Spatial and seasonal variability of temperature responses of biochemical photosynthesis parameters and leaf nitrogen content within a Pinus densiflora crown

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Received July 3, 2003; accepted November 21, 2003; published on May 3, 2004

Summary  We measured seasonal variation in area-based nitrogen concentration (N), maximum rate of carboxylation (Vcmax) and maximum rate of electron transport (Jmax) in 1-year-old needles along four first-order branches within a Pinus densiflora Sieb. & Zucc. crown, and analyzed their relationships to growth irradiance and temperature. Each leaf light environment was expressed as a ratio of the monthly mean of daily integrated photosynthetically active irradiance (Irel) for the particular needle to Irel above the canopy (Irel). Needle N decreased in the upper crown during the development of new needles, whereas it remained fairly constant in the lower crown, reflecting differences between upper and lower crown needles in their contribution to the nitrogen of new needles. Gradients of N within the crown were correlated with Irel in all seasons (r2 = 0.40–0.78). Seasonal variation in N was weakly correlated with mean daily air minimum temperatures. Both Vcmax and Jmax showed seasonal variation in all first-order branches, and decreased to their lowest values in winter. The gradients of Vcmax and Jmax within the crown were not correlated with Irel in some seasons, but were correlated with changes in N in most months (r2 = 0.33–0.75), except in the winter. Furthermore, the regression slope of the relationship between N and Vcmax and the temperature response of Vcmax and Jmax exhibited seasonal variation.

Keywords: acclimation, maximum rate of carboxylation, maximum rate of electron transport, seasonal change, translocation.

Introduction

Many models estimating forest carbon exchange employ the biochemical model of Farquhar et al. (1980) at the leaf level, which has two key parameters: the maximum rate of carboxylation (Vcmax) and the maximum rate of electron transport (Jmax). It has been well documented that variation in Vcmax and Jmax within canopies is strongly correlated with leaf nitrogen concentration on an area basis (N) (Wilson et al. 2000, Warren and Adams 2001, Meir et al. 2002) because of the large proportion of N in the photosynthetic machinery (Evans 1989). Leaf N is often found to correlate with photosynthetically active irradiance (Irel) within canopies. The associations between Irel, N and Vcmax within canopies is now widely used in scaling estimates of leaf photosynthesis to the canopy level, although most of these correlations have been observed only during the growing season (Baldocchi and Harley 1995, de Pury and Farquhar 1997). A recent report found that neglecting seasonal variations in photosynthesis at the leaf level when scaling up from biochemical models may result in overestimation of the annual net ecosystem carbon exchange by as much as 50% (Wilson et al. 2001). This indicates a need to examine how Vcmax and Jmax vary with seasonal changes in growth temperature.

The model of Farquhar et al. examines photosynthetic acclimation to growth irradiance (Niinemets and Tenhunen 1997, Le Roux et al. 1999, Niinemets et al. 1999, Meir et al. 2002) and growth temperature (Hikosaka et al. 1999, Bunce 2000). Photosynthetic responses to temperature may vary according to the time scale over both short-term and seasonal scales (Wilson et al. 2000). Photosynthetic capacity exhibits a seasonal pattern (Reich et al. 1991, Ellsworth 2000, Jach and Ceulemans 2000) because of changes in leaf age (Wilson et al. 2000), stomatal responses to vapor pressure deficits (Medlyn et al. 2002b) and temperature acclimation (Battaglia et al. 1996). However, few studies have examined the dependencies of Vcmax and Jmax on growth temperature (Hikosaka et al. 1999, Dreyer et al. 2001, Medlyn et al. 2002b, Dungan et al. 2003). Seasonal variations in Vcmax and Jmax and their relationships to leaf N have been examined in several herbs and shrubs (Wohlfahrt et al. 1999), and broadleaf tree species (Wilson et al. 2000, Dungan et al. 2003), but few studies have focused on coniferous species (Medlyn et al. 2002b).

In a previous study, we measured variation in Vcmax and Jmax in a crown of Pinus densiflora Sieb. & Zucc. and analyzed the relationship between these variables and with leaf N and acclimation to irradiance in the summer (Han et al. 2003a). In this study, we continued these investigations by measuring the sea-
sonal changes in leaf N, $V_{\text{max}}$ and $J_{\text{max}}$. We also analyzed the relationships between these variables and environmental factors such as growth irradiance and temperature.

Materials and methods

Study site
A representative $P$. densiflora tree was selected for study in an approximately 80-year-old forest in which CO$_2$ fluxes have been investigated since 1999 (35°45′ N, 138°80′ E; elevation 1,030 m). $Pinus$ densiflora, a shade-intolerant pioneer species, is the dominant overstory tree species. The crown of the selected tree (height = 19.8 m, diameter at breast height = 27.4 cm) was accessed from a tower. Mean annual precipitation and temperature near the site for the period 1973–1996 were 1597 mm and 10.3 °C, respectively (Japanese Bureau of Meteorology). In the same period, mean daily, mean daily maximum and mean daily minimum temperatures were 20.7, 25.6 and 17.4 °C in July, and −0.8, 5.0 and −6.4 °C in January, respectively. The mean extreme high temperature in July was 30.8 °C and the mean extreme low temperature in January was −12.7 °C.

Crown light and gas exchange measurements
We selected four first-order branches, 18, 16.0, 15.5 and 14.5 m above ground, which were 9, 16, 16 and 22 years old, respectively, in 2001, and are hereafter called the upper, middle1, middle2 and lower branches with an azimuth of 160, 180, 320 and 260°, respectively. We selected 3–4 points on each first-order branch, that were representative of the foliated portion of the branch, to measure photosynthetically active irradiance ($I$) with LI-190SA sensors (Li-Cor, Lincoln, NE). This arrangement is described more fully in Han et al. (2003a). Gas exchange in needles adjacent to the LI-190SA sensors was measured in situ with a portable photosynthesis system (LI-6400, Li-Cor) every other month from July 2001 to May 2002. For this purpose, 1-year-old hemiclindrical needles developed on 2-, 4–6- and 7–9-year-old twigs in 2001 were chosen from the outer, middle and inner crowns of the first-order branches, respectively. In each case, these needles were about 2 years old at the time of the last measurement. Three replicates were measured around each LI-190SA sensor. For each replicate, five fascicles, consisting of 10 needles, were placed in the Li-Cor standard chamber with an adaptor to arrange each needle separately. The curved needle surfaces were exposed to the 6400-02B LED light source. Photosynthetic response curves to intercellular CO$_2$ concentration ($A/C_i$) were measured at 10 CO$_2$ concentrations, ranging from 50 to 1800 µmol mol$^{-1}$ as described by Han et al. (2003a). Leaf temperatures, calculated from their energy balance (Ehleringer 1989), were kept at 25 ± 0.5 °C in July and September, 21 ± 0.6 °C in November, 14 ± 0.5 °C in January, 16 ± 0.3 °C in March and 22 ± 0.2 °C in May. The vapor pressure deficit in the chamber was less than 1.5 kPa during all measurements. In addition, $A/C_i$ curves were measured at four to five temperatures ranging from 15 to 35 °C (July, November and May) or from 2 to 17 °C (January) in the outer crown of the upper branch, and the vapor pressure deficit was less than 2.0 kPa for all measurements.

Needles were harvested after the gas exchange measurements and their projected areas measured with a portable area meter (Li-3000A, Li-Cor). Needles were then dried at 80 °C for 48 h, and their nitrogen content determined by gas chromatography (GC-8A, Shimadzu, Kyoto, Japan) after combustion with circulating O$_2$ using an NC analyzer (Sumigraph NC-900, SCAS, Osaka, Japan).

Calculation of photosynthetic parameters
We estimated $V_{\text{max}}$, $J_{\text{max}}$ and the rate of dark respiration ($R_d$) from $A/C_i$ curves based on the model of Farquhar et al. (1980) by nonlinear regression following the procedures of Harley and Baldocchi (1995). Values of $V_{\text{max}}$ and $R_d$ were estimated from the $A/C_i$ curve at $C_i < 200$ µmol mol$^{-1}$, assuming that the only factors limiting the assimilation of CO$_2$ at low $C_i$ were the amount, activity and kinetic properties of Rubisco (Wullschleger 1993). The value of $J_{\text{max}}$ was estimated from the $A/C_i$ curve when $C_i > 700$ µmol mol$^{-1}$, with a fixed light energy conversion value ($\alpha = 0.24$) and the $R_d$ value obtained from the $V_{\text{max}}$ regression. An example showing the fit of the measured data to the Farquhar et al. model is given in Figure 1. Values for Michaelis constants at 25 °C ($K_c = 27.5$ Pa and $K_o = 42.0$ kPa), activation energies ($H_c(K_c) = 80.5$ kJ mol$^{-1}$ and $H_o(K_o) = 14.5$ kJ mol$^{-1}$), and the specificity for Rubisco ($\tau = 232.1$) were used in the fit. $R_d(t) = −29.0$ kJ mol$^{-1}$ were taken from Harley and Baldocchi (1995). The temperature dependence of $K_c$, $K_o$ and $\tau$ is described by:

$$f(T_k) = f(298)\exp((T_k−298)H_c/(298RT_k))$$

where $f(T_k)$ is the value of a given parameter at leaf temperature $T_k$ (K) and $f(298)$ is the value of the parameter at 25 °C, and $R$ is the gas constant (0.00831 kJ mol$^{-1}$).

Figure 1. Representative photosynthetic response curves to intercellular CO$_2$ concentration ($A/C_i$) showing the fit of the data to the Farquhar et al. (1980) model. Measurements were made in July 2001 on needles of the outer crown of the upper branch at three leaf temperatures.
The temperature dependence of \( V_{\text{cmax}} \) and \( J_{\text{max}} \) is described by the equation (Harley et al. 1992):

\[
\text{Parameter}(V_{\text{cmax}}, J_{\text{max}}) = \frac{\exp(c - H_d / RT_{\text{max}})}{1 + \exp((\Delta S_H - H_d) / RT_{\text{max}})}
\]

where \( H_d \) is the deactivation energy, \( c \) is a scaling constant, and \( \Delta S \) is an entropy term, 0.65 kJ K\(^{-1}\) mol\(^{-1}\). Temperature response parameters of \( H_d, H_a \) and \( c \) were obtained by fitting the above equation to response curves of \( V_{\text{cmax}} \) and \( J_{\text{max}} \) to leaf temperature with SigmaPlot (SPSS, Chicago, IL). Optimal temperatures (\( T_{\text{opt}} \)) for \( V_{\text{cmax}} \) and \( J_{\text{max}} \) were calculated by solving the following equation, as described by Dreyer et al. (2001) and Medlyn et al. (2002a):

\[
T_{\text{opt}} = \frac{H_d}{\Delta S - R \ln \left( \frac{H_d}{H_d - H_a} \right)}
\]

**Results**

**Temperature responses of \( V_{\text{cmax}} \) and \( J_{\text{max}} \)**

The temperature responses of \( V_{\text{cmax}} \) and \( J_{\text{max}} \), which closely fitted the peaked function as described by Harley et al. (1992), exhibited seasonal variation (Figures 2a and 2b). Both \( V_{\text{cmax}} \) and \( J_{\text{max}} \) decreased significantly in January. Parameters of the temperature response of \( V_{\text{cmax}} \) and \( J_{\text{max}} \) are given in Table 1. The optimal temperature for both \( V_{\text{cmax}} \) and \( J_{\text{max}} \) decreased in winter. Activation energy (\( H_d \)) for \( J_{\text{max}} \) decreased more than \( H_d \) for \( V_{\text{cmax}} \) in winter. Mean \( H_d \) throughout the season was 74 kJ mol\(^{-1}\) for \( V_{\text{cmax}} \) and 52 kJ mol\(^{-1}\) for \( J_{\text{max}} \). The temperature responses of \( V_{\text{cmax}} \) and \( J_{\text{max}} \) were normalized to 1 at 25 °C based on the estimated parameters (Figures 2c and 2d), which led to relatively little variation in the data at temperatures lower than 25 °C except for the data collected in January. Therefore, we further analyzed the seasonal variations in \( V_{\text{cmax}} \) and \( J_{\text{max}} \) throughout the crown at a common temperature of 25 °C.

**Seasonal variations in \( N, V_{\text{cmax}} \) and \( J_{\text{max}} \) within the crown**

In some months, \( A/C_i \) curves were not measured at 25 °C. Therefore, we recalculated \( V_{\text{cmax}} \) and \( J_{\text{max}} \), standardized to a temperature of 25 °C, according to the activation and deactivation energy parameters presented in Table 1. The patterns of seasonal changes in \( N, V_{\text{cmax}} \) and \( J_{\text{max}} \) were similar at all positions along each first-order branch and the results from the outer crown are shown in Figure 3. The needles maintained a constant \( N \) in the lower crown (\( P > 0.11 \)) throughout the season, but exhibited seasonal variations in \( N \) in the upper and middle crowns (Figure 3a). In the upper crown, \( N \) decreased significantly in September and increased again in November when current-year leaves fully developed and old leaves fell, after which \( N \) was relatively constant, until a significant decrease occurred in May when new shoots flushed. Needles in the upper crown had higher \( N \) than needles in the lower crown.
on most occasions except in September, when \( N \) became almost constant throughout the crown.

Photosynthetic parameters varied from 1.7 to 77.7 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) for \( V_{\text{cmax}} \), from 8.4 to 225.9 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) for \( J_{\text{max}} \), and showed seasonal patterns in all measured crown positions (Figures 3b and 3c). Values of \( V_{\text{cmax}} \) and \( J_{\text{max}} \) were lowest in January and increased afterwards in all crown positions. In most seasons, \( V_{\text{cmax}} \) was higher in the upper crown than in the lower crown except in September and January. There were no significant differences in values of \( V_{\text{cmax}} \) among crown positions in September and January (\( P > 0.29 \)).

### Table 1. Parameters of the temperature responses of maximum rate of carboxylation (\( V_{\text{cmax}}; \mu \text{mol m}^{-2} \text{s}^{-1} \)) and maximum rate of electron transport (\( J_{\text{max}}; \mu \text{mol m}^{-2} \text{s}^{-1} \)) for the data shown in Figure 2: activation energy (\( H_a; \text{kJ mol}^{-1} \)), deactivation energy (\( H_d; \text{kJ mol}^{-1} \)), scaling constant (\( c; \text{no units} \)) and optimal temperature (\( T_{\text{opt}}; ^\circ \text{C} \)).

<table>
<thead>
<tr>
<th>Month</th>
<th>( V_{\text{cmax}} )</th>
<th>( J_{\text{max}} )</th>
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<tr>
<td></td>
<td>( H_a )</td>
<td>( H_d )</td>
</tr>
<tr>
<td>July</td>
<td>81.0</td>
<td>200.0</td>
</tr>
<tr>
<td>November</td>
<td>84.8</td>
<td>194.7</td>
</tr>
<tr>
<td>January</td>
<td>55.5</td>
<td>189.9</td>
</tr>
<tr>
<td>May</td>
<td>76.5</td>
<td>196.1</td>
</tr>
</tbody>
</table>

Figure 3. Seasonal variations in (a) area-based nitrogen concentration (\( N \)), (b) maximum rate of carboxylation (\( V_{\text{cmax}} \)) and (c) maximum rate of electron transport (\( J_{\text{max}} \)) measured at 25 °C in needles of the outer foliated terminals along first-order branches of \( P. \text{densiflora} \) in the: upper (azimuth 160°, \( \Box \)); middle1 (azimuth 180°, \( \square \)); middle2 (azimuth 320°, \( \Delta \)); and lower (azimuth 260°, \( \bigcirc \)) crown. Measurements were made from July 2001 and May 2002. Each value is the mean ± SE of three replicate shoots.

**Seasonal patterns in the relationships between \( I_{\text{rel}}, N, V_{\text{cmax}} \) and \( J_{\text{max}} \)**

The relationships between \( I_{\text{rel}}, N, V_{\text{cmax}} \) and \( J_{\text{max}} \) were analyzed by linear regression of the monthly data. Leaf \( N \) was correlated with \( I_{\text{rel}} \) in all months in which these parameters were measured (Figure 4a). The regression slope exhibited lower values in September and January than in other months, but these differences were not statistically significant (Table 2). The maximum rate of carboxylation was correlated with \( I_{\text{rel}} \) in March, May and July, but not in September, November or January (Figure 4b, Table 2). Similar results were also observed for the relationship between \( I_{\text{rel}} \) and \( J_{\text{max}} \) (data not shown). Both \( V_{\text{cmax}} \) and \( J_{\text{max}} \) were related to \( N \) in most months, except in January for both parameters and in November for \( J_{\text{max}} \) (Figures 4c and 4d, Table 3). The regression slopes of the relationship between \( N \) and \( V_{\text{cmax}} \) did not differ significantly, although \( V_{\text{cmax}} \) was lower in January and November than in other months (March, May, July and September) for a given value of leaf \( N \). In addition, the regression between \( V_{\text{cmax}} \) and \( N \) could be expressed by a single equation for all data except January and November (Figure 4c, bold line). Similar trends were observed in the regression slope of the relationship between \( N \) and \( J_{\text{max}} \), but it was difficult to divide these data into meaningful groups because of their greater scatter.

Maximum rates of carboxylation and \( J_{\text{max}} \) were strongly correlated in all months (Figure 5, Table 4). The slopes of the linear regressions of \( J_{\text{max}} \) to \( V_{\text{cmax}} \) varied from 3.20 to 1.20, with a higher value in January than in the other months (Table 4). When all data were pooled, the slope of the linear regression of \( J_{\text{max}} \) to \( V_{\text{cmax}} \) was 2.46 (Figure 5, bold line).

Air temperatures measured above the canopy at the experimental site (Y. Ohtani, Forestry and Forest Product Research Institute, unpublished data) were used in the regression analysis. Temperature differences within the crown at each measuring occasion were not taken into account. Higher coefficients
of determination for all parameters were found with the mean daily minimum temperature of the respective months ($T_{\text{min}}$) than with the corresponding mean daily maximum or mean daily temperatures. Leaf $N$ was weakly related to $T_{\text{min}}$ ($N = 3.11 - 0.021T_{\text{min}}, r^2 = 0.13, P = 0.003$). Neither $V_{\text{cmax}}$ nor $J_{\text{max}}$ was correlated with $T_{\text{min}}$ ($P > 0.12$). We found that $T_{\text{min}}$ correlated with the optimal temperature for $V_{\text{cmax}}$ ($r^2 = 0.85, P = 0.05$), but not for $J_{\text{max}}$ ($r^2 = 0.69, P = 0.17$). The activation energy for $J_{\text{max}}$ was correlated with $T_{\text{min}}$ ($r^2 = 0.90, P = 0.05$), but not for $V_{\text{cmax}}$ ($r^2 = 0.32, P = 0.43$).

**Discussion**

Both of the photosynthetic parameters we monitored ($V_{\text{cmax}}$ and $J_{\text{max}}$) and leaf $N$ concentration ($N$) displayed seasonal fluctuations in all crown positions of *P. densiflora*. In addition, the close relationships between $I_{\text{rel}}, N, V_{\text{cmax}}$ and $J_{\text{max}}$, which are often found in canopies during the growing season (Meir et al. 2002), were found to have seasonal patterns, or were uncorrelated at certain times of year. Furthermore, the temperature response of $V_{\text{cmax}}$ and $J_{\text{max}}$ exhibited seasonal changes, indicating acclimation to growth irradiance and temperature (Niinemets et al. 1999). The nitrogen concentration in 1-year-old needles exhibited seasonal fluctuations in the upper crown (Figure 3a), reflecting a cyclical pattern with prominent phases of accumulation, retranslocation and replenishment (Nambiar and Fife 1991, Bauer et al. 1997). In contrast, $N$ in 1-year-old needles was relatively constant in the lower crown (Figure 3a), indicating that no retranslocation occurred here during the development of new needles, which started in May.
Table 3. Regression results \(y = ax + b\) for the data shown in Figure 4, which describe the seasonal variations in the relationships between leaf nitrogen concentration \(N\) and the maximum rate of carboxylation \((V_{\text{cmax}}; \mu\text{mol} \text{ m}^{-2} \text{s}^{-1})\) and the maximum rate of electron transport \((J_{\text{max}}; \mu\text{mol} \text{ m}^{-2} \text{s}^{-1})\). The statistical significances of the slopes are indicated as: ns = \(P > 0.05\); * = \(P < 0.05\); ** = \(P < 0.01\); and *** = \(P < 0.001\). Intercepts were not significantly different to zero for any of the regressions \((P > 0.05)\). There were no significant differences between the slopes \((P > 0.05, \text{ANCOVA test})\). The number of replicate shoots was 33 for all months.

<table>
<thead>
<tr>
<th>Month</th>
<th>(V_{\text{cmax}})</th>
<th>(J_{\text{max}})</th>
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<tbody>
<tr>
<td></td>
<td>(a)</td>
<td>(b)</td>
</tr>
<tr>
<td>July</td>
<td>15.02 **</td>
<td>5.17</td>
</tr>
<tr>
<td>September</td>
<td>15.71 *</td>
<td>–3.04</td>
</tr>
<tr>
<td>November</td>
<td>15.59 *</td>
<td>–18.31</td>
</tr>
<tr>
<td>January</td>
<td>–1.97 ns</td>
<td>9.54</td>
</tr>
<tr>
<td>March</td>
<td>29.45 *</td>
<td>–45.40</td>
</tr>
<tr>
<td>May</td>
<td>15.94 **</td>
<td>6.75</td>
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</table>

and ended in October 2001. Variations in \(N\) within the \(P. \text{densiflora}\) crown were correlated with \(I_{\text{el}}\) for all months in which the relationship was measured (Table 2), suggesting that \(N\) allocation is predominantly determined by growth irradiance. In addition, seasonal fluctuations in \(N\) were weakly correlated with \(T_{\text{min}}\), reflecting acclimation to growth temperature (Medlyn et al. 2002b).

Both \(V_{\text{cmax}}\) and \(J_{\text{max}}\) exhibited seasonal fluctuations, decreasing in the winter (Figures 3b and 3c). However, the within-crown variations in \(V_{\text{cmax}}\) and \(J_{\text{max}}\) were not correlated with \(I_{\text{el}}\) in September, November and January. Nitrogen concentrations in 1-year-old leaves have been observed to decrease to a threshold value during the development of new leaves in \(P. \text{abies}\) (L.) Karst. (Bauer et al. 1997). Retranslocation of \(N\) to newly expanded needles, which were fully developed in October, resulted in the observed reductions in \(V_{\text{cmax}}\) and \(J_{\text{max}}\) in the upper crown. Therefore, the correlation between \(I_{\text{el}}\) and \(V_{\text{cmax}}\) and \(J_{\text{max}}\) may have been mediated by changes in leaf \(N\), as both \(V_{\text{cmax}}\) and \(J_{\text{max}}\) were correlated with \(N\) in September (Figures 4c and 4d, Table 3). However, \(V_{\text{cmax}}\) and \(J_{\text{max}}\) were not correlated with \(N\) in winter. Photoinhibition and down-regulation of photosynthesis often occur in conifers subjected to freezing temperatures, which prevents photodamage to the photosynthetic apparatus (Ottander et al. 1995, Ivanov et al. 2001, Han et al. 2003b). Light-harvesting complexes of photosystem II were reorganized from a low quenching state to a highly quenched state in \(P. \text{sylvestris}\) L. to maximize dissipation of excitation energy during winter (Ottander et al. 1995). The amount of Rubisco in \(C. \text{japonica}\) D. Don in winter decreased more than threefold compared with the amount of Rubisco in summer (Han et al. 2003b). A fraction of Rubisco was inactive in \(P. \text{pinaster}\) Ait. and increased in winter, thus apparently functioning as a storage protein (Warren and Adams 2001). This long-term acclimation to freezing temperatures resulted in low \(V_{\text{cmax}}\) and \(J_{\text{max}}\) in winter with the result that these variables were not correlated with leaf \(N\).

At other times of the year \(V_{\text{cmax}}\) and \(J_{\text{max}}\) were significantly related to \(N\), but for a given \(N\) value, these parameters differed up to twofold between seasons. These findings are consistent

Table 4. Regression results \(y = ax + b\) for the data in Figure 5, which show seasonal variations in the relationship between the slope of the maximum rate of electron transport \((J_{\text{max}}; \mu\text{mol} \text{ m}^{-2} \text{s}^{-1})\) and the maximum rate of carboxylation \((V_{\text{cmax}}; \mu\text{mol} \text{ m}^{-2} \text{s}^{-1})\). The statistical significances of the slopes are indicated as: ns = \(P > 0.05\); * = \(P < 0.05\); ** = \(P < 0.01\); and *** = \(P < 0.001\). Different letters adjacent to each slope indicate a significant difference \((P < 0.05, \text{ANCOVA test})\). The number of replicate shoots was 33 for all months.

| Month   | \(a\)    | \(b\)  | \(r^2\) |
|---------|-----------|---------------------|
| July    | 1.20 ** a | 59.56 ** | 0.71   |
| September | 2.02 *** a | 25.60 ns | 0.72   |
| November | 1.61 ** a | 61.47 ** | 0.56   |
| January | 3.20 *** b | 5.28 * | 0.83   |
| March   | 2.02 *** a | 7.98 ns | 0.84   |
| May     | 3.07 ** b | 29.03 ns | 0.56   |
with the results of Wilson et al. (2000) and Dungan et al. (2003). One possible explanation is that the fraction of N partitioned into Rubisco decreases with leaf age (Wilson et al. 2000), and this decrease is often accompanied by an increase in starch accumulation (Rey and Jarvis 1998). These authors suggested that starch accumulation may create a feedback limitation to Rubisco synthesis. We found continuous decreases in $V_{\text{cmax}}$ for a given $N$ value from March to November (Figure 4c), supporting this explanation. The $V_{\text{cmax}}$ increased in March and May, but the needles were about 2-years-old at the time of those measurements. This increase was probably caused by starch consumption during bud break and initiation of shoot growth (Hansen et al. 1996), and thus the feedback limitation to Rubisco synthesis was relieved. Ellsworth (2000) found that maximum photosynthesis occurred in May, concurrent with the emergence of new foliage in $P.$ taeda L. The second possibility is that a fraction of Rubisco was inactive and that this fraction increased in winter and functioned as storage protein (Warren and Adams 2001). This hypothesis is in accordance with the changes in $N$ and $V_{\text{cmax}}$ from September to November. Compared to $N$ values in September, $N$ increased in November as a result of resorption from fallen leaves and replenishment after the full development of current-year needles (Figure 3a), but $V_{\text{cmax}}$ did not increase (Figures 3b and 4c). The seasonal fluctuation in the inactive fraction may reflect an ontogenetic process relating to long-term temperature acclimation.

When standardized to total leaf surface area, the values of $V_{\text{cmax}}$ and $J_{\text{max}}$ that we measured in $P.$ densiflora fall within the range for conifers reported by Wullschleger (1993). Our values of $H_2$ and $H_4$ for both $V_{\text{cmax}}$ and $J_{\text{max}}$ were within the range of values for conifers reported by Medlyn et al. (2000a), however, our measurements were made on only one leaf for each season. The $T_{\text{opt}}$ values for both $V_{\text{cmax}}$ and $J_{\text{max}}$ in this study declined in the winter, which was not observed in $P.$ pinaster (Medlyn et al. 2000b). This deviation may reflect differences between the species in long-term acclimation to chilling and freezing temperatures. Our measurements were conducted on needles in situ, whereas Medlyn et al. (2000b) made measurements in detached shoots. Therefore, the deviation may also be related to stomatal limitation because of low soil temperature (Schwarz et al. 1997). The slopes of the linear regressions of $J_{\text{max}}$ to $V_{\text{cmax}}$ increased in winter, because $H_2(J_{\text{max}})$ decreased more than $H_4(V_{\text{cmax}})$. These results are consistent with previous measurements taken at different leaf or growth temperatures (Walcroft et al. 1997, Bunce 2000, Dungan et al. 2003) and could be explained by acclimatory responses to low temperature including changes in the following: N partitioning among photosynthetic components (Wilson et al. 2000), the sensitivity of stomata to vapor pressure deficits (Medlyn et al. 2002b), and cell-wall CO$_2$ conductance (Makino et al. 1994).

In conclusion, seasonal patterns in the relationship between $J_{\text{rib}}$, leaf $N$ and $V_{\text{cmax}}$ and $J_{\text{max}}$ at the leaf level should be considered when modeling canopy carbon exchange. In addition, seasonal variation in the temperature responses of $V_{\text{cmax}}$ and $J_{\text{max}}$ indicates that modeling canopy carbon exchange based on a single response curve for all seasons may lead to significant errors. However, cost may sometimes preclude the intensive data collection necessary in order to estimate these parameters. Normalizing the temperature response of $V_{\text{cmax}}$ and $J_{\text{max}}$ to 1 at 25 °C led to relatively little seasonal variation at temperatures less than 25 °C except for data collected in winter, indicating that seasonal variation could be incorporated simply by varying the scale constant if a single temperature response curve is used for all except the coldest month. The relationship between $V_{\text{cmax}}$ and $N$ could also be expressed as a single equation for all data except for those collected in January and November.

Acknowledgments

The authors thank Dr. Y. Ohtani of FFPRI for supplying unpublished temperature data. The authors are grateful to the Japan Science and Technology Corporation (JST) for the Cooperative System for Supporting Priority Research Fund. This research was supported by the following grants: (1) Establishment of an AsiaFlux Network and its Operation for Carbon Sequestration Estimation in the Eastern Asian Monsoon Ecosystems, funded by the Global Environment Research Fund of the Ministry of the Environment of Japan; (2) Global Carbon Cycle and Related Mapping Based on Satellite Imagery Program, funded by the Special Coordination Fund of the Ministry of Education, Culture, Sports, Science and Technology of Japan; and (3) Development of Detailed Methods to Evaluate CO$_2$ Budgets in Forest and Ocean Ecosystems, funded by the Ministry of Agriculture, Forestry and Fisheries of Japan.

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