Is the parameter electron transport rate useful as a predictor of photosynthetic carbon assimilation rate?

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Abstract

We compared the photosynthetic carbon assimilation rate \( A \) and the simultaneously measured electron transport rate (ETR) through photosystem II in order to examine the reliability of the \( A \) estimation method based on the ETR values and \( A \)-ETR relationships under field conditions. We first compared the ETR with the \( A \) values, which were calculated using biochemical models, under different temperature conditions typical in August, November and February. We established the regression lines of \( A \) with ETR values at reference conditions (mean leaf temperature and leaf intercellular CO\(_2\) partial pressure, \( C_i \), in each season). When leaf temperatures and \( C_i \) changed from the reference conditions according to diurnal changes in the environment, the relationship between \( A \) and ETR changed from the regression lines, but the error of the estimated \( A \) values was modest in each season's biochemical calculations. The correlation of \( A \) with the ETR values derived from the data in the field measurements was, however, too weak to precisely regress the \( A \) values based on the ETR values. This weak correlation would be due to the low leaf internal conductance in the measured leaves and large differences in physiological traits, such as temperature dependence, among the leaves. Thus, we concluded that in a field with spatially and temporally heterogeneous environmental conditions, \( A \) estimation based on the ETR values would be difficult.

Keywords: Electron transport rate, photosynthetic carbon assimilation rates, estimated \( A \) value, biochemical model, field-measured \( A \)-ETR relation

Introduction

Photosynthetic traits differ among leaves of different species, light environments and leaf age. Within a crown, a plant arranges leaves with high photosynthetic capacity under sunny conditions and leaves with low photosynthetic capacity under shaded conditions in order to attain maximal carbon gain by using a limited amount of resources, particularly nitrogen (Field, 1983; Hirose and Werger, 1987). Since the distribution of leaves with different photosynthetic traits along the light gradient strongly influences the crown-level carbon gain, we need to understand the photosynthetic traits of leaves within a crown in order to comprehend plant growth and plant performance for increasing carbon gain.

There are two methods for the investigation of photosynthetic rates and photosynthetic capacity. One involves the use of a gas-exchange system for the measurement of the light-saturated rate of the net

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photosynthetic carbon assimilation per unit leaf area \( (A) \). The value of \( A \) has been used as a parameter for the photosynthetic rate; further, \( A \) is known to be correlated with the relative growth rate (Lusk and Del Pozo, 2002) and coincides with the successional stage of a species (Koike, 1990). The other method uses a chlorophyll fluorescence measurement system (Genty et al., 1989) and measures the electron transport rate (ETR) at light saturation, which is also called the photosynthetic capacity. In many research institutions, researchers use the chlorophyll fluorescence measuring system and measure the ETR in order to express the photosynthetic rates and the physiological traits of the focal leaves. In this method, the value of \( A \) is estimated based on the ETR value and the regression equation of \( A \) with the ETR values.

There are, however, problems in the estimation of values of \( A \) using ETR values and the relationship between \( A \) and the ETR value because the \( A \)-ETR relationship is thought to change under different conditions. Under a light intensity that is lower than the light compensation point required for carbon assimilation, ETR values are positive, while \( A \) values are negative. The transported electron is not only utilized for ribulose biphosphate (RuBP) carboxylation and oxygenation but also for cyclic electron transport and the water-water cycle (Asada, 1999; Farage et al., 2006). In these cases, ETR values would not precisely represent \( A \) \((= \text{RuBP carboxylation rate} – 0.5 \times \text{RuBP oxygenation rate})\). Further, because the ratio of RuBP carboxylation and RuBP oxygenation changes with leaf temperature and the partial pressure of CO\(_2\) at the reaction site (chloroplast CO\(_2\) concentration and intercellular CO\(_2\) concentration, \( C_i \)), leaves having the same ETR value would have various \( A \) values due to their different leaf temperatures or \( C_i \) values (Farquhar et al., 1980). If the relationship between \( A \) values and ETR values changes in response to environmental changes (i.e. leaves with the same ETR value have greatly different \( A \) values under different environmental conditions), the estimation of the \( A \) value using the ETR value would be difficult in field measurements where leaves experience different environmental conditions both within a day and within a year. In order to examine the reliability of the estimation of \( A \) values using ETR values, we should first review the relationship between ETR and \( A \) values by using a biochemical model and establish a theoretical regression equation of \( A \) values with ETR values. Further, we should evaluate any possible errors in the estimated \( A \) values due to changes in leaf temperature and \( C_i \), within their range of the daily and seasonal course.

In this study, we examined the relationship between ETR values and \( A \) values under various environmental conditions in the field. We carried out the following researches: (1) review of the regression equation of \( A \) values with ETR values for the estimation of \( A \) values, based on previous biochemical models; (2) evaluation of the error in the estimated \( A \) values by comparison with the actual \( A \) values due to the changes in leaf temperature and \( C_i \) conditions; and (3) comparison of ETR values with \( A \) values by using the in vivo data measured under a wide range of leaf temperatures and \( C_i \) conditions in the field. Finally, we addressed the reliability of \( A \) values and the estimation of photosynthetic capacity by using ETR values in field measurements.
Materials and methods

**Calculation of ETR and A values using biochemical models**

In this study, we assumed that the rate of electron transport (J, μmol·m⁻²·s⁻¹) required for RuBP regeneration and photosynthesis reactions equalled the value of ETR (i.e., ETR = J). We further assumed that the number of electrons transported during the water-water cycle (Asada 1999) and cyclic electron transport would be negligible in the calculation of A-ETR relations. In addition, we assumed that RuBP is saturated at light saturation in ambient CO₂ and temperature conditions, similar to previous studies (Hikosaka *et al*., 1999; Miyazawa and Kikuzawa, 2006b). The value of J is calculated using the biochemical model of Farquhar *et al*., (1980) when the leaf assimilates CO₂ at the rate A (μmol·m⁻²·s⁻¹);

\[
J = \text{ETR} = (A + R_d) \left(4C_i + 8\Gamma^* \right) / \left(C_i - \Gamma^* \right)
\]

where \(C_i\) is the pCO₂ of the intercellular space of the leaf (Pa), \(\Gamma^*\) is the CO₂ compensation point (Pa) and \(R_d\) is the day respiration rate (μmol·m⁻²·s⁻¹). In this study, we assumed infinite internal CO₂ conductance (see Epron *et al*., 1995 and Niinemets *et al*., 2006).

Under warm conditions at 25 °C, A is described as the maximum capacity of the leaf for RuBP carboxylation at a leaf temperature of 25 °C; it is denoted by \(V_{\text{cmax}}^{25}\) (Farquhar *et al*., 1980);

\[
A = V_{\text{cmax}}^{25} \left(C_i - \Gamma^* \right) / (C_i + K_c / (1 + O / K_o) - R_d)
\]

where \(K_c\) and \(K_o\) are the Michaelis-Menten constants for the enzyme Rubisco, and \(O\) is the partial pressure of O₂ in ambient air (21000 Pa). The values of the parameters of Equations [1] and [2] are shown in Table 1.

The values of the parameters in Equations [1] and [2] change with leaf temperature, and we assumed that these parameters change with the following equation:

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

where \(P(T)\) is \(K_c, K_o\) or \(\tau (\Gamma^* = O/2\tau)\), and \(P^{(25)}\) is the value at a leaf temperature of 25 °C. To determine the temperature dependency of the rate of RuBP carboxylation (\(V_{\text{cmax}}\)), we used the following equation (Harley *et al*., 1992b):

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**Table 1.** List of Michaelis-Menten constants, the specific factor of Rubisco and the activation energy used in the Equations [1], [2] and [3].

<table>
<thead>
<tr>
<th></th>
<th>unit</th>
<th>Values</th>
<th>(E_a) (J mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_c) (25°C)</td>
<td>μmol mol⁻¹</td>
<td>404*</td>
<td>80500†</td>
</tr>
<tr>
<td>(K_o) (25°C)</td>
<td>mol mol⁻¹</td>
<td>248*</td>
<td>14500†</td>
</tr>
<tr>
<td>(\tau) (25°C)</td>
<td></td>
<td>2846*</td>
<td>−29000†</td>
</tr>
</tbody>
</table>

*: von Caemmerer *et al*., (1994); †: Harley *et al*., (1992b)
$V_{\text{cmax}}(T) = \frac{V_{\text{cmax}}^{(25)} \exp \left( \frac{E_a}{298.15 R} \left( 1 - \frac{298.15}{T} \right) \right)}{1 + \exp \left( \frac{\Delta S - H_d}{RT} \right)} \left[ 1 + \exp \left( \frac{298.15 \Delta S - H_d}{298.15 R} \right) \right] [4]$ 

where $H_d$ is the deactivation enthalpy and $\Delta S$ is the entropy term. We used the values of $E_a$, $H_d$ and $\Delta S$ of RuBP carboxylation in previous studies, and these values are listed in Table 2.

We first calculated the $A$ and ETR values under the ‘reference conditions’ ($C_i = 21$ Pa and leaf temperature = the average value in daytime in each measurement season) in August, November and February. We then calculated the $A$ and ETR values for the hypothetical leaves with the photosynthetic capacity $V_{\text{cmax}}^{25} = 10, 30, 50$ and $70 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, under temperature conditions typical in August, November and February (leaf temperature = 7–15 °C, 13–27 °C and 25–37 °C, respectively) and $C_i = 15–26$ Pa, based on previous studies (Miyazawa and Kikuzawa 2006a). We calculated the values in order to evaluate the error due to a shift in the measurement conditions from the reference conditions.

**Evaluation of the error between the estimated photosynthetic parameters and the actual values by using biochemical models**

We established the regression lines of $A$ with ETR values by using Equation [1] for the reference conditions in August, November and February based on the data in a previous study (Miyazawa and Kikuzawa 2006a). We calculated the estimated $A$ value ($a$, $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) of a leaf based on the in situ measured ETR value of the leaf and the regression lines of $A$ with ETR values under the conditions in each season. Similarly, we established the regression equation of the photosynthetic capacity, $V_{\text{cmax}}^{25}$ with ETR values. In this analysis, we obtained the estimated $V_{\text{cmax}}^{25}$ values ($V_{\text{cmax}}^{25}$), which were calculated based on the regression equation and the in situ measured ETR values. We then calculated the ETR values and $A$ values at $C_i$ and leaf temperature conditions different from the reference conditions, within the range of the daily courses of $C_i$ and leaf temperatures. Under the specified conditions, we calculated the error of the $a$ value from the $A$ value ($A - a$) in order to evaluate the reliability of $a$ and this estimation method. Under conditions different from the reference ones, we also calculated the error of $V_{\text{cmax}}^{25}$ from $V_{\text{cmax}}^{25}$ ($V_{\text{cmax}}^{25} - V_{\text{cmax}}^{25}$) and evaluated the reliability of the $V_{\text{cmax}}^{25}$ estimation method.

**Table 2.** List of Michaelis-Menten constants, the specific factor of Rubisco and the activation energy used in the Equation [4].

<table>
<thead>
<tr>
<th></th>
<th>unit</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{cmax}}$</td>
<td>$\mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$</td>
<td>10 - 50</td>
</tr>
<tr>
<td>$H_a$</td>
<td>KJ mol$^{-1}$</td>
<td>60.8</td>
</tr>
<tr>
<td>$H_{d_i}$</td>
<td>KJ mol$^{-1}$</td>
<td>401</td>
</tr>
<tr>
<td>$\Delta S$</td>
<td>JK mol$^{-1}$</td>
<td>1285</td>
</tr>
</tbody>
</table>

‡: Dreyer et al. (2001)
Fig. 1. Intercellular CO partial pressure ($C_i$, Pa) and leaf temperatures of the light-saturated leaves of saplings of *Quercus glauca* in Kamigamo Experimental Forest, Kyoto, Japan in (a) August, (b) November and (c) February.
**Field measurements**

In order to examine the range of leaf temperatures and $C_i$ in the field, we used the data of a previous study (Miyazawa and Kikuzawa, 2006a) in order to determine the daily courses of the photosynthetic rates of saplings of *Quercus glauca* Thunb, measuring 1.5 m in height, in summer (August), autumn (October) and winter (February). Saplings were planted along logging roads in Kamigamo Experimental Forest of Kyoto University, Kyoto, Japan (35°04'N, 13°43'E). The mean annual temperature from 1971 to 2001 was 14.6 °C, with temperatures being the highest in August (31.6 °C) and the lowest in January (−0.9 °C). The mean annual precipitation was 1582 mm. The details of the climate and vegetation of the study site are described elsewhere (Miyazawa and Kikuzawa, 2005). We selected 10 – 20 leaves for each measurement. In the course of the photosynthesis measurement, we measured the leaf temperature and transpiration rate for the calculation of the stomatal conductance of H$_2$O ($g_s$, mol·m$^{-2}$·s$^{-1}$) and $C_i$. We measured the photosynthetic rates using a well-calibrated Li-6400 portable photosynthesis system (Li-cor, Lincoln, NE, USA). Leaves were kept in a cuvette with the light source supplying saturating light.

In order to examine the relationship between $A$ values and ETR values, we simultaneously measured ETR values during the photosynthesis measurement by using the chlorophyll fluorescence measurement system Li-6400-40 (Li-cor).

**Statistical analysis**

We examined the correlation of ETR values with the simultaneously measured $A$ values using the field-measured data. In the calculation of ETR and $A$ values based on the biochemical models, we calculated the error in the estimation as $(A - a)$ and $(V_{cmax}^{25} - v_{cmax}^{25})$ for each leaf temperature and $C_i$ condition in order to examine the error due to the differences in the measurement conditions from the reference conditions between $a$ and $v_{cmax}^{25}$ and $A$ and $V_{cmax}^{25}$, respectively.

**Results**

In the daytime, the $C_i$ at light saturation changed and was different among leaves; however, the difference was within the range calculated in previous studies (Fig. 1). The average $C_i$ value was approximately 21 Pa. We did not find significant differences between the average values and the range of $C_i$ values obtained from the data of each measurement season. The data included those obtained from leaves with different values of photosynthetic capacity $V_{cmax}^{25}$ (e.g. the initial slope of the $A - C_i$ relation), but no trend was observed in the values of $C_i$ among the leaves. Leaf temperatures changed with the seasons and showed similar average values as those obtained in previous studies (Fig. 1). However, they showed a narrower range in daily variations with regard to the average values compared with previous studies, in which photosynthetic rates were measured while maintaining the environmental conditions of the leaf cuvette similar to ambient ones.

In biochemical simulations, the maximum rates of RuBP carboxylation and electron transport ($V_{cmax}$ and $J_{max}$, respectively) increased with leaf temperatures until they reached their peak values (Fig. 2). Since the specific factor of Rubisco to CO$_2$ decreased with leaf temperature, the light-saturated rate of
Fig. 2. Temperature dependencies of photosynthetic parameters: the maximum rates of RuBP carboxylation and electron transport ($V_{\text{cmax}}$ and $J_{\text{max}}$, $\mu$mol·m$^{-2}$·s$^{-1}$, respectively), the specificity factor of Rubisco for CO$_2$ ($\tau$), the light-saturated rate of net photosynthesis ($A$, $\mu$mol·m$^{-2}$·s$^{-1}$) and the electron transport rate calculated based on chlorophyll fluorescence data (ETR, $\mu$mol·m$^{-2}$·s$^{-1}$) in this study.

Is electron transport rate useful for predicting carbon assimilation rate?
Fig. 3. The relationship between $A$ values and ETR values calculated using the biochemical models under various leaf temperature conditions typical in (a) August, (b) November and (c) February. The lines indicate the regression line of the $A$-ETR relationships at ‘reference conditions’ (leaf temperature = the average values in each season and $C_i$ = 21 Pa). The symbols in each figure represent the data of leaves with the same photosynthetic capacity ($V_{cmax}$). The points of each symbol represent the ETR and $A$ values at seven steps of the measurement of the leaf temperatures under the specified range in August, November and February (leaf temperature = 7–15 °C, 13–27 °C and 25–37 °C, respectively). The data at higher leaf temperatures are arranged in the direction of the arrows.
Is electron transport rate useful for predicting carbon assimilation rate?

Fig. 4. Errors between the estimated $A$ values (a) and the actual $A$ values ($\hat{A}$) for the range of leaf temperatures in (a) August, (b) November and (c) February. Each symbol represents the data of leaves with different $V_{\text{cmax}}$. 

(a) 

(b) 

(c)
net photosynthesis \( (A, \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}) \) reached a peak at lower leaf temperatures than \( V_{\text{cmax}} \) and \( J_{\text{max}} \), and decreased with higher leaf temperatures. The ETR \( (\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}) \), which was calculated based on biochemical models, was similarly dependent on temperature.

**The regression lines of \( A \) and \( V_{\text{cmax}}^{25} \) with ETR under different leaf temperature conditions in a biochemical simulation**

The values of \( A \) for leaves with different values of photosynthetic capacity \( (V_{\text{cmax}}^{25}) \) were positively correlated with the ETR values under various temperature conditions; however, the regression line of \( A \) with the ETR values differed from season to season due to differences in the leaf temperature (Fig. 3). The regression line well represented the \( A \)-ETR relationship when data were collected under the temperature conditions in November. In each season, leaves with different leaf temperatures had ETR and \( A \) values that differed from the values obtained from the regression line, which were determined for the mean leaf temperature in each season. Data at low leaf temperature had higher \( A/\text{ETR} \) ratio, and those at high leaf temperature had lower \( A/\text{ETR} \) ratio than \( A/\text{ETR} \) ratio of the regression line.

The error in the estimated \( A \) values \((a)\) increased as the leaf temperatures increased or decreased from the reference leaf temperatures at which the regression line was established in each season (Fig. 4). Similarly, the difference between \( V_{\text{cmax}}^{25} \) and the estimated one \( (v_{\text{cmax}}^{25}) \) was large at low and high temperatures, and the error was larger than the error in the estimation of \( A_{\text{max}} \) (Fig. 4). The error between the \( v_{\text{cmax}}^{25} \) and the \( V_{\text{cmax}}^{25} \) values increased as leaf temperatures increased or decreased from the reference leaf temperatures. The error between the values of \( a \) and \( v_{\text{cmax}}^{25} \) was high in leaves with a high photosynthetic capacity than in leaves with a low photosynthetic capacity.

**The regression lines of ETR with \( A \) and \( V_{\text{cmax}}^{25} \) under different leaf temperature conditions**

The estimated \( A \) values \((a)\) differed greatly from the actual \( A \) values as the \( C_i \) (Pa) of leaves increased or decreased from the leaf temperatures considered for the establishment of the regression line. Further, this difference was high in August and January (Fig. 5), but the error was modest. As \( C_i \) decreased or increased from the reference \( C_i \) value considered for the regression line of ETR with \( A \), the error between \( a \) and \( v_{\text{cmax}}^{25} \) increased (Fig. 6). High errors were observed in the value of \( a \) in leaves with a high photosynthetic capacity.

**Comparison of ETR values with \( A \) values measured in the field**

The values of \( A \) were positively correlated with the ETR values, but the estimation of \( A \) values by using ETR values was difficult due to the large variations in the ETR values (Fig. 7). In each season, leaves had \( A \) values lower than the \( a \) values of the regression line of the biochemical models, and the difference was high in leaves with high \( A \) values. When data were grouped based on the measurement of leaf temperatures, we could not obtain clear differences in the \( A \)-ETR relationship among leaves in August and November. In January, the data at lower leaf temperatures had a steeper slope (higher \( A/\text{ETR} \) values) than the data collected at higher leaf temperatures (lower \( A/\text{ETR} \) values).
Fig. 5. The relationship between $A$ values and ETR values calculated using the biochemical models under various leaf $C_i$ conditions and mean leaf temperature in (a) August, (b) November and (c) February (9 °C, 26 °C and 31 °C, respectively). The lines indicate the regression line of the $A$-ETR relationship at ‘reference conditions’. Symbols in each figure represent the data of the leaves with the same $V_{c_{\text{max}}}$. The points of each symbol represent the ETR and $A$ values at eight steps of the measurement of $C_i$ under the range $15 \text{ Pa} < C_i < 26 \text{ Pa}$. The data at higher $C_i$ are arranged in the direction of the arrows.

Is electron transport rate useful for predicting carbon assimilation rate?
Fig. 6. Errors between the estimated $A$ values (a) and the real $A$ values for the range of $C_i$ values and the mean leaf temperatures in (a) August, (b) November and (c) February. Each symbol represents the data of leaves with different $V_{\text{cmax}}$.
Discussion

Although a positive correlation was observed between ETR values and $A$ and $V_{\text{max}}^{25}$ in model simulations based on several assumptions (e.g. infinite intercellular CO$_2$ conductance and the temperature dependence of photosynthetic parameters as determined by previous studies) (Figs. 3 and 5), we found that the estimation of $A$ values and $V_{\text{max}}^{25}$ values based on ETR values would be difficult during field measurement. Model simulations revealed that daily and seasonal variations in $C_i$ values and leaf temperatures caused errors in the estimation of $A$ values using ETR values ($A - a$ and $V_{\text{max}}^{25} - V_{\text{max}}^{25}$) (Fig. 3), but the variation observed in the field measurement was much higher than the degree of the expected error. In November and August, the differences in the $A$ values among leaves having the same ETR value were higher than the leaf-to-leaf variation in $A$ values in previous studies that described the interspecific differences in $A$ values among species of different successional stages (Koike, 1990) and among leaves under different light environments within a crown (Ellsworth and Reich, 1993).

$A$ values lower than the $a$ values which were calculated using ETR values and the biochemical models indicated several physiological backgrounds. First, certain electrons, which were transported through the thylakoid membrane, would not be utilized for RuBP carboxylation and oxygenation. The assumption $J = \text{ETR}$ in Equation [1] assumes the utilization of all the transported electrons; however, several studies revealed passes for the transported electrons other than RuBP carboxylation and oxygenation, such as the water-water cycle (Asada, 1999) and cyclic electron transport (Farage et al., 2006). Second, leaf internal conductance would not be infinite in *Q. glauca*, in contrast to our assumption. If the internal conductance was low, more electrons would be utilized for RuBP oxygenation due to lower CO$_2$ concentration at the site of RuBP carboxylation and oxygenation than $C_i$ (Harley et al., 1992a). In this case, $A$ would be lower in the leaf with infinite internal conductance, under similar levels of electron transport for RuBP carboxylation and oxygenation. Previous studies revealed low internal conductance.

![Fig. 7](image-url)

**Fig. 7.** $A$ values and the simultaneously measured ETR values in the field in (a) August, (b) November and (c) February. The lines represent the regression line of the $A$-ETR relationship at reference conditions in the seasons, based on the biochemical models. The symbols represent the data collected at different dates under different temperature conditions (high: closed circles, medium: closed triangles and low: open circles).
for sclerophyll species (Hanba et al., 1999; Miyazawa and Terashima, 2001). Because internal conductance greatly differs among leaves of different ages or under different light environments (Niinemets et al., 2006), the application of Equation [1] to the leaves in the field would be a less reliable method for $A$ estimation.

Thus, the estimation of photosynthetic traits using the chlorophyll fluorescence measurement system would be a useful method only in the comparison of photosynthesis among leaves with similar physiological traits, such as the intraspecific comparison of leaves of different ages or the interspecific comparison of leaves under the same light environments. Moreover, the simultaneous measurement of ETR and $A$ would allow us to investigate other physiological traits, including CO$_2$ concentration in chloroplasts and the evaluation of the limitation of leaf internal conductance on carbon assimilation (Harley et al., 1992a).

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References


Koike, T. (1990) Autumn coloring, photosynthetic performance and leaf development of deciduous broad-leaved trees in...
relation to forest succession. Tree Physiol. 7: 21–32.
Miyazawa, Y. and K. Kikuzawa (2006b) Physiological basis of seasonal trend in leaf photosynthesis of five evergreen broad-leaved species in a temperate deciduous forest. Tree Physiol. 26: 249–256.