Physiological basis of seasonal trend in leaf photosynthesis of five evergreen broad-leaved species in a temperate deciduous forest

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Received December 8, 2004; accepted May 7, 2005; published online November 8, 2005

Summary The physiological basis of photosynthesis during winter was investigated in saplings of five evergreen broad-leaved species (Camellia japonica L., Cleyera japonica Thunb., Photinia glabra (Thunb.) Maxim., Castanopsis cuspidata (Thunb.) Schottky and Quercus glauca Thunb.) co-occurring under deciduous canopy trees in a temperate forest. We focused on temperature dependence of photosynthetic rate and capacity as important physiological parameters that determine light-saturated rates of net photosynthesis at low temperatures during winter. Under controlled temperature conditions, maximum rates of ribulose bisphosphate carboxylation and electron transport (V\text{max} and J\text{max}, respectively) increased exponentially with increasing leaf temperature. The temperature dependence of photosynthetic rate did not differ among species. In the field, photosynthetic capacity, determined as V\text{max} and J\text{max} at a common temperature of 25 °C(V\text{max}(25) and J\text{max}(25)), increased until autumn and then decreased in species-specific patterns. Values of V\text{max}(25) and J\text{max}(25) differed among species during winter. There was a positive correlation of V\text{max}(25) with area-based nitrogen concentration among leaves during winter in Camellia and Photinia. Interspecific differences in V\text{max}(25) were responsible for interspecific differences in light-saturated rates of net photosynthesis during winter.

Keywords: electron transport rate, nitrogen content, light-saturated rate of net photosynthesis, RuBP carboxylation, temperature dependency of photosynthetic rate.

Introduction

Photosynthesis in woody evergreen saplings growing beneath deciduous canopy trees in a temperate forest occurs in two contrasting light environments. In the primary growing season (spring to autumn) leaves are shaded by the canopy trees, but during winter they are exposed to high solar irradiances through the bare canopy. Previously, we found that carbon gain during winter was greater than during the growing season in the evergreen broad-leaved tree species, Camellia japonica L., Ilex pedunculosa Miq. and Photinia glabra (Thunb.) Maxim., beneath deciduous canopy trees (Miyazawa and Kikuzawa 2005). In three other co-occurring species (Cleyera japonica Thunb., Castanopsis cuspidata (Thunb.) Schottky and Quercus glauca Thunb.), however, the light-saturated rate of photosynthesis at ambient carbon dioxide (CO\textsubscript{2}) and temperature (A) was greatly reduced in winter, when carbon gain was as low as during the growing season in the shade. Thus, under deciduous canopy trees, winter photosynthesis of evergreen species is a major determinant of annual carbon gain and hence an important factor affecting tree survival. However, little is known about the physiological basis for interspecific differences in winter photosynthesis.

We hypothesized that interspecific differences in winter photosynthesis of evergreen broad-leaved species result from interspecific differences in (1) the temperature dependence of photosynthesis among species and (2) photosynthetic capacity. Species with a similar photosynthetic rate (A) in warm conditions may differ in A under cold conditions (Hikosaka et al. 1999). Previous studies have shown differences in the temperature dependence of A among leaves of different species and among leaves of the same species in different growth environments (Ferrar et al. 1989, Bunce 2000a, 2000b, Dreyer et al. 2001, Medlyn et al. 2002b). To test our first hypothesis, the temperature dependence of A, such as the maximum rate of ribulose bisphosphate (RuBP) carboxylation (V\text{max}) and the maximum rate of electron transport (J\text{max}), were determined for each species.

Interspecific differences in photosynthetic capacity result in differences in A among species at low temperatures (Farquhar et al. 1980, Harley et al. 1992b). In evergreen trees of temperate forests, photosynthetic capacity changes both during (Miyazawa et al. 1998, Miyazawa and Terashima 2001) and after (Kume and Ino 1993, Medlyn et al. 2002b) leaf maturation. Therefore, to test our second hypothesis, we monitored photosynthetic capacity, which is represented by J\text{max} and V\text{max} scaled to a common leaf temperature (25 °C in this study) (V\text{max}(25) and J\text{max}(25), respectively) and compared wintertime photosynthetic capacity among species.

The specific objectives of this study were to: (1) investigate...
interspecific differences in the temperature dependence of \( V_{\text{cmax}} \) and \( J_{\text{max}} \) and daytime respiration rate (\( R_d \)) among species; (2) monitor seasonal \( V_{\text{cmax}}^{(25)} J_{\text{max}}^{(25)} \) and foliar nitrogen content of leaves in the field; and (3) investigate interspecific differences in \( V_{\text{cmax}}^{(25)} J_{\text{max}}^{(25)} \) and \( R_d^{(25)} \). In pursuing objective (2), we focused on the seasonal changes in nitrogen content, because it is closely related to photosynthetic capacity (Evans 1989) and changes seasonally (Medlyn et al. 2002b). We discuss the physiological basis for the interspecific differences in winter photosynthesis.

**Materials and methods**

**Measurements of photosynthesis in the field**

The study was conducted in a secondary forest in Kamigamo Experimental Forest Station, Kyoto University (35°04’ N, 135°43’ E). Mean annual air temperature from 1971 to 2001 was 14.6 °C, with the highest temperatures in August (31.6 °C) and the lowest temperatures in January (–0.9 °C). Mean annual precipitation was 1582 mm. The forest overstory is composed of an evergreen conifer species (Chamaecyparis obtusa Sieb. & Zucc.), an evergreen broad-leaved species (I. pedunculosa), and a deciduous oak (Quercus serrata Sieb. & Zucc.), which is the dominant canopy species in the stand. Leaf area index (LAI) of the overstory of the Q. serrata stand was 2.59 ± 0.47, and stand height was about 12 m. We calculated LAI from hemispheric photographs taken in the understory and analyzed with HemiView software (HemiView Canopy Analysis Software Version 2.1, Delta-T Devices, Cambridge, U.K.). Further details of the climate and vegetation of the study site are described elsewhere (Miyazawa and Kikuzawa 2005).

We studied five evergreen species, including small to sub-canopy tree species (Camellia japonica, Cleyera japonica and Photinia glabra) and tall trees species (Castanopsis cuspidata and Q. glauca). Saplings of these species co-occur beneath the crowns of the dominant canopy species Q. serrata, which is leafless from December through April. All of the species undergo bud flushing, leaf expansion and shoot elongation within a short period from mid April to early May and then rarely expand new leaves until the next spring. Under deciduous canopy trees, leaves of the study species are retained for 2 to 3 years.

Light-saturated rate of net photosynthesis (\( A \)) at ambient temperature was measured at different CO\(_2\) partial pressures in 2003 (January–April, June, August, November and December) and in 2004 (January, February, April and June). Under Q. serrata canopy trees, we selected one study sapling (1.5–2.0 m in height) per species for periodic measurements of photosynthesis in the field in October 2002. On each occasion, we selected current-year leaves \((n = 7–13)\) in the outer crown of the sample sapling of each species and measured the \( A \) versus intercellular \( \text{CO}_2 \) partial pressure \((c_i, \text{Pa})\) relationship (\( A-c_i \) relationship) of each leaf. Photosynthesis was measured with an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE). Photosynthetic photon flux (PPF) and vapor pressure deficit in the cuvette were held at 1300 μmol m\(^{-2}\) s\(^{-1}\) (at which photosynthetic rates reached saturation; Miyazawa and Kikuzawa 2005) and less than 1 kPa, respectively. Light was supplied by the attached Li-Cor light source (LI-6400-40). We first set air temperature in the cuvette to ambient temperature and kept it constant throughout the measurement. We stabilized the photosynthetic rate at a cuvette CO\(_2\) partial pressure of 35 Pa and then measured photosynthetic rates at 10 cuvette CO\(_2\) partial pressures from 0 to 200 Pa. It took about 4 min to obtain a stable photosynthetic rate with each increase in cuvette CO\(_2\) partial pressure. Stomatal conductance of H\(_2\)O at light saturation \((g_s, \text{mol m}^{-2} \text{s}^{-1})\) was measured at the same time as photosynthesis. Because the sample tree of each species showed photosynthetic traits similar to those of other nearby saplings measured in our previous work (see Miyazawa and Kikuzawa 2005), photosynthetic traits of the sample saplings can be generalized for saplings in the same environment.

After sunset, we measured night respiration rates \((R_n, \text{μmol m}^{-2} \text{s}^{-1})\) of the sample leaves at ambient air temperature.

Based on the \( A-c_i \) relationship, we calculated the maximum rate of RuBP carboxylation, \( V_{\text{cmax}} (\text{μmol m}^{-2} \text{s}^{-1}) \) and the maximum electron transport rate, \( J_{\text{max}} (\text{μmol m}^{-2} \text{s}^{-1}) \) of each leaf. The measured \( A \) at each \( c_i \), is expressed as:

\[
A = \min\{A_c, A_i\} \tag{1}
\]

where \( A_c (\text{μmol m}^{-2} \text{s}^{-1}) \) is net photosynthetic rate at RuBP-saturation (Rubisco limitation) and \( A_i (\text{μmol m}^{-2} \text{s}^{-1}) \) is net photosynthetic rate at RuBP-limitation at each \( c_i \). We calculated \( V_{\text{cmax}} \) and \( J_{\text{max}} \) from the \( A-c_i \) relationship as (Farquhar et al. 1980, von Caemmerer and Farquhar 1981):

\[
A_c = \frac{V_{\text{cmax}}(c_i - \Gamma^*)}{(c_i + K_c(1 + o/K_c))} - R_d \tag{2}
\]

\[
A_i = \frac{J(c_i - \Gamma^*)}{(4c_i + 8I^*)} - R_d \tag{3}
\]

where parameters \( K_c \) and \( K_o \) are the Rubisco Michaelis-Menten constants for CO\(_2\) and O\(_2\), respectively, and \( \Gamma^* \) is the CO\(_2\) compensation point without daytime respiration. We assumed that the values of these parameters were similar to those obtained in vivo (\( K_c = 40.4 \text{ Pa}, K_o = 24,800 \text{ Pa} \) and \( \Gamma^* = 3.69 \text{ Pa} \) at 25 °C; von Caemmerer et al. 1994). Parameter \( J (\text{μmol m}^{-2} \text{s}^{-1}) \) is the electron transport rate (maximum \( J \) is \( J_{\text{max}} \)), \( R_d \) is daytime respiration rate at light saturation (\( \text{μmol m}^{-2} \text{s}^{-1} \)) and \( o \) is the O\(_2\) partial pressure in the chloroplast (21,000 Pa). In these calculations, we assumed that \( c_i = \text{CO}_2 \) partial pressure at the site of RuBP carboxylation (but see Harley et al. 1992a, Epron et al. 1995, Ethier and Livingston 2004). We calculated \( R_d \) of each individual leaf from \( R_n \) as (Lloyd et al. 1995):

\[
R_d = (0.5 - 0.05 \ln I) R_n \tag{4}
\]

where \( I \) is incident PPF (\( \text{μmol m}^{-2} \text{s}^{-1} \)). In the calculation of \( V_{\text{cmax}} \) and \( J_{\text{max}} \), \( R_d \) was calculated by Equation 4 because these values were not significantly different from the \( R_d \) calculated.
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from the \( A-c_i \) relationship and \( \Gamma^* \) in the sample leaves (repeated measures of ANOVA, SPSS release 7.5.1, SPSS, Chicago, IL).

We monitored the electron transport rate of photosystem II (ETR, \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) during measurement of the \( A-c_i \) relationship by simultaneously measuring the photochemical efficiency of photosystem II (\( \Phi \text{II} \)) by determining chlorophyll fluorescence with a fluorometer attached to the cuvette (LI-6400-40, Li-Cor). We calculated ETR as (Genty et al. 1989):

\[
\text{ETR} = 0.5 \Phi \text{II} / \alpha
\]

where \( \alpha \) is leaf absorbance and 0.5 is the fraction of light absorbed by photosystem II. Though \( \alpha \) differed among leaves, differences in \( \alpha \) do not affect the comparison of ETR at different \( \text{CO}_2 \) partial pressures. In this study, ETR was considered equal to \( J \). We determined whether ETR was saturated (\( J = J_{\text{max}} \)) at each \( c_i \).

Temperature dependence of photosynthesis in the laboratory

In autumn of 2001, we collected 1- to 3-year-old saplings of the five species at the study site. The saplings were taken to Kyoto University and grown in washed sand in 8-l, clay pots in Honbu Experimental Garden of Kyoto, Kyoto University, Japan (35°01’ N, 135°47’E). The saplings were grown under light and temperature conditions similar to those of the sample saplings in the field. Each week they were watered to field capacity with nutrient solution (400 mg nitrogen, Hyponex, 5:10:5 N,P,K, Murakami-Bussan, Kamigori, Japan).

In April 2004, the temperature dependencies of \( V_{\text{max}} \) and \( J_{\text{max}} \) were determined by measuring the \( A-c_i \) relationship of each leaf at different temperatures. In March 2004, we selected current-year leaves of the potted saplings (five leaves per species) for measurements of temperature dependence of photosynthesis. Because photosynthetic rates did not stabilize until more than 4 h after the sapling had been transferred to a low temperature, each sapling was first placed overnight in an incubator at 7 °C in the dark (LP-200S, Nippon Medical and Chemical Instruments, Osaka, Japan). Outdoor air was supplied with a pump to keep the \( \text{CO}_2 \) partial pressure in the incubator equal to the atmospheric \( \text{CO}_2 \) partial pressure. On the morning of the measurement day, A of the leaf in the incubator was measured with an LI-6400, in saturating PPF (1300 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) and at a cuvette temperature of 7 °C. We waited at least 60 min until the photosynthetic rate had become stable after leaves were exposed to high light. The \( A-c_i \) relationship was measured as described previously, after which the air temperature in both the cuvette and the incubator was increased in five steps (15, 20, 25, 30 and 35 °C). After each increase in temperature, we waited for 30–60 min until the photosynthetic rate had stabilized before making measurements. When we measured photosynthesis at a high temperature, high humidity was maintained with a humidifier (Li-610, Li-Cor). After completing a set of \( A-c_i \) measurements, we measured \( \text{CO}_2 \) flux from an empty cuvette to estimate leakage. Photosynthetic measurements at high and low \( \text{CO}_2 \) partial pressures were then corrected for \( \text{CO}_2 \) leakage. We found no significant differences in the light-saturated rate of net photosynthesis at \( \text{CO}_2 \) partial pressure = 35 Pa and leaf temperature = 25 °C before and during determination of the temperature dependence of photosynthesis in each leaf, indicating that the handling of the saplings during measurement did not affect photosynthesis.

We determined the temperature dependencies of \( V_{\text{max}}, J_{\text{max}} \) and \( R_n \) (Farquhar et al. 1980, Medlyn et al. 2002b) according to the equation:

\[
P_T = P^{(25)} \exp \left( \frac{E_a}{298.15} \left( 1 - \frac{T}{298.15} \right) \right)
\]

where \( P_T \) is either \( V_{\text{max}}, J_{\text{max}} \) or \( R_n \) at leaf temperature \( T \) (K), \( P^{(25)} \) is the value of \( P_T \) scaled to a common temperature (25 °C = 298.15 K) and \( E_a \) is the activation energy of \( P_T \) (J mol\(^{-1}\)), which was calculated by nonlinear regression of \( P_T \) with \( T \). The temperature dependencies of \( K_c, K_o, \) and a relative specific factor for Rubisco (\( \tau \)) which represents \( \Gamma^{*0} = \alpha \Omega2 \tau \), were similarly expressed. The values of \( E_a \) for temperature dependencies of \( K_c, K_o, \) and \( \tau \) were taken from Harley et al. (1992b) and are shown in Table 1. We calculated the \( E_a \) for each leaf used to measure the temperature dependence of photosynthesis in the laboratory (\( n \) per species).

Calculation of \( V_{\text{max}}, J_{\text{max}} \) and \( R_n \) of leaves in the field

We calculated the values of \( V_{\text{max}}, J_{\text{max}} \) and \( R_n \) of sample leaves in the field by substituting \( V_{\text{max}}, J_{\text{max}} \) and \( R_n \) and the mean value of leaf temperature during the measurement of the leaf into Equation 6 for each species (Leuning 1997). Values of \( E_a \) for \( V_{\text{max}}, J_{\text{max}} \) and \( R_n \) of the sample leaves in the field were assumed to be similar to those of leaves of the same species measured in the temperature dependency study. Values of \( E_a \) were assumed constant during the measurement period.

Measurements of LMA and nitrogen concentration and content of leaves in the field

After determining the \( A-c_i \) relationship of leaves in the field, the leaves were harvested (\( n = 7–13 \) per species at each measurement time) and taken to the laboratory for determination of leaf mass per area (LMA, g m\(^{-2}\)) and mass-based nitrogen concentration (\( N_{\text{max}}, \text{g g}^{-1}\)). Images of freshly collected leaves were taken with an image scanner (Epson GT-5500 Art, Seiko

<table>
<thead>
<tr>
<th>Unit</th>
<th>Values</th>
<th>( E_a ) (J mol(^{-1}))</th>
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<tbody>
<tr>
<td>( K_c ) (25 °C)</td>
<td>Pa 40.4 (^1)</td>
<td>80,500 (^2)</td>
</tr>
<tr>
<td>( K_o ) (25 °C)</td>
<td>Pa 24,800 (^1)</td>
<td>14,500 (^2)</td>
</tr>
<tr>
<td>( \tau ) (25 °C)</td>
<td>2846 (^1)</td>
<td>-29,000 (^2)</td>
</tr>
</tbody>
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\(^1\) von Caemmerer et al. 1994.
\(^2\) Harley et al. 1992b.

Table 1. List of Michaelis-Menten constants, the specific factor for Rubisco (\( \tau \)) and the activation energy used in Equations 2, 3 and 6. Abbreviations: \( E_a \) = activation energy; and \( K_c \) and \( K_o \) = Rubisco Michaelis-Menten constants for \( \text{CO}_2 \) and \( \text{O}_2 \), respectively.
Epson Corporation, Japan) and processed with NIH Image Software Version 1.62 (RSB, National Institute of Health, Bethesda, MD) and then each leaf was dried to a constant mass at 80 °C to determine LMA. The dried leaves were ground and analyzed for nitrogen (N_{mass}) with a CN-coder MT-600 (Yanaco, Kyoto, Japan). We calculated leaf nitrogen content on an area basis (N_{area} g m^{-2}) as the product of LMA and N_{mass}.

Statistical analysis

To examine interspecific differences in the temperature dependence of photosynthetic rate, we compared E_a among species by analysis of variance. To examine interspecific differences in photosynthetic capacity of the sample leaves in the field (n = 7–13 per species at each measurement time), we compared the values of g_s, J_{max}(25) and V_{cmax}(25) among species by Tukey’s test. The variances of J_{max}(25) and V_{cmax}(25) were calculated by the method of Beaudet et al. (2000) by taking into account the variance of E_a as the source of the variance of J_{max} and V_{cmax}. Correlation of N_{area} with photosynthetic capacity was examined among leaves at different measurement times. We examined the temporal changes in photosynthetic capacity by comparing the values of a given pair of measurement times with the t test.

Results

Temperature dependency of photosynthetic rate

Values of V_{cmax}, J_{max} and R_n increased exponentially with increasing leaf temperature (Figure 1). At leaf temperatures ≥ 30 °C, V_{cmax} and J_{max} of most leaves were lower than the values predicted by the regression equation because the equations did not take into account the decrease in V_{cmax} and J_{max} caused by deactivation of Rubisco and the electron transport systems at high temperature (Sharpe and DeMichele 1977, Harley et al. 1992b). To calculate E_a of each leaf, we therefore regressed V_{cmax} and J_{max} with leaf temperatures below 30 °C. The effect of leaf temperature on E_a for V_{cmax} did not differ significantly among species (E_a = 61.71 ± 6.08 SE kJ mol^{-1} when E_a values of the five species were pooled). Similarly, values of E_a for J_{max} were not significantly different among species (E_a = 41.43 ± 6.147 SE kJ mol^{-1}).

Interspecific difference in photosynthetic capacity of leaves in the field

Photosynthetic capacity, represented by V_{cmax} and J_{max} scaled at a common leaf temperature of 25 °C, V_{cmax}(25) (µmol m^{-2} s^{-1}) and J_{max}(25) (µmol m^{-2} s^{-1}), respectively, increased significantly after August except in Clearya (n = 7–11 per species at each measurement time). Values of V_{cmax}(25) reached an annual peak in

![Figure 1. Relationships between leaf temperature and V_{cmax}, J_{max} and R_n (µmol m^{-2} s^{-1}) (n = 5). Curves were generated with Equation 6 and the mean E_a values (kJ mol^{-1}) of five evergreen understory species. Abbreviations: V_{cmax} = maximum rate of ribulose bisphosphate carboxylation; J_{max} = maximum rate of electron transport; R_n = night respiration rate; and E_a = activation energy.](https://academic.oup.com/treephys/article-abstract/26/2/249/1676817)
November or December (Figure 2). During most periods from January to March, \( V_{\text{cmax}}^{(25)} \) values of *Castanopsis*, *Cleyera* and *Quercus* were lower than in August, but increased again after February or March. In *Camellia* and *Photinia*, \( V_{\text{cmax}}^{(25)} \) values also decreased with time during winter, but were significantly higher in most periods during winter than in August \( (P < 0.05, t \text{ test}) \). In August, \( V_{\text{cmax}}^{(25)} \) differed slightly among species \( (P = 0.039, \text{ANOVA}) \). Large interspecific differences in \( V_{\text{cmax}}^{(25)} \) were observed from January to March \( (*Camellia*, *Photinia* > *Quercus*, *Castanopsis*, *Cleyera*, \( P < 0.01, \text{Tukey's test} \)). The patterns of changes in \( J_{\text{max}}^{(25)} \) were similar to those in \( V_{\text{cmax}}^{(25)} \). There was a positive correlation between \( V_{\text{cmax}}^{(25)} \) and \( J_{\text{max}}^{(25)} \) when all data were pooled \( (r^2 = 0.648, P < 0.01, J_{\text{max}}^{(25)}/V_{\text{cmax}}^{(25)} = 2.13 \pm 0.47 \text{ SE}) \). The \( J_{\text{max}}^{(25)}/V_{\text{cmax}}^{(25)} \) ratio increased with decreasing leaf temperature. Light-saturated stomatal conductance \( (g_s) \) of \( \text{H}_2\text{O} \) at ambient \( \text{CO}_2 \) concentration and temperature decreased after November, reaching a minimum in February. Night respiration scaled to 25 °C \( (R_n^{(25)}, \mu\text{mol m}^{-2} \text{s}^{-1}) \) reached an annual peak in December or January. Leaves had higher values of \( R_n^{(25)} \) in winter than in summer.

Throughout the measurement period, photosynthesis of the sample leaves in the field was regulated by the rate of RuBP carboxylation, i.e., \( A = A_r \) at a cuvette \( \text{CO}_2 \) partial pressure = 35 Pa. Chlorophyll fluorescence revealed that ETR did not reach the maximum rate \( (J_{\text{max}}) \) at a cuvette \( \text{CO}_2 \) partial pressure of 35 Pa, suggesting that \( A < A_r \) throughout the measurement period (data not shown). We found no evidence of triose phosphate limitation of photosynthesis (Sharkey 1985).

![Figure 2](https://academic.oup.com/treephys/article-abstract/26/2/249/1676817)
Correlation between leaf nitrogen content and photosynthetic capacity

Values of $V_{\text{cmax}}^{(25)}$ were positively correlated with $N_{\text{area}}$ (g m\(^{-2}\)) on the various measurement occasions in Camellia ($r^2 = 0.62, P < 0.01$) and Photinia ($r^2 = 0.59, P < 0.01$), but not in Castanopsis, Clevera and Quercus (Figure 2). In Camellia and Photinia, both $V_{\text{cmax}}^{(25)}$ and $N_{\text{area}}$ were higher in winter than in summer. However, in Castanopsis, Clevera and Quercus, significantly lower values of $V_{\text{cmax}}^{(25)}$ were observed in January and February than in November and December, though $N_{\text{area}}$ changed little after November. Among leaves on some measurement occasions, there was a significant positive correlation of $V_{\text{cmax}}^{(25)}$ and $N_{\text{area}}$ in the five species; however, on other measurement occasions, $N_{\text{area}}$ was not significantly correlated with $V_{\text{cmax}}^{(25)}$ partly because of small variations in $V_{\text{cmax}}^{(25)}$ and $N_{\text{area}}$ among the leaves. There was a positive correlation between $J_{\text{max}}^{(25)}$ and $N_{\text{area}}$ in Photinia ($r^2 = 0.47, P < 0.01$), but not in the other species (data not shown). We did not find a significant correlation between $R_{\text{a}}^{(25)}$ and $N_{\text{area}}$.

Discussion

Interspecific differences in the temperature dependence of $A$ were small and were not responsible for the interspecific differences in light-saturated net photosynthetic rates at ambient CO\(_2\) concentration during winter (Miyazawa and Kikuzawa 2005). Values of $E_a$ for $V_{\text{cmax}}$ and $J_{\text{max}}$ of the five species were within the ranges previously reported (Farquhar et al. 1980, Bunce 2000a, Dreyer et al. 2001, Medlyn et al. 2002a, Medlyn et al. 2002b). Variation in the temperature dependence of $V_{\text{cmax}}$ and $J_{\text{max}}$ with prevailing climate have been reported (Ferrar et al. 1989, Bunce 2000a) as well as among leaves grown at different temperatures (Ferrar et al. 1989, Hikosaka et al. 1999, Bunce 2000a). However, we found no significant differences in the temperature dependence of $A$ among saplings of our study species, which differ in geographical distribution (Hori-kawa 1972).

During winter, $V_{\text{cmax}}^{(25)}$ was either strongly enhanced or depressed by the species-specific changes in $V_{\text{cmax}}^{(25)}$ at the end of the summer (Figure 2), and the interspecific differences in $V_{\text{cmax}}^{(25)}$ were responsible for the different values of wintertime $A$ among species. Patterns of seasonal changes in $V_{\text{cmax}}^{(25)}$ and $J_{\text{max}}^{(25)}$ (i.e., increase after summer and decrease after autumn) were evident even when $V_{\text{cmax}}^{(25)}$ was calculated on the basis of the lowest or highest values of $E_a$ in previous studies (Medlyn et al. 2002a). Under deciduous canopy trees, maintenance of $V_{\text{cmax}}$ at high values in the sun during winter would be an important factor determining plant growth and survival (Miyazawa and Kikuzawa 2005).

Synchronous seasonal trends in $N_{\text{area}}$ and $V_{\text{cmax}}^{(25)}$ after leaf maturation indicated that $N_{\text{area}}$ was responsible for changes in $V_{\text{cmax}}^{(25)}$ in Camellia and Photinia after summer (Figure 3). High $N_{\text{area}}$ during winter reflects the accumulation of nitrogen necessary to support growth of new leaves the next spring (Millard and Proe 1991, Proe and Millard 1994). The seasonal changes in $N_{\text{area}}$ would have involved changes in the content of the photosynthetic apparatus and changed $V_{\text{cmax}}^{(25)}$ in Camellia and Photinia as has been shown in Pinus sylvestris and Pinus pinaster (Medlyn et al. 2002b, Warren et al. 2003). Similar close correlations of $N_{\text{area}}$ with $A$ and the content of the photosynthetic apparatus, such as Rubisco, have been reported (Evans and Terashima 1988, Evans 1989, Hikosaka and Hirose 2000, Evans and Poorter 2001).

Increases in photosynthetic capacity in response to a sudden increase in light availability have been reported for mature leaves (Yamashita et al. 2000, Frak et al. 2001, Oguchi et al. 2003). In our study, however, increases in $V_{\text{cmax}}^{(25)}$ in response to increased light availability were small: values of $V_{\text{cmax}}^{(25)}$ increased greatly in autumn when the canopy was still closed, but increased only slightly after overstory leaf fall in late November. The decrease and increase in $V_{\text{cmax}}^{(25)}$ during winter in Castanopsis, Clevera and Quercus were attributed to physiological mechanisms other than the change in $N_{\text{area}}$. There are many possible mechanisms that could account for temporary fluctuations in $V_{\text{cmax}}^{(25)}$ at low temperatures without changes in $N_{\text{area}}$. These include decreased $V_{\text{cmax}}$ per Rubisco content (Warren et al. 2003), embolism of vessels in the leaves (Brodribb and Holbrook 2003), depressed internal conductance at low temperatures (Bernacchi et al. 2002) and effects of cold hardening during the series of subfreezing nights before the measurements. We were unable to pinpoint the physiological mecha-

![Figure 3. Correlations between nitrogen content on an area basis, $N_{\text{area}}$ (g m\(^{-2}\)), and $V_{\text{cmax}}$ at a common temperature of 25 °C ($V_{\text{cmax}}^{(25)}$) for the five species in August (○), November (■), December (●), January (▲), February (◇) and April (○). The lines represent the linear regression of $V_{\text{cmax}}^{(25)}$ with $N_{\text{area}}$ for Camellia ($r^2 = 0.62$) and Photinia ($r^2 = 0.59$) leaves at different measurement times ($n = 7–13$ per species per measurement time).](https://academic.oup.com/treephys/article-abstract/26/2/249/1676817)
nisms that determine the photosynthetic capacity of these species during cold months.

We calculated V_{\text{cmax}}^{(25)} and J_{\text{max}}^{(25)} based on the assumption that CO₂ transfer conductance from the intercellular space to the site of RuBP carboxylation is infinite. Internal conductance, however, is low in evergreen tree species: 0.05–0.14 mol m⁻² s⁻¹ for Q. glauca, 0.06–0.12 mol m⁻² s⁻¹ at leaf maturation for Castanopsis species and 0.05–0.15 mol m⁻² s⁻¹ for Camellia (Hanba et al. 1999, Miyazawa and Terashima 2001). Interspecific differences in the values of V_{\text{cmax}}^{(25)} and J_{\text{max}}^{(25)} observed in our study were associated with interspecific difference in internal conductance at low temperature as well as with interspecific differences in photosynthetic capacity. Further studies on internal conductance as well as on photosynthetic capacity during winter are needed to understand the physiological basis of winter photosynthesis of evergreen tree species.

Acknowledgments

This study was partly supported by the 21st Century COE Program, Kyoto University “Innovative Food and Environmental Studies pioneered by Entomomimetic Sciences.” We thank Dr. S.-I. Miyazawa for his constructive comments on an earlier version of this paper. We thank Dr. M.J. Lechowicz, A. Takayanagi, R. Yamasaki and members of the Laboratory of Forest Biology of Kyoto University for their discussion and assistance in this research, and the staff of Kamigamo Experimental Forest Station of Kyoto University.

References


