

## Seasonal changes in the temperature response of photosynthesis in canopy leaves of *Quercus crispula* in a cool-temperate forest

KOUKI HIKOSAKA,<sup>1,2</sup> ERI NABESHIMA<sup>3,4</sup> and TSUTOM HIURA<sup>3</sup>

<sup>1</sup> Graduate School of Life Sciences, Tohoku University, Sendai 980-8578, Japan

<sup>2</sup> Corresponding author (hikosaka@mail.tains.tohoku.ac.jp)

<sup>3</sup> Tomakomai Research Station, Field Science Center for Northern Biosphere, Hokkaido University, Tomakomai 053-0035, Japan

<sup>4</sup> Present address: Tokyo University of Agriculture and Technology, Harumi-cho 3-8-1, Fuchu, Tokyo 183-8538, Japan

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**Summary** Understanding seasonal changes in photosynthetic characteristics of canopy leaves is indispensable for modeling the carbon balance in forests. We studied seasonal changes in gas exchange characteristics that are related to the temperature dependence of photosynthesis in canopy leaves of *Quercus crispula* Blume, one of the most abundant species in cool-temperate forests in Japan. Photosynthetic rate and ribulose-1,5-bisphosphate (RuBP) carboxylation capacity ( $V_{\text{cmax}}$ ) at 20 °C increased from June to August and then decreased in September. The activation energy of  $V_{\text{cmax}}$ , a measure of the temperature dependence of  $V_{\text{cmax}}$ , was highest in summer, indicating that  $V_{\text{cmax}}$  was most sensitive to leaf temperature at this time. The activation energy of  $V_{\text{cmax}}$  was significantly correlated with growth temperature. Other parameters related to the temperature dependence of photosynthesis, such as intercellular  $\text{CO}_2$  partial pressure and temperature dependence of RuBP regeneration capacity, showed no clear seasonal trend. It was suggested that leaf senescence affected the balance between carboxylation and regeneration of RuBP. The model simulation showed that photosynthetic rate and its optimal temperature were highest in summer.

**Keywords:** activation energy,  $J_{\text{max}}$ , temperature acclimation, temperature dependence of photosynthesis,  $V_{\text{cmax}}$ .

### Introduction

Photosynthesis by canopy leaves is a major determinant of the carbon cycle in forests (Baldocchi and Meyers 1998, Wilson et al. 2001). Understanding seasonal changes in photosynthetic characteristics of canopy leaves is indispensable for predicting responses of carbon flow in ecosystems to climate change. Because of increasing concern about global warming, the temperature response of photosynthesis has become an important focus of study (Medlyn et al. 2002a, 2002b, Hikosaka et al. 2006).

In the field, leaves are subjected to changes in air temperature at various time scales. As a short-term response (seconds to minutes) to leaf temperature, the light-saturated rate of pho-

tosynthesis ( $P_{\text{max}}$ ) is reduced at the low and high temperature extremes and has an optimum at intermediate temperature (Berry and Björkman 1980). As a long-term response (days to months) to growth temperature, the temperature dependence of photosynthesis changes. In many plants, the optimal temperature for  $P_{\text{max}}$  increases with growth temperature (Berry and Björkman 1980, Hikosaka et al. 2006).

According to the biochemical model of Farquhar et al. (1980), photosynthetic rate is limited either by RuBP (ribulose-1,5-bisphosphate) carboxylation or by the rate of RuBP regeneration. Thus, changes in the temperature dependence of photosynthesis are attributable to changes in four traits: (1)  $\text{CO}_2$  partial pressure at the site of carboxylation; (2) temperature dependence of the maximum rate of RuBP carboxylation ( $V_{\text{cmax}}$ ); (3) temperature dependence of the maximum rate of RuBP regeneration, expressed as the rate of electron transport ( $J_{\text{max}}$ ); and (4) the balance between  $V_{\text{cmax}}$  and  $J_{\text{max}}$  (Hikosaka 1997, Hikosaka et al. 1999, 2006). Responses of these traits to growth temperature, however, seem to differ among species (Hikosaka et al. 2006). For example, some species alter the  $J_{\text{max}}$  to  $V_{\text{cmax}}$  ratio in response to growth temperature (Hikosaka et al. 1999, Hikosaka 2005, Onoda et al. 2005a, Yamori et al. 2005), but others do not (Bunce 2000, Medlyn et al. 2002a, Onoda et al. 2005b). Some species alter the temperature dependence of  $J_{\text{max}}$  (Hikosaka et al. 1999, Bunce 2000), but others do not (Borjigidai et al. 2006). Several studies have reported that  $V_{\text{cmax}}$  is more sensitive to temperature when the leaf is acclimated to higher temperatures (Hikosaka et al. 1999, Bunce 2000, Yamori et al. 2005, Borjigidai et al. 2006). Factors other than growth temperature may alter the traits related to temperature dependence of photosynthesis. For example, Onoda et al. (2005b) found that the  $J_{\text{max}}$  to  $V_{\text{cmax}}$  ratio in leaves of *Fagus crenata* Blume seedlings decreased from summer to autumn, which may be a result of leaf senescence rather than an environmental acclimation.

In this decade, the photosynthetic parameters of canopy leaves in forests, including the temperature dependence of  $V_{\text{cmax}}$  and  $J_{\text{max}}$ , have been investigated (Harley and Baldocchi 1995, Walcroft et al. 1997, Kosugi et al. 2003, Kosugi and

Matsuo 2006). Medlyn et al. (2002a) and Han et al. (2004) reported that the temperature dependence of  $V_{\text{cmax}}$  and  $J_{\text{max}}$  changed seasonally in leaves of *Pinus* species. However, it is still unclear if the change is related to environmental acclimation or to ontogeny. We studied canopy leaves of *Quercus crispula* Blume, one of the most abundant deciduous trees in cool-temperate forests in Japan, to determine CO<sub>2</sub>- and temperature-dependence of photosynthetic rates in spring, summer and autumn of two years. We sought answers to two questions. First, which of the four traits (intercellular CO<sub>2</sub> partial pressure, temperature dependence of  $V_{\text{cmax}}$ , temperature dependence of  $J_{\text{max}}$  and the  $J_{\text{max}}$  to  $V_{\text{cmax}}$  ratio) is involved in the seasonal response of leaf photosynthesis in *Q. crispula*? Second, is the seasonal response of leaf photosynthesis determined by growth temperature?

### Materials and methods

We studied a mature, deciduous broad-leaved forest stand in the Tomakomai Experimental Forest (TOEF; 42°40' N, 141°36' E). Annual precipitation at TOEF is 1304 mm, most of which occurs in summer, and the mean summer temperature is 18 °C. The dominant species are *Acer mono* Maxim., *Acer palmatum* Thunb. var. *amoenum* Ohwi, *Cercidiphyllum japonicum* Siebold et Zucc., *Ostrya japonica* Sarg., *Prunus ssiori* Friedr. Schmidt and *Quercus crispula* (Hiura et al. 1998). We selected a canopy tree of *Q. crispula* (22.4 m in height and 76.3 cm in diameter at breast height) at the canopy crane site in TOEF. Meteorological data were collected at a flux tower 1 km from the canopy crane.

In the TOEF forest, *Q. crispula* leaves complete their expansion by the end of May and senesce in late October. Photosynthetic measurements were made on June 6–11, August 6–10 and September 25–29 in 2001 and June 26–29, July 25–29 and September 25–28 in 2002. We used attached unshaded leaves. Photosynthetic rates were measured with open gas exchange systems (Model LI-6400, Li-Cor, Lincoln, NE) with an LED light source (Li-Cor LI-6400-02B) and a dual Peltier device to regulate photosynthetic photon flux (PPF) and temperature in the chamber (3 × 2 cm). The CO<sub>2</sub>-response curves of photosynthesis were obtained at various leaf temperatures and a PPF of 1000 μmol m<sup>-2</sup> s<sup>-1</sup>. Vapor pressure deficit was unregulated. For each CO<sub>2</sub>-response curve, photosynthesis was measured from low (10 Pa) to high CO<sub>2</sub> partial pressures (100 or 150 Pa). Dark respiration rate was measured at ambient CO<sub>2</sub> partial pressure (36 Pa). In some cases, stomatal conductance showed a large decrease during determination of the CO<sub>2</sub> dependence of photosynthesis and we obtained insufficient data points at high CO<sub>2</sub> partial pressures. In July and September 2002, we conducted photosynthetic measurements for some leaves only at high CO<sub>2</sub> partial pressures. After the measurements, four leaf discs of 1 cm diameter were punched from one leaf, oven-dried and analyzed with an NC-analyzer (NC-80, Shimadzu, Kyoto).

The CO<sub>2</sub>-dependence curve of photosynthesis was fitted with the biochemical model of Farquhar et al. (1980). At lower

CO<sub>2</sub> partial pressures, the following curve was applied, assuming that RuBP carboxylation is the limiting step of photosynthesis:

$$P_c = \frac{V_{\text{cmax}}(C_i - \Gamma^*)}{C_i + K_c \left(1 + \frac{O}{K_o}\right)} - R_d \quad (1)$$

where  $P_c$  is the carboxylation-limited photosynthetic rate,  $K_c$  and  $K_o$  are the Michaelis-Menten constants of rubisco (RuBP carboxylase/oxygenase) for CO<sub>2</sub> and O<sub>2</sub>, respectively,  $C_i$  and  $O$  are the intercellular partial pressures of CO<sub>2</sub> and O<sub>2</sub>, respectively,  $\Gamma^*$  is the CO<sub>2</sub> compensation point in the absence of day respiration and  $R_d$  is the rate of day respiration. At higher CO<sub>2</sub> partial pressures, the following curve was applied assuming that the photosynthetic rate is limited by RuBP regeneration:

$$P_r = \frac{J_{\text{max}}(C_i - \Gamma^*)}{4C_i + 8\Gamma^*} - R_d \quad (2)$$

where  $P_r$  is the regeneration-limited rate of photosynthesis. We assumed that photosynthesis at high CO<sub>2</sub> partial pressure was limited only by RuBP regeneration, and limitation by triose-phosphate utilization (Sharkey 1985) was ignored.

Temperature dependence of parameter values was fitted using the Arrhenius model:

$$f = f(25) \exp\left(\frac{E_a(T_k - 298)}{298RT_k}\right) \quad (3)$$

where  $f(25)$  is the value of  $f$  at 25 °C,  $E_a$  is the activation energy of  $f$ ,  $R$  is the universal gas constant (8.314 J mol<sup>-1</sup> K<sup>-1</sup>) and  $T_k$  is leaf temperature in °K.

We calculated values of  $K_c$ ,  $K_o$  and  $\Gamma^*$  with Equation 3, where their values at 25 °C and  $E_a$  were derived from Harley and Tenhunen (1991). Values of  $V_{\text{cmax}}$  and  $R_d$  were obtained by fitting Equation 1 to the CO<sub>2</sub>-dependence curves at low CO<sub>2</sub> partial pressures (< 30 Pa). Then, using the  $R_d$  value,  $J_{\text{max}}$  was obtained by fitting Equation 2 to the CO<sub>2</sub>-dependence curves at high CO<sub>2</sub> partial pressures (> 50 Pa). Curve fitting was performed with Kaleida graph (Synergy Software, Reading, PA).

### Results

Climate conditions before the measurements are shown in Table 1. Daily temperature was highest in summer and was similar between June and September. Irradiance was highest in June. Relative humidity tended to be high in July and August.

Figure 1 shows seasonal changes in leaf characteristics. Leaf mass per area (LMA) increased rapidly in June and then remained virtually unchanged until September (Figure 1a). Leaf nitrogen concentration per unit mass ( $N_{\text{mass}}$ ) was stable from June to September. Leaf nitrogen concentration per unit area ( $N_{\text{area}}$ ), the product of LMA and  $N_{\text{mass}}$ , showed a similar seasonal change with LMA (Figure 1c).

Table 1. Climate conditions. Mean daily values for the 10 days prior to the measurements. Abbreviations: PPF, photosynthetic photon flux; and RH, relative humidity.

	Temperature (°C)	PPF (mol m <sup>-2</sup> day <sup>-1</sup> )	RH (%)
<i>2001</i>			
May 27–June 5	12.3	36.1	81.3
July 27–August 15	16.9	26.0	85.4
September 15–24	13.4	25.6	81.6
<i>2002</i>			
June 16–25	12.4	34.4	82.6
July 15–24	18.1	16.8	93.1
September 15–24	12.4	26.7	79.6

The light-saturated rate of photosynthesis at a leaf temperature of 20 °C and ambient CO<sub>2</sub> partial pressure ( $P_{\max}$ ) showed a parabolic curve against day of year, with highest rates from July to August (Figure 2a), whereas the dark respiration rate was highest in June and subsequently decreased during the course of the summer (Figure 2b). Both  $J_{\max}$  and  $V_{\max}$  showed parabolic relationships with day of year (Figures 2c and 2d). However, the autumnal decrease was greater in  $V_{\max}$  than in  $J_{\max}$ , leading to an increase in the  $J_{\max}$  to  $V_{\max}$  ratio from August to September (Figure 2e). Intercellular CO<sub>2</sub> partial pressure ( $C_i$ ) at ambient CO<sub>2</sub> partial pressure was similar across the season (Figure 2f).

Both  $J_{\max}$  and  $V_{\max}$  increased exponentially with increasing temperature, without deactivation of either  $J_{\max}$  or  $V_{\max}$  at high temperatures, and the Arrhenius model fitted well (Figure 3). Figure 4 shows the activation energies for respiration ( $E_{\text{aR}}$ ),  $J_{\max}$  ( $E_{\text{aJ}}$ ) and  $V_{\max}$  ( $E_{\text{aV}}$ ). Values of  $E_{\text{aR}}$  increased seasonally,  $E_{\text{aJ}}$  values showed no clear seasonal trend and  $E_{\text{aV}}$  followed a parabolic course over time, with highest values from July to August.

Both  $P_{\max}$  and stomatal conductance at 20 °C were significantly correlated with mean daily temperature ( $P < 0.05$ ), whereas  $V_{\max}$  at 20 °C showed only a weak correlation ( $P < 0.1$ ; Table 2). The activation energy of  $V_{\max}$  was significantly correlated with mean daily temperature (Figure 5), whereas those of respiration and  $J_{\max}$  were not (Table 2).

Stomatal conductance was highly sensitive to changes in vapor pressure deficit (VPD) (data not shown). Because we did not control water vapor concentration during the measurements, VPD in the measurement chamber tended to increase with increasing leaf temperature, especially when the air temperature was low. Consequently,  $C_i$  decreased with increasing leaf temperature (data not shown) and affected the temperature dependence of photosynthetic rate. To avoid this artifact, we calculated photosynthetic rates assuming that  $C_i$  was constant at 22.6 Pa (mean value at 20 °C throughout the experiment, Figure 2f). Figure 6 shows the calculated photosynthetic rate plotted against leaf temperature. In all curves,  $P_c$  was lower than  $P_r$  at any temperature, i.e., photosynthetic rates were always limited by RuBP carboxylation. The optimal temperature was higher in summer than in spring and autumn.

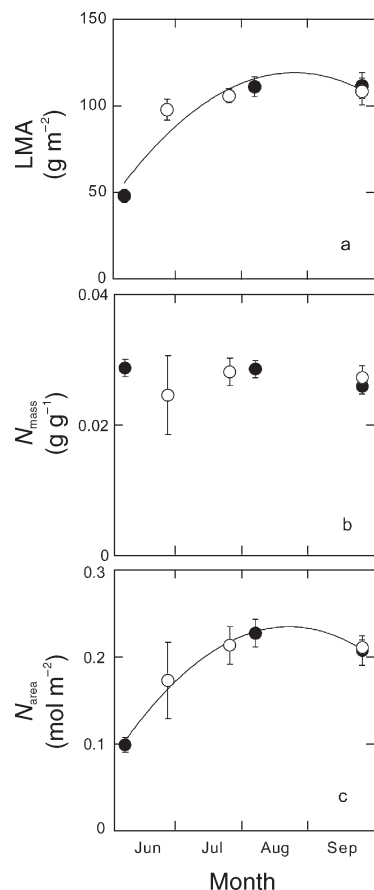


Figure 1. Seasonal changes in (a) mean leaf mass per unit area (LMA), (b) leaf nitrogen concentration per unit mass ( $N_{\text{mass}}$ ) and (c) leaf nitrogen concentration per unit area ( $N_{\text{area}}$ ) in canopy leaves of *Quercus crispula* in 2001 (●) and 2002 (○). Bars are standard deviations. Polynomial curves are fitted for (a) ( $r^2 = 0.95$ ,  $P < 0.05$ ) and (c) ( $r^2 = 0.99$ ,  $P < 0.05$ ).

## Discussion

Throughout the measurement period,  $P_{\max}$  was always limited by RuBP carboxylation because  $J_{\max}$  was relatively high and  $C_i$  did not change greatly (Figure 2f). Therefore,  $V_{\max}$  was the factor responsible for the seasonal change in  $P_{\max}$ . Early in the season,  $V_{\max}$  at 20 °C increased, as did  $N_{\text{area}}$  (Figures 1 and 2), suggesting that rubisco concentration increased from June to July. In late season, on the other hand,  $V_{\max}$  decreased though  $N_{\text{area}}$  remained constant. Age-dependent decreases in photosynthetic nitrogen-use efficiency (PNUE, photosynthetic capacity per unit leaf nitrogen) have been observed in several herbaceous (Hikosaka 1996), deciduous (Wilson et al. 2000, Onoda et al. 2005b) and evergreen species (Kitajima et al. 2002, Escudero and Mediavilla 2003, Miyazawa et al. 2004, Niinemets et al. 2005). Such a decrease may be explained by: (1) selective degradation of rubisco; (2) inactivation of rubisco; or (3) decreased CO<sub>2</sub> diffusion in old leaves (Hikosaka et al. 1998, Hikosaka 2004). Hikosaka (1996) found that, in *Ipomoea tricolor* Cav. leaves that were grown without shading, the amounts of rubisco, cytochrome *f* and photosystem I and II decreased even though  $N_{\text{area}}$  remained constant.

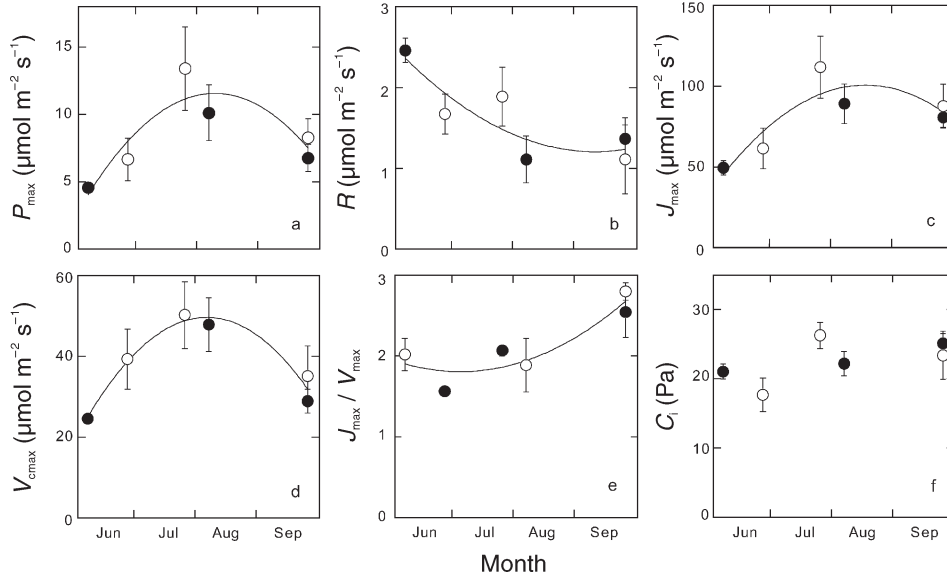


Figure 2. Seasonal changes in gas exchange characteristics of canopy leaves of *Quercus crispula* determined at a leaf temperature of 20 °C. (a) Light-saturated rate of photosynthesis at 37 Pa CO<sub>2</sub> ( $P_{max}$ ), (b) dark respiration rate ( $R$ ), (c) RuBP regeneration capacity expressed as the electron transport rate ( $J_{max}$ ), (d) RuBP carboxylation capacity ( $V_{cmax}$ ), (e) ratio of  $J_{max}$  to  $V_{cmax}$  and (f) intercellular CO<sub>2</sub> partial pressure ( $C_i$ ). All values are means obtained in 2001 (●) and 2002 (○). Bars are standard deviations. Polynomial curves are fitted if significant ( $r^2 > 0.76, P < 0.05$ ).

Niinemets et al. (2005) showed that the CO<sub>2</sub> partial pressure in chloroplasts decreased with leaf age in several Mediterranean evergreen broad-leaved species. However, there are several reports showing that photosynthetic capacity or  $V_{cmax}$  decreases in parallel with  $N_{area}$  in deciduous woody species (Reich et al. 1991, Yasumura et al. 2006). These findings suggest that age dependence in PNUE differs among deciduous species.

The temperature dependence of  $V_{cmax}$  was responsible for that of  $P_{max}$ . According to the model of Farquhar et al. (1980), as  $E_{aV}$  increases, the optimal temperature for  $P_{max}$  increases at a rate of  $0.54 \text{ °C kJ}^{-1} \text{ mol}^{-1} E_{aV}$  (Hikosaka et al. 2006). Because  $E_{aV}$  in *Q. crispula* leaves was significantly correlated with mean daily temperature (Table 2, Figure 5), the optimal temperature for  $P_{max}$  was predicted to be highest during sum-

mer (Figure 6). This is in accord with previous studies showing higher optimal temperature of  $P_{max}$  in leaves grown at higher temperatures (Slatyer 1977, Berry and Björkman 1980, Badger et al. 1982, Hikosaka et al. 1999, Yamori et al. 2005). In their literature survey, Hikosaka et al. (2006) suggested that the increase in  $E_{aV}$  with increasing growth temperature is a general response in C<sub>3</sub> plants.

What mechanisms are involved in the change in  $E_{aV}$ ? It should be noted that  $V_{cmax}$  obtained with the gas exchange method is determined not only by rubisco kinetics but also by the rubisco activation state and the internal conductance for CO<sub>2</sub> diffusion (Salvucci and Crafts-Brandner 2004, Hikosaka et al. 2006). Yamori et al. (2006) showed that temperature dependence of rubisco activation state in spinach leaves differs

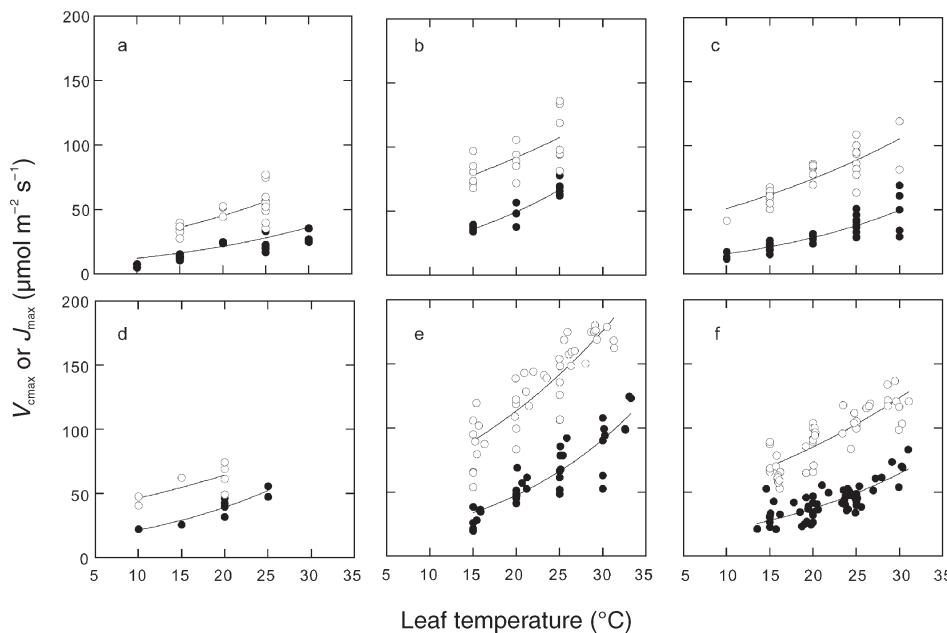


Figure 3. Temperature dependence of RuBP regeneration capacity expressed as electron transport rate ( $J_{max}$ , ○) and RuBP carboxylation capacity ( $V_{cmax}$ , ●) in canopy leaves of *Quercus crispula*. Measurements were made in June (a), August (b) and September (c) in 2001 and June (d) and September (e) in 2002. Arrhenius curves are fitted ( $r^2 > 0.39, P < 0.05$ ; see Figure 4 for the activation energies).

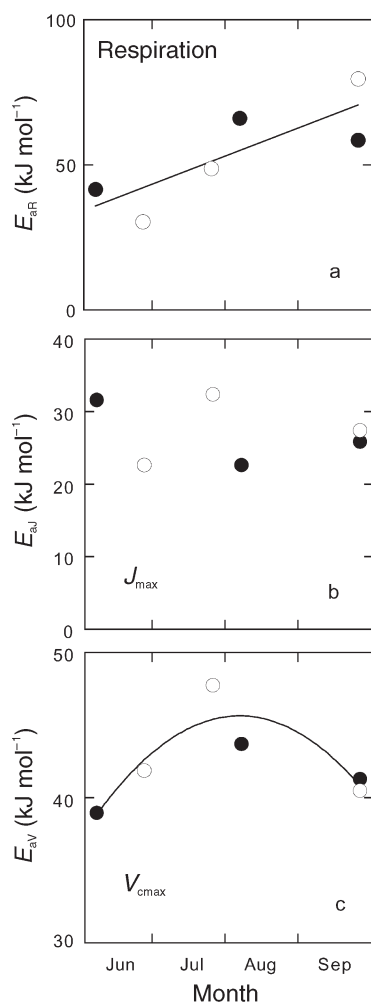


Figure 4. Seasonal changes in the activation energies of (a) dark respiration ( $E_{aR}$ ), (b) RuBP regeneration expressed as the electron transport rate ( $E_{aJ}$ ) and (c) RuBP carboxylation ( $E_{aV}$ ) in canopy leaves of *Quercus crispula*. Closed (●) and open (○) circles denote mean values obtained in 2001 and 2002, respectively. Linear ( $r^2 = 0.66$ ,  $P < 0.05$ ) and polynomial ( $r^2 = 0.79$ ,  $P < 0.05$ ) curves are fitted for (a) and (c), respectively.

depending on growth temperature; for example, the activation state of rubisco in leaves grown at 15 °C was 60% at 30 °C, whereas that in leaves grown at 30 °C was fully activated until 30 °C. Yamori et al. (2006) also found that the kinetics of rubisco changed with growth temperature. In our study, temperature dependence of  $V_{cmax}$  was fitted well by the Arrhenius model and there was no apparent thermal depression in  $V_{cmax}$ , implying that there was no great change in the activation state across measurement temperatures.

The activation energy for  $J_{max}$  showed no seasonal trend or any correlation with growth temperature. This finding contrasts with results obtained from plants growing under controlled conditions, where  $J_{max}$  of leaves grown at lower temperatures tends to have a lower temperature optimum (Badger et al. 1982, Mitchell and Barber 1986, Yamori et al. 2005) or a smaller temperature dependence at low leaf temperatures

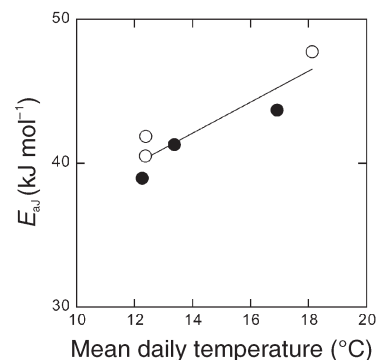


Figure 5. Relationship between the activation energy of RuBP carboxylation ( $E_{aJ}$ ) and daily temperature in canopy leaves of *Quercus crispula*. Daily temperature was calculated as the mean daily temperature for the 10 days before measurement. See Table 2 for regression analysis.

(Armond et al. 1978, Hikosaka et al. 1999, Yamasaki et al. 2002). However, in the literature survey by Hikosaka et al. (2006), there was no trend in the response of  $E_{aJ}$  to growth temperature, suggesting that it is species-dependent. The  $J_{max}$  to  $V_{cmax}$  ratio at 20 °C increased in September (Figure 2e) but was not correlated with growth temperature (Table 2). This change may simply reflect the decrease in  $V_{cmax}$  in autumn. If true, this implies that the seasonal change in the ratio of  $J_{max}$  to  $V_{cmax}$  in *Q. crispula* was an age-dependent change rather than an acclimation response (Onoda et al. 2005b). This contrasts with findings for some perennial herbs and evergreen trees showing a temperature-dependent change in the  $J_{max}$  to  $V_{cmax}$  ratio (Hikosaka et al. 1999, Onoda et al. 2005a, 2005b, Yamori et al. 2005). Atkin et al. (2006) suggested that phenotypic plasticity to growth temperature is greater in fast-growing species than in slow-growing species.

Table 2. Regression analysis for the relationship between model parameters (dependent variable) and mean daily temperature (°C). Daily temperature was calculated as the mean of daily temperature for 10 days before measurement. Values of  $E_{aR}$ ,  $E_{aJ}$  and  $E_{aV}$  are the activation energies of dark respiration rate, RuBP regeneration expressed as the electron transport rate ( $J_{max}$ ) and RuBP carboxylation ( $V_{cmax}$ ), respectively. Significance values: ns,  $P > 0.1$ ; \*,  $P < 0.1$ ; and \*\*,  $P < 0.05$  ( $n = 6$ ). Abbreviation:  $C_i$ , intercellular partial pressure of  $CO_2$ .

Parameter	Intercept	Slope	$r$
<i>Value at 20 °C leaf temperature</i>			
Photosynthetic rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	-7.07	1.08	0.90**
Respiration rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	1.99	-0.027	0.14ns
$J_{max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	18.4	4.15	0.63ns
$V_{cmax}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	0.69	2.55	0.74*
$J_{max}/V_{cmax}$	2.57	-0.03	-0.17ns
$C_i$ (Pa)	13.5	0.643	0.54ns
<i>Activation energy</i>			
$E_{aR}$ ( $\text{kJ mol}^{-1}$ )	142.4	0.830	0.12ns
$E_{aJ}$ ( $\text{kJ mol}^{-1}$ )	23.6	0.248	0.15ns
$E_{aV}$ ( $\text{kJ mol}^{-1}$ )	27.0	1.079	0.91**

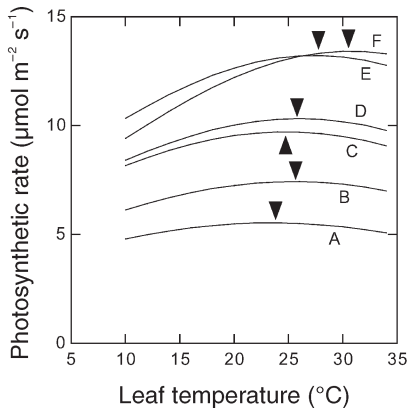


Figure 6. Simulated temperature-dependence of photosynthetic rate in canopy leaves of *Quercus crispula*. Values of gas exchange parameters followed experimental results except for intercellular CO<sub>2</sub> partial pressure, which was kept constant at 22.6 Pa. Simulations are for June (A), August (E) and September (B) in 2001 and June (D), July (F) and September (C) in 2002. Arrowheads denote the optimal temperature for maximum photosynthetic rate.

We assumed that photosynthesis at high CO<sub>2</sub> partial pressures was always limited by RuBP regeneration and ignored any limitation by triose-phosphate utilization (TPU). However, TPU may have limited photosynthesis at high CO<sub>2</sub> concentration, in which case we might have underestimated  $J_{\max}$ . However, because photosynthetic rates at ambient CO<sub>2</sub> partial pressure were always lower than the rates expected based on the assumption of TPU limitation (data not shown), ignoring TPU limitation may not affect our conclusion that photosynthesis at ambient CO<sub>2</sub> partial pressure was always limited by RuBP carboxylation.

In conclusion, the temperature response of photosynthesis changed in two ways through the season:  $P_{\max}$  and its optimal temperature both increased with increasing ambient temperature. Changes in  $E_{aV}$  regulated the optimal temperature, whereas changes in  $V_{\max}$  regulated  $P_{\max}$ . These changes may contribute to the increase in photosynthetic production at the respective growth environment. It was also suggested that some characteristics related to the temperature-response curve of photosynthesis were age-dependent, thus temperature dependence of photosynthesis may not be a simple function of growth temperature. Further studies are needed to obtain a comprehensive understanding of the temperature response of photosynthesis in canopy leaves.

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