-fir trees. *Tree Physiology* **22**, 763–774.
- McDowell N.G., Barnard H.R., Bond B.J., *et al.* (2002b) The relationship between tree height and leaf area: sapwood area ratio. *Oecologia* **132**, 12–20.
- Meinzer F.C. & Grantz D.A. (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport. *Plant, Cell and Environment* **13**, 383–388.
- Mencuccini M. & Grace J. (1996a) Hydraulic conductance, light interception and needle nutrient concentration in Scots pine stands and their relation with net primary productivity. *Tree Physiology* **16**, 459–468.
- Mencuccini M. & Grace J. (1996b) Developmental patterns of aboveground hydraulic conductance in a Scots pine (*Pinus sylvestris* L.) age sequence. *Plant, Cell and Environment* **19**, 939–948.
- Mielke M.S., Oliva M.A., de Barros N.F., Penchel R.M., Martinez C.A. & de Almeida A.C. (1999) Stomatal control of transpiration in the canopy of a clonal *Eucalyptus grandis* plantation. *Trees-Structure and Function* **13**, 152–160.
- Olbrich B.W. (1994) *The Application of the Heat Pulse Velocity Technique to the Study of Transpiration from Eucalyptus grandis*. PhD Dissertation, University of Natal, Durban, South Africa.
- Oren R., Sperry J.S., Katul G.G., Pataki D.E., Ewers B.E., Phillips N. & Schafer K.V.R. (1999) Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell and Environment* **22**, 1515–1526.
- Pataki D.E., Oren R. & Phillips N. (1998) Responses of sap flux and stomatal conductance of *Pinus taeda* L. Trees to stepwise reductions in leaf area. *Journal of Experimental Botany* **49**, 871–878.
- Phillips N., Bond B.J., McDowell N.G. & Ryan M.G. (2002) Canopy and hydraulic conductance in young, mature and old Douglas-fir trees. *Tree Physiology* **22**, 205–211.
- Phillips N., Bond B.J. & Ryan M.G. (2001) Gas exchange and hydraulic properties in the crowns of two tree species in a Panamanian moist forest. *Trees* **15**, 123–130.
- Phillips N., Nagchaudhuri A., Oren R. & Katul G. (1997) Time constant for water transport in loblolly pine trees estimated from time series of evaporative demand and stem sapflow. *Trees* **11**, 412–419.
- Pothier D., Margolis H.A. & Waring R.H. (1989) Patterns of change of saturated sapwood permeability and sapwood conductance with stand development. *Canadian Journal of Forest Research* **19**, 432–439.
- Roberts S., Vertessy R. & Grayson R. (2001) Transpiration from *Eucalyptus sieberi* (L. Johnson) forests of different age. *Forest Ecology and Management* **143**, 153–161.
- Ryan M.G. & Waring R.H. (1992) Maintenance respiration and stand development in a subalpine lodgepole pine forest. *Ecology* **73**, 2100–2108.
- Ryan M.G. & Yoder B.J. (1997) Hydraulic limits to tree height and tree growth. *Bioscience* **47**, 235–242.
- Ryan M.G., Binkley D. & Fownes J.H. (1997) Age-related decline in forest productivity: pattern and process. *Advances in Ecological Research* **27**, 213–262.
- Ryan M.G., Bond B.J., Law B.E., Hubbard R.M., Woodruff D., Cienciala E. & Kucera J. (2000) Transpiration and whole-tree conductance in ponderosa pine trees of different heights. *Oecologia* **124**, 553–560.

- Saliendra N.Z., Sperry J.S. & Comstock J.P. (1995) Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. *Planta* **196**, 357–366.
- Schäfer K.V.R., Oren R. & Tenhunen J.D. (2000) The effect of tree height on crown level stomatal conductance. *Plant, Cell and Environment* **23**, 365–375.
- Smith F.W. & Resh S.C. (1999) Age-related changes in production and below-ground carbon allocation in *Pinus contorta* forests. *Forest Science* **45**, 333–341.
- Sperry J.S. & Pockman W.T. (1993) Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **16**, 279–287.
- Sperry J.S., Alder N.N. & Eastlack S.E. (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *Journal of Experimental Botany* **44**, 1075–1082.
- Sprugel D.G. (1990) Components of woody-tissue respiration in young *Abies amabilis* trees. *Trees* **4**, 88–98.
- Teskey R.O., Hinckley T.M. & Grier C.C. (1983) Effect of interruption of flow path on stomatal conductance of *Abies amabilis*. *Journal of Experimental Botany* **34**, 1251–1259.
- Thom A.S. (1972) Momentum, mass, and heat exchange of vegetation. *Quarterly Journal of the Royal Meteorological Society* **98**, 124–134.
- Tyree M.T. & Ewers F.W. (1991) The hydraulic architecture of trees and other woody plants (Tansley Review, 34). *New Phytologist* **119**, 345–360.
- Tyree M.T. & Sperry J.S. (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? *Plant Physiology* **88**, 574–580.
- Tyree M.T., Snyderman D.A., Wilmot T.R. & Machado J.L. (1991) Water relations and hydraulic architecture of a tropical tree (*Schefflera morototoni*) – Data, models, and a comparison with 2 temperate species (*Acer saccharum* and *Thuja occidentalis*). *Plant Physiology* **96**, 1105–1113.
- Waring R.H. & Running S.W. (1978) Sapwood water storage: its contribution to transpiration and effect upon water conductance through the stems of old-growth Douglas-fir. *Plant, Cell and Environment* **1**, 131–140.
- Waring R.H., Whitehead D. & Jarvis P.G. (1979) The contribution of stored water to transpiration in Scots pine. *Plant, Cell and Environment* **2**, 309–317.
- White D.A., Beadle C.L. & Worledge D. (2000) Control of transpiration in an irrigated *Eucalyptus globulus* Labill. plantation. *Plant, Cell and Environment* **23**, 123–134.
- Whitehead D., Edwards W.R.N. & Jarvis P.G. (1984) Conducting sapwood area, foliage area, and permeability in mature trees of *Picea sitchensis* and *Pinus contorta*. *Canadian Journal of Forest Research* **14**, 940–947.
- Whitehead D., Livingston N.J., Kelliher F.M., Hogan K.P., Peppin S., McSeveny T.M. & Byers J.N. (1996) Response of transpiration and photosynthesis to transient change in illuminated foliage area for a *Pinus radiata* D. Don tree. *Plant, Cell and Environment* **19**, 949–957.
- Yoder B.J., Ryan M.G., Waring R.H., Schoettle A.W. & Kaufmann M.R. (1994) Evidence of reduced photosynthetic rates in old trees. *Forest Science* **40**, 513–527.

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## APPENDIX

### Symbols

Symbol	Term	Units
$g_s$	Stomatal conductance	$\text{mmol m}^{-2} \text{s}^{-1}$
$A$	Photosynthesis	$\mu\text{mol m}^{-2} \text{s}^{-1}$
$C_p, C_a$	Inter-cellular, ambient $\text{CO}_2$ concentration	$\mu\text{mol mol}^{-1}$
$A_{\text{max}}$	Photosynthesis with $D < 0.8 \text{ kPa}$ , $I_p = 1300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , $C_a \sim 360$	$\mu\text{mol m}^{-2} \text{s}^{-1}$
$A_s : A_l$	Sapwood area to leaf area	$\text{m}^2 \text{m}^{-2}$
$V_{c,\text{max}}$	Maximum carboxylation velocity	$\mu\text{mol m}^{-2} \text{s}^{-1}$
$\Psi_{\text{LEAF}}$	Leaf water potential	MPa
$\Psi_{\text{MIN}}$	Minimum leaf water potential	MPa
$Q_l$	Water flux through xylem (sapflow) per unit leaf area	$\text{mmol m}^{-2} \text{s}^{-1}$
$Q_s$	Water flux through xylem (sapflow) per unit sapwood area	$\text{mol m}^{-2} \text{s}^{-1}$
$G_T$	Total canopy conductance to water vapour	$\text{mmol m}^{-2} \text{s}^{-1}$
$G_C$	Canopy stomatal conductance to water vapour	$\text{mmol m}^{-2} \text{s}^{-1}$
$G_A$	Canopy aerodynamic conductance to water vapour	$\text{mmol m}^{-2} \text{s}^{-1}$
$K_L$	Hydraulic conductance from root-to-leaf, calculated with Eqn 3	$\text{kg m}^{-2} \text{h}^{-1} \text{MPa}^{-1}$
$D$	Vapour pressure deficit	kPa
$I_p$	Photosynthetically active radiation	$\mu\text{mol m}^{-2} \text{s}^{-1}$
$T_a$	Air temperature	$^{\circ}\text{C}$