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BRIEF COMMUNICATION

Photosynthetic limitation of similar-height *Cryptomeria japonica* trees growing at different rates

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Abstract

We compared light-saturated photosynthetic rates and their stomatal limitations among *Cryptomeria japonica* trees with a similar height but different current growth rates. Although slow-growing trees had a lower stomatal conductance and a higher carbon isotope ratio in shoots, the stomatal limitations in photosynthesis were not different. Large mesophyll CO_2 diffusion resistance contributed to the low photosynthetic rate of the slow-growing trees.

Additional key words: P_N-C_i curve; stable carbon isotope ratio; water potential.

The depression in a growth and photosynthesis of aged trees has been explained by the hydraulic limitation hypothesis (Ryan and Yoder 1997, Day *et al.* 2001, Ryan *et al.* 2006, Matsuzaki *et al.* 2005), in view of this, a water stress caused by a height of tall trees decreases a stomatal conductance (g_s) and thus depresses photosynthesis. However, low stomatal conductance (g_s) did not fully explain the low photosynthetic rates of tall, old *Picea abies* and *Pinus sylvestris* that might be also affected by a large mesophyll CO_2 diffusion resistance (r_m) (Niinemets 2002). r_m is a sum of a gas phase diffusion resistance from sub-stomatal cavities to a surface of mesophyll cell wall and a liquid phase diffusion resistance from a cell wall to chloroplast stroma. Because of much slower diffusion in liquid phase compared to gas phase, r_m is affected by a liquid phase diffusion distance such as cell wall thickness (Evans *et al.* 2009). Leaf water condition could affect leaf morphological development and thus also r_m. The mesophyll conductance (the reciprocal of r_m) decreased in saplings grown under dry soil conditions (Duan *et al.* 2009). The mesophyll cell walls of Douglas fir became thicker as a tree height increased, and the thickness was negatively correlated with the mesophyll conductance and photosynthetic rate (Woodruff *et al.* 2009). We often see trees of a similar height growing at different rates. The explanation for the difference is that leaf water condition is not determined solely by a tree height (*i.e.*, a hydraulic length), but it is also affected by environmental factors, such as soil water conditions or soil depth. Here, we examined three hypotheses: (*1*) that the leaf water condition of slow-growing (SG) trees is drier than those of similar height but fast-growing (FG) trees; (*2*) that the photosynthetic rate of SG trees is lower than that of FG trees; and (*3*) that r_m decreases the photosynthetic rate of SG trees.

The sample stands were a 73-year-old SG tree stand and a 20-year-old FG tree stand at the Experimental Station at Tanashi, University of Tokyo, on the Musashino

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Abbreviations: P_N – net photosynthetic rate; FG – fast-growing; SG – slow-growing; C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; C_c – CO_2 concentration in chloroplast stroma; g_s – stomatal conductance; r_m – mesophyll CO_2 diffusion resistance; CE – carboxylation efficiency on a nitrogen content basis; PPFD – photosynthetic photon flux density; $\delta^{13}C$ – stable carbon isotope ratio; ψ_w – water potential.

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Plateau, where the topography is almost flat. The soil is an Andosol formed from volcanic ash. We examined four trees in each stand. The mean heights of the sample trees were 19.8 ± 0.5 m in SG trees and 18.5 ± 0.1 min FG trees.The annual height growth rates in 2008 and 2009 were 0.23 ± 0.10 and 0.19 ± 0.04 m a^{-1} , respectively, in SG trees and 1.15 ± 0.33 and 0.84 ± 0.03 m a^{-1} in FG trees. The thickness of the layer of soft soil (the penetration resistance < 5.8 × 10^6 kg m^{-1} s^{-2}, measured by a soil penetrometer) was about 1.0 m in SG tree stand and 1.7 m in FG tree stand. Air temperature, relative humidity, and photosynthetic photon flux density (PPFD) were measured at an open area near the sample stands.

We measured diurnal changes of water potential (ψ_w) and gas-exchange rates in current-year shoots close to the tops of crowns with a pressure chamber (*Model 3000*, *Soilmoisture Equipment Corp.*, Santa Barbara, CA, USA) and a portable photosynthesis system (*LI-6400*, *Li-Cor Inc.*, Lincoln, NE, USA), on 12–15 August 2009. Three shoots were measured on each tree. The gas-exchange rates were expressed on a shoot dry mass basis. The CO_2 concentration in the leaf chamber was maintained at about 360 ppm. Air temperature and relative humidity in the chamber were similar to ambient, except in the early morning, when the ambient relative humidity was over 90% RH. Nitrogen contents of the shoots sampled above ($n = 6$) and from current-year shoots from the same trees in September 2008 ($n = 3$) were analysed with an *NCS analyser* (*NA1500*, *Thermo Finnigan*, San Jose, CA, USA). Stable carbon isotope ratios ($\delta^{13}C$) were analysed with an elemental analyser/mass spectrometer (*NC2500/ Delta Plus System*, *Thermo Finnigan*, San Jose, CA, USA). To estimate carboxylation efficiency, we measured the net photosynthetic rate (P_N) of current-year shoots at a range of low CO_2 concentrations in detached 20-cm-long branches ($n = 2$) with their cut ends immersed in water (Farquhar *et al.* 1980). The CO_2 concentration of the inlet air was controlled at 30, 50, or 100 ppm. P_N was measured under PPFD higher than 1,000 µmol m^{-2} s^{-1}. The efficiency was calculated as the slope of the linear regression ($r > 0.99$, $P<0.01$) between P_N and intercellular CO_2 concentration (C_i). To eliminate the effect of photosynthetic capacity, we divided the carboxylation efficiency by the nitrogen concentration (CE) of the sample shoots (Matsuzaki *et al.* 2005). The ψ_w of the detached shoots were confirmed to be above –0.02 MPa after each gas-exchange measurement.

The ψ_w of current year shoots was significantly more negative in SG trees than in FG trees at midday (Table 1, $P<0.05$). The daily minimum ψ_w were –1.61 ± 0.06 MPa in SG trees and –1.37 ± 0.04 MPa in FG trees. Light-saturated P_N at midday (10:00–15:00) was lower in SG trees than in FG trees (Table 1, $P<0.05$). The maximum g_s was much lower in SG trees than in FG trees (Fig. 1, $P<0.05$). $\delta^{13}C$ (–26.3 ± 0.6‰) was significantly higher in SG trees than in FG trees (–29.2 ± 0.1). $\delta^{13}C$ in 2008 showed the same tendency (–25.7 ± 0.9‰ in SG trees, –28.2‰ ± 0.3‰ in FG trees). Although there was a clear difference in the light-saturated P_N between SG and FG trees (Fig. 1), there were no clear differences in C_i or in stomatal limitations of photosynthesis (ratio of C_i to ambient CO_2 concentration, C_a) (Table 1). CE of SG trees (9.1 ± 3.0 mmol g N^{-1} s^{-1}) was significantly lower than that of FG trees (17.1 ± 1.2 mmol g N^{-1} s^{-1}).

As a tree height increases, leaf ψ_w gets more negative at a range of –0.01 to –0.02 MPa m^{-1} (Woodruff and Meinzer 2011). The difference in the tree height between SG and FG trees was too small to explain the difference in the daily minimum shoot ψ_w. The thinner soft-soil depth in SG tree stand could be a factor influencing the shoot ψ_w. The largest $\delta^{13}C$ occurs during a fixation of CO_2 by Rubisco in the chloroplast (Lanigan *et al.* 2008). The significantly higher $\delta^{13}C$ of SG trees means that the CO_2 concentration at the fixation site in chloroplast stroma (C_c) was lower in SG trees than in FG trees. C_c decreases as C_i decreases or as r_m increases. Although g_s was lower in SG trees compared with FG trees, no difference was found in C_i (Table 1, Fig. 1). These results indicate that r_m was larger in SG trees than in FG trees. The lower CE in SG trees suggests the same. On the assumption that there was a similar carboxylation capacity per unit of Rubisco in SG and FG trees, the

Table 1. Net photosynthesis, stomatal conductance, water potential, and stomatal limitation based on the intercellular and ambient CO_2 concentrations at midday (10:00–15:00) in current-year shoots of slow-growing (SG) and fast-growing (FG) *Cryptomeria japonica* trees. Values are means ± SD. T_{air} – air temperature (°C, $n = 11$); VPD – vapour pressure deficit (kPa, $n = 11$); PPFD – photosynthetic photon flux density (µmol m^{-2} s^{-1}, $n = 600$); ψ_w – water potential (MPa, $n = 20–24$); g_s – stomatal conductance (mmol g^{-1}(DM)s^{-1}, $n = 20–24$); P_N – net photosynthetic rate (nmol g^{-1}(DM) s^{-1}, $n = 20–24$); C_i – CO_2 concentration in intercellular space (ppm, $n = 20–24$); C_i/C_a – ratio of intercellular to ambient CO_2 concentration as an index of stomatal limitation of photosynthesis ($n = 20–24$). n = number of measurements × number of sample trees. Different letters denote significant differences between measurement days (*t*-test with *Bonferroni'*s correction, $P<0.05$).

Stand	Date	T_{air}	VPD	PPFD	Ψ_w	g_s	P_N	C_i	C_i/C_a
SG	Aug. 12	32.5 ± 1.5^b	2.1 ± 0.4^a	1481 ± 464^a	-1.50 ± 0.15^b	0.26 ± 0.13^b	14.3 ± 5.3^c	260 ± 17^{ab}	0.72 ± 0.05^{ab}
	Aug. 15	33.7 ± 1.7^a	2.9 ± 0.5^a	1524 ± 434^a	-1.46 ± 0.08^b	0.25 ± 0.08^b	17.6 ± 5.3^c	242 ± 9^c	0.67 ± 0.03^c
FG	Aug. 13	32.6 ± 2.3^{ab}	1.9 ± 0.6^b	1192 ± 462^b	-1.26 ± 0.13^a	0.57 ± 0.16^a	42.2 ± 8.6^a	269 ± 12^a	0.74 ± 0.03^a
	Aug. 14	33.3 ± 1.4^{ab}	2.4 ± 0.5^{ab}	1096 ± 463^c	-1.29 ± 0.09^a	0.32 ± 0.06^b	30.2 ± 3.5^b	243 ± 13^{bc}	0.67 ± 0.04^{bc}

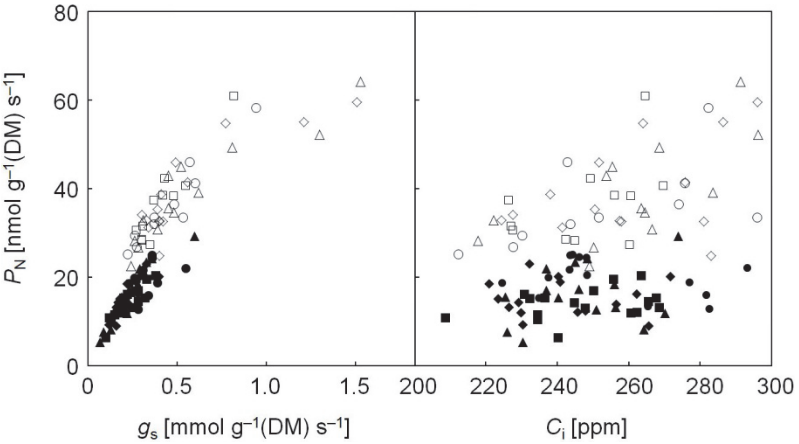

Fig. 1. Relationships between net photosynthetic rate (P_N) and (*left*) stomatal conductance (g_s) or (*right*) CO_2 concentration in intercellular space (C_i) in slow-growing (SG; *black*) and fast-growing (FG; *white*) trees; symbols denote different trees. Data were taken under light-saturated conditions (PPFD > 600 μmol m^{-2} s^{-1}) on two days for each tree. The vapour-pressure deficit of the ambient air was 0.74–3.51 kPa in SG trees and 0.89–3.13 kPa in FG trees.

light-saturated P_N (adjusted for foliage N) was determined by C_c. Thus, the difference in the CE reflects the difference in r_m . r_m is not affected only by CO_2 channel (aquaporin) activity on the cell membrane (Hanba *et al.* 2004), but also by anatomical properties of mesophyll cells such as cell wall thickness (Evans *et al.* 2009). Delfine *et al.* (1999) reported that the activity of CO_2 channels in the cell membrane of spinach leaves was depressed by water stress caused by high salt concentration. We measured CE in well-watered detached shoots. The difference in r_m between SG and FG trees at midday should be larger than that estimated from CE of the detached shoots, because the leaf ψ_w at midday was much more negative in SG trees than in FG trees (Table 1). The lack of correlation between C_i and light-saturated P_N in SG trees (Fig. 1) suggests that r_m was a dominant factor in the photosynthetic rate in SG trees. As anatomical properties of mesophyll cell were affected by turgor-driven cell expansion under water stress, the low photosynthetic rate of SG trees might be a result and not a cause of their slow height growth.

Our results showed that the current-year shoots of the slow-growing trees experienced more water stress and had lower photosynthetic rates than the fast-growing trees. The lower photosynthetic rate seemed to be determined by the larger r_m. Further studies of the change in r_m with a tree height and a growth status are necessary in order to clarify the photosynthetic characteristics of older trees.

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