Leaf Respiration of Snow Gum in the Light and Dark. Interactions between Temperature and Irradiance\(^1\)

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We investigated the effect of temperature and irradiance on leaf respiration (\(R\), non-photorespiratory mitochondrial \(\text{CO}_2\) release) of snow gum (\(Eucalyptus pauciflora\) Sieb. ex Spreng). Seedlings were hydroponically grown under constant 20°C, controlled-environment conditions. Measurements of \(R\) (using the Laisk method) and photosynthesis (at 37 \(\text{Pa CO}_2\)) were made at several irradiances (0–2,000 \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\)) and temperatures (6°C–30°C). At 15°C to 30°C, substantial inhibition of \(R\) occurred at 12 \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\), with maximum inhibition occurring at 100 to 200 \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\). Higher irradiance had little additional effect on \(R\) at these moderate temperatures. The irradiance necessary to maximally inhibit \(R\) at 6°C to 10°C was lower than that at 15°C to 30°C. Moreover, although \(R\) was inhibited by low irradiance at 6°C to 10°C, it recovered with progressive increases in irradiance. The temperature sensitivity of \(R\) was greater in darkness than under bright light. At 30°C and high irradiance, light-inhibited rates of \(R\) represented 2% of gross \(\text{CO}_2\) uptake (\(\nu_s\)), whereas photorespiratory \(\text{CO}_2\) release was approximately 20% of \(\nu_s\). If light had not inhibited leaf respiration at 30°C and high irradiance, \(R\) would have represented 11% of \(\nu_s\). Variations in light inhibition of \(R\) can therefore have a substantial impact on the proportion of photosynthesis that is respired. We conclude that the rate of \(R\) in the light is highly variable, being dependent on irradiance and temperature.

Leaf respiration provides ATP, reducing equivalents, and carbon skeletons necessary for biosynthetic reactions. Leaf respiration may also help protect the photosynthetic apparatus from photoinhibitory damage by oxidizing excess photosynthetic reducing equivalents (Raghavendra et al., 1994; Saradadevi and Raghavendra, 1994; Hurry et al., 1995; Atkin et al., 2000b). Moreover, leaf respiration can provide ATP for Suc synthesis (Krömer, 1995) and may help repair photosynthetic proteins degraded by photoinhibition (in particular, the D1 protein of photosystem II) (Hoefnagel et al., 1998, and refs. therein). Leaf respiration is therefore a vital component of plant metabolism. However, leaf respiration also represents a major source of \(\text{CO}_2\) release in plants. Up to 35% of the \(\text{CO}_2\) fixed by photosynthesis each day is released back into the atmosphere by leaf respiration in plants grown under controlled-environment, constant-temperature conditions (Van Der Werf et al., 1994; Atkin and Lambers, 1998). Variations in the magnitude of leaf respiration could therefore have an important impact on the carbon economy of a plant.

While leaf respiration (\(R\), non-photorespiratory mitochondrial \(\text{CO}_2\) release) occurs both in the light and in darkness, the extent to which it continues in the light appears to be highly variable. Most studies have reported that the rate of leaf respiration in the light (\(R_l\) or day respiration) is less than that in darkness (\(R_d\) or night respiration) (Brooks and Farquhar, 1985; Avelange et al., 1991; Krömer, 1995; Atkin et al., 1997, 1998a, 1998b), with the degree of inhibition ranging from 16% to 77%. The inhibition of \(R\) by light is rapid (within approximately 50 s) and occurs at irradiances as low as 3 \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\) (Atkin et al., 1998a).

Most studies that have investigated the degree to which \(R\) is inhibited by light have done so at a single temperature (typically 25°C). In their natural habitat, plants are exposed to large temperature fluctuations, with leaf temperatures during the day often being 20°C to 30°C higher than those at night. It is not clear, however, if the degree of light inhibition is constant across a wide range of temperatures. Although Brooks and Farquhar (1985) reported that variations in temperature did not affect the degree of inhibition, they did not determine respiratory flux in the light at temperatures below 15°C. It is also not known if the effect of light on \(R\) at each temperature varies with irradiance; exposure to low temperatures and bright light may well have very different effects on \(R\) than exposure to low temperatures at low irradiance, particularly if mitochondrial oxidize excess photosynthetic reducing equivalents under cold, bright conditions (Raghavendra et al., 1994; Saradadevi and Raghavendra, 1994; Hurry et al., 1995; Atkin et al., 2000a). To fully elucidate the degree to which respiration continues in the light, we need to determine the effect of temperature and irradiance on leaf respiration.

Our study investigates the interactive effects of temperature and irradiance on leaf respiration in snow gum (\(Eucalyptus pauciflor\)....)
*Eucalyptus pauciflora* Sieb. ex Spreng). We used the Laisk (1977, as extended by Brooks and Farquhar, 1985) method to obtain estimates of $R_d$ at each temperature and irradiance. The study also determines the impact of temperature/irradiance induced variations in $R_d$ on net CO2 uptake in the light. Our results indicate that the degree of inhibition of $R$ varies with both temperature and irradiance. The temperature sensitivity of leaf respiration at high irradiance is substantially lower than in darkness. Moreover, in leaves exposed to high temperatures, variations in the degree of light inhibition play an important role in determining the proportion of gross photosynthetic CO2 uptake that is respired.

**MATERIALS AND METHODS**

Snow gum (*Eucalyptus pauciflora* Sieb. ex Spreng) seedlings were raised from seed from a population collected in Gudgenby Valley in Namadgi National Park in southeastern Australia (35°45'S/148°59'E). The seeds were transported to Utrecht University in the Netherlands, vernalized at 4°C for 4 weeks, and then germinated on seed trays under controlled-environment conditions (constant 20°C temperature; 14 h/10 h day/night rhythm; 520 μmol photons m$^{-2}$ s$^{-1}$ photosynthetically active radiation [PAR]; 70% relative humidity). Germinants were transplanted 6 weeks later to 32-L hydroponics tanks containing a fully aerated modified Hoagland nutrient solution. Full details on the growth conditions and nutrient solution are given in Atkin et al. (1996). The seedlings were grown for a further 10 to 14 weeks. The plants reached a height of approximately 0.3 m.

Measurements of CO2 uptake and release in intact, attached leaves were conducted using an IR gas analyzer (LI-6262, LI-COR, Lincoln, NE) in the differential mode in an open system (Keithley 575, Cleveland) and measured simultaneously. Air in each chamber was mixed with a fan, which resulted in boundary layer conductances of approximately 6 to 10 mmol m$^{-2}$ s$^{-1}$. Different light intensities were obtained by placing small-mesh wire netting filters in front of slide projector lamps mounted above each cuvette (Atkin et al., 1997). Leaf temperatures were measured using two 0.08-mm type K thermocouples per cuvette, which were appressed to the underside of the leaves. Temperature was controlled by a thermostat-controlled circulating water bath. Water vapor pressure and CO2 partial pressures were controlled as previously described (Atkin et al., 1997). Gas-exchange parameters were calculated according to the method of von Caemmerer and Farquhar (1981).

Determinations of leaf gas exchange commenced after at least 2 h of photosynthesis in the growth cabinets. One of the labeled leaves on each of the three 20°C-grown plants was inserted into each temperature-controlled leaf chamber of the gas exchange system. Each of the three leaves was then allowed to equilibrate for 30 min, during which time they were exposed to a moderate irradiance (400 μmol photons m$^{-2}$ s$^{-1}$ PAR). The leaves were then exposed to a range of irradiances (0, 12, 100, 200, 400, 800, and finally 2,000 μmol photons m$^{-2}$ s$^{-1}$ PAR), and then left to adjust for 15 to 20 min at each new irradiance before the CO2 response was measured. The first measurements of $R_d$ were conducted after 30 min of darkness; it takes 10 to 25 min for post-illumination respiration to stabilize in snow gum, with the time increasing with decreasing temperature (Atkin et al., 1998b). At each irradiance, net CO2 exchange rates were measured at four to eight decreasing internal CO2 partial pressure ($p_i$) values (in the range of approximately 10–2.5 Pa CO2).

Leaves were then exposed to an atmospheric CO2 partial pressure of 37 Pa and the rate of net CO2 exchange determined. A linear regression of net CO2 exchange versus $p_i$ for the low CO2 partial pressure range (10–2.5 Pa) was then calculated for each irradiance. The point at which three regressions intersected was used to determine $\Gamma$, whenever possible. $\Gamma$ is the $p_i$ where CO2 uptake by carboxylation is matched by photorespiratory CO2 release, and where the rate of CO2 release is $R_d$ (Laisk, 1977). In our study, the three linear regressions that were used to calculate the $\Gamma$ values were taken from leaves exposed to 100, 200, and 400 μmol photons m$^{-2}$ s$^{-1}$ for 6°C, 10°C, 15°C, 20°C, and 25°C. At 6°C and 10°C, the point at which the three regressions intersected yielded negative respiration values, i.e. CO2 uptake. $\Gamma$ could not, therefore, be determined at 6°C and 10°C. At 30°C, 200, 400, and 800 μmol photons m$^{-2}$ s$^{-1}$ data were used, as $R_d$ was not constant until 200 μmol photons m$^{-2}$ s$^{-1}$. An assumption underlying the Laisk (1977) method is that $R$ does not change with irradiance.

The above measurements were conducted at a single temperature on each measuring day, after which time the plants were returned to the controlled-environment growth cabinet. The measurement procedure was then repeated on the next day at a new temperature. The sequence of measurement temperatures was 25°C, 6°C, 30°C, 10°C, 20°C, and 15°C. Checks of gas exchange characteristics were made after the 3rd and 6th measuring day by measuring gas exchange at a common temperature (25°C); exposure to the different temperatures did not have any significant effect on the rates of respiration in darkness or the light-saturated rate of net photosynthesis at 25°C (data not shown).

The rate of leaf respiration in the light at each measurement temperature and irradiance was determined using the regressions for the net CO2 exchange versus $p_i$ over the low CO2 partial pressure range (see above). $R_d$ was taken as the rate of CO2 efflux at $\Gamma$. Rates of carboxylatory CO2 uptake ($v_c$) and photorespiratory CO2 release (i.e. 0.5$v_o$) were calculated according to the method of Farquhar and von Caemmerer (1982):

\[
v_c = (A_{max} + R_d) / [1 - (\Gamma / p_i)]
\]

(1)

and

\[
0.5v_o = 0.5 \times [v_c(2\Gamma / p_i)]
\]

(2)

where $A_{max}$ is the rate of net photosynthetic CO2 uptake in the presence of an atmospheric CO2 partial pressure of 37 Pa (von Caemmerer and Farquhar, 1981). Data from the CO2-response curves under light saturation were used to calculate $V_{cmax}$ values according to the method of von
Caemmerer and Farquhar (1981) using Michaelis-Menten constants for CO₂ and O₂ reported by von Caemmerer et al. (1994). \( V_{cmax} \) was calculated under the assumption that at low \( p_i \), photosynthesis was limited by Rubisco only.

The impact of CO₂ partial pressure and temperature on leaf respiration rates measured in darkness was assessed using a two-way analysis of variance (Zar, 1996).

**RESULTS**

Figure 1 shows an example of the net CO₂ exchange over the \( p_i \) range of 3 to 10 Pa at several irradiances for a single leaf exposed to three temperatures (6°C, 15°C, and 25°C). Similar results were observed for the other three temperatures (10°C, 20°C, and 30°C; data not shown). The response

![Figure 1](image)

**Figure 1.** Example of the effect of irradiance on net CO₂ exchange (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \)) versus \( p_i \) of a single leaf at three temperatures: 6°C (A), 15°C (B), and 25°C (C). Measurements were also conducted at 10°C, 20°C, and 30°C (not shown). The symbols represent the irradiances under which each set of measurements was made (in \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \)). Lines represent the linear regressions at each irradiance.

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<tr>
<th>Temperature (°C)</th>
<th>( V_{cmax} )</th>
<th>( R_n )</th>
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<tr>
<td>6</td>
<td>21.1 ± 3.5</td>
<td>0.24 ± 0.03</td>
<td>0.44 ± 0.04</td>
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<td>10</td>
<td>27.5 ± 4.6</td>
<td>0.37 ± 0.03</td>
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<td>15</td>
<td>47.6 ± 11.3</td>
<td>0.64 ± 0.07</td>
<td>0.74 ± 0.08</td>
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<td>20</td>
<td>62.7 ± 13.6</td>
<td>1.06 ± 0.03</td>
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<td>25</td>
<td>72.6 ± 2.0</td>
<td>1.61 ± 0.11</td>
<td>1.72 ± 0.13</td>
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<td>30</td>
<td>105.6 ± 2.04</td>
<td>2.48 ± 0.21</td>
<td>2.67 ± 0.04</td>
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The \( V_{cmax} \) values were estimated from fitted CO₂-response curves similar to those shown in Figure 1 for measurements done at 2,000 \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \). \( V_{cmax} \) values for each temperature were calculated according to the method of Von Caemmerer and Farquhar (1981), using data from the CO₂-response curves (e.g. Fig. 1) and the Michaelis-Menten constants for CO₂ and O₂ described in the method of Von Caemmerer et al. (1994). The \( G* \) used in these calculations was 4.31 Pa (see “Results”). \( V_{cmax} \) was calculated under the assumption that at the low \( p_i \) values shown in Figure 1, photosynthesis was limited by Rubisco only. Values are means of three replicate measurements (±SE).

**Table 1.** Effect of temperature on maximum carboxylation rates \( (V_{cmax}) \) and \( R_n \) measured at ambient atmospheric CO₂ partial pressure \( (p_i) \) of 37 Pa and at a low (4–5 Pa) \( p_i \). The \( V_{cmax} \) values were estimated from fitted CO₂-response curves similar to those shown in Figure 1 for measurements done at 2,000 \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \). \( V_{cmax} \) values for each temperature were calculated according to the method of Von Caemmerer and Farquhar (1981), using data from the CO₂-response curves (e.g. Fig. 1) and the Michaelis-Menten constants for CO₂ and O₂ described in the method of Von Caemmerer et al. (1994). The \( G* \) used in these calculations was 4.31 Pa (see “Results”). \( V_{cmax} \) was calculated under the assumption that at the low \( p_i \) values shown in Figure 1, photosynthesis was limited by Rubisco only. Values are means of three replicate measurements (±SE).

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**Figure 2.** Effect of temperature on \( \Gamma \), \( \bar{\Gamma} \), \( \bar{\Gamma} \), values calculated using the intercept of three linear regressions of net CO₂ exchange data versus \( p_i \) (e.g. Fig. 1) for leaves of 20°C-grown plants exposed to 15°C, 20°C, 25°C, and 30°C (e.g. Fig. 1, B and C). The three linear regressions used to calculate \( \Gamma \), were for 100, 200, and 400 \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \) for all temperatures except 30°C, where 200, 400, and 800 \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \) were used. Values represent the mean of three individual leaves (±SE); where the SE values are not visible, they are smaller than the shown symbol. The erroneous \( \bar{\Gamma} \), values for leaves exposed to 6°C and 10°C are shown for comparison (●); it was not possible to accurately calculate the \( \bar{\Gamma} \), values at 6°C and 10°C because the common regression intercept for measurements at three irradiances yielded a negative \( R \) value. The solid line represents the temperature dependence of \( \bar{\Gamma} \), of spinach calculated from the data of Jordan and Ogren (1984) using our estimate of \( \bar{\Gamma} \), at 25°C (4.31 ± 0.04 Pa).
at each irradiance was linear for all temperatures over the range of low $p_i$ values (e.g. Fig. 1, A–C). Exposure to very low irradiance (12 $\mu$mol photons m$^{-2}$ s$^{-1}$) resulted in a substantial decrease in the net release of CO$_2$ at all temperatures (relative to darkness), suggesting that leaf respiration was inhibited even by this low irradiance. At 6°C (Fig. 1A), the intersection of the 100, 200, and 400 $\mu$mol photons m$^{-2}$ s$^{-1}$ regressions yielded negative respiration values (i.e. positive net CO$_2$ exchange). Leaf respiration in darkness was significantly greater when measured at low (4–5 Pa) CO$_2$ partial pressure compared with measurements at 37 Pa ($F_{1, 36} = 35.9; P < 0.01; \text{Table I}$).

Figure 2 shows the temperature dependence of our experimentally derived $\Gamma_*$ values over the 15°C to 30°C range where the regressions of the net CO$_2$ exchange versus $p_i$ at three irradiances intersected. The erroneous $\Gamma_*$ values at 6°C and 10°C are shown for comparison. The solid line shows the temperature dependence of $\Gamma_*$ calculated from data of Jordan and Ogren (1984) by Brooks and Farquhar (1985):

$$
\Gamma_T = \Gamma_{25} + [0.188 \times (T - 25)] + [0.0036 \times (T - 25)^{2}] \quad (3)
$$

where $\Gamma_T$ is the $\Gamma_*$ value at a set temperature ($T$) and $\Gamma_{25}$ is $\Gamma_*$ at 25°C. With the exception of 15°C, our $\Gamma_*$ values were almost identical to those predicted by Jordan and Ogren (1984) as long as we used our experimentally derived $\Gamma_{25}$ value (i.e. 4.31 ± 0.04 Pa; $n = 5$; ±SE). Given this match, and the erroneous nature of our $\Gamma_*$ values at 6°C and 10°C (Fig. 2), which yielded negative respiration values, we decided to estimate $R$ values for all temperatures using $\Gamma_*$ values predicted by Equation 3 and our experimentally derived $\Gamma_{25}$ value of 4.31 Pa. Doing so provided positive estimates of $R$ for both 6°C and 10°C cases.

Figure 3 shows the effect of temperature and irradiance on leaf respiration. $R_n$ increased with increasing temperature. At low temperatures, (i.e. 6°C and 10°C; Fig. 3A), $R_n$ was inhibited by low quantum flux density, but then recovered with progressive increases in irradiance. $R_n$ was also inhibited by low irradiance at moderate-to-high temperatures (i.e. 15°C–30°C; Fig. 3, B and C); however, higher irradiance had little additional effect on $R$ at these temperatures. The irradiance necessary to maximally inhibit $R$ increased with increases in leaf temperature (e.g. 12 $\mu$mol photons m$^{-2}$ s$^{-1}$ at 15°C [Fig. 3B] and 400 $\mu$mol photons m$^{-2}$ s$^{-1}$ at 30°C [Fig. 3C]).

Was the apparent irradiance-dependent increase in $R$ at 6°C and 10°C (Fig. 3A) real, or was it the result of errors in the value of $\Gamma_*$? If the $\Gamma_*$ value for snow gum leaves in our system at 6°C were higher than that predicted by Jordan and Ogren (1984), then we would have overestimated the

![Figure 3](image.png)

**Figure 3.** Relationship between $R$ and irradiance at various temperatures. Values are ±SE; $n = 3$. Values of $R$ were calculated using the linear regressions of net CO$_2$ exchange versus $P_i$ at each irradiance (e.g. Fig. 1), our estimate of $\Gamma_{25}$ (4.31 Pa), and the temperature dependence of $\Gamma_*$ given in Equation 3.

![Figure 4](image.png)

**Figure 4.** Determining the effect of different $\Gamma_*$ values on the relationship between $R$ and irradiance at 6°C using the temperature dependence of $\Gamma_*$ given in Equation 3 (±SE $n = 3$). Three different estimates of $\Gamma_*$ at 6°C were used in the calculations.
actual $R$ value at each irradiance. To assess the impact of errors in $G^*$ on our estimates of $R$, we determined the impact of $G^*$ values at 6°C that were 0.3 Pa higher and 0.3 Pa lower (i.e. a 15% change) than that used in our calculations (2.04 Pa) on the irradiance dependence of $R$ at 6°C (Fig. 4). Figure 4 demonstrates that $R$ increased in an irradiance-dependent manner when $G^*$ at 6°C was assumed to be 2.04 or 1.74 Pa. When $G^*$ was assumed to be 2.34 Pa (i.e. $G^*_{25} = 4.61$), little increase in $R$ occurred until 400 μmol photons m$^{-2}$ s$^{-1}$; the $G^*$ value therefore has a substantial impact on the degree to which the calculated rates of $R$ increase with increasing irradiance.

What effect did the interaction of irradiance and temperature have on the temperature response curves of leaf respiration? Figure 5, A and B, shows the temperature response of leaf respiration for leaves exposed to 0, 12, and 100 μmol photons m$^{-2}$ s$^{-1}$ (Fig. 5A) and 200, 400, 800, and 2,000 μmol photons m$^{-2}$ s$^{-1}$ (Fig. 5B). The $Q_{10}$ (the proportional increase in respiration for each 10°C rise in temperature) of $R_n$ was 2.21; a common $Q_{10}$ could be applied over the range of temperatures used in our study, as plots of log$_{10}$transformed $R_n$ against leaf temperature were linear. The degree of temperature sensitivity decreased, however, when leaves were exposed to irradiances greater than 12 μmol photons m$^{-2}$ s$^{-1}$. For example, the $Q_{10}$ values over the 6°C to 25°C range (assuming a constant $Q_{10}$) were 1.61 and 1.57 at 800 and 2,000 μmol photons m$^{-2}$ s$^{-1}$, respectively (Fig. 5B). Moreover, there was little difference in the rates of $R$ at 6°C and 30°C in leaves exposed to 800 to 2,000 μmol photons m$^{-2}$ s$^{-1}$ (Fig. 5B).

Figure 5 also shows the rate of leaf respiration at each irradiance and temperature expressed as a percentage of the rate in darkness; a low percentage value indicates a high degree of light inhibition of $R$. The degree of inhibition at a set irradiance was therefore highly variable.

Figure 6 shows the effect of temperature and irradiance on gross photosynthetic CO$_2$ uptake (i.e. $n_c$) or the percentage of $n_c$ that is respired at each temperature and irradiance. In leaves exposed to 200 μmol photons m$^{-2}$ s$^{-1}$, increasing the temperature increased $n_c$ (Fig. 6A) but had little effect on the percentage of $n_c$ that was respired (Fig. 6C). Leaf respiration represented 2% to 5% of gross CO$_2$ assimilation in leaves exposed to 200 to 2,000 μmol photons m$^{-2}$ s$^{-1}$ (Fig. 6C). This contrasts with the approximately 5% to 20% (at 6°C to 30°C, respectively) of $n_c$ that was
was greatest at high irradiance and moderate-to-high temperatures, and lowest at high irradiance and low temperatures (Figs. 3 and 5). Using a $^{14}$C pulse-chase method to determine rates of R in the light and in darkness, Hurry et al. (1996) and Pärnik et al. (1998) also reported differences in the degree of light inhibition at different temperatures in controlled-environment-grown winter rye. In contrast, Brooks and Farquhar (1985) reported that the degree of inhibition at a set irradiance did not vary with temperature in spinach. Kirschbaum and Farquhar (1984) reported that light inhibited leaf respiration by a constant 40% in controlled-environment-grown snow gum when measured across a temperature range of 15°C to 35°C. Clearly, the effect of temperature on light inhibition of R does not always vary with temperature. Several factors may be responsible for the contrasting results, including the differences in plant species, growth conditions, and experimental protocols.

What effect do variations in irradiance and temperature have on the percentage of photosynthetic CO$_2$ uptake released by leaf respiration compared with that released by photorespiration? Photosynthetic CO$_2$ release can represent a large percentage of $v_c$, particularly at high temperatures (Fig. 6B; Sage, 1995). In contrast, $R_d$ represents a minor proportion of $v_c$ at all temperatures in leaves exposed to high irradiance values (e.g. only 2% at 30°C and 2,000 $\mu$mol photons m$^{-2}$ s$^{-1}$; Fig. 6C). A substantially greater proportion of $v_c$ would have been respired at high temperatures and high irradiance if leaf respiration had not been inhibited by light (e.g. at 30°C, leaf respiration rates in darkness were 11% of $v_c$ at 2,000 $\mu$mol photons m$^{-2}$ s$^{-1}$). At 40°C and high irradiance, this value would have been substantially higher if respiration continued to increase with temperature to a greater extent than $v_c$. Incomplete inhibition of R by light contributed to the high percentage of $v_c$ that was respired (23%) in leaves exposed to 30°C and 100 $\mu$mol photons m$^{-2}$ s$^{-1}$ (Fig. 6C). Clearly, a high degree of light inhibition of R at high temperatures and high irradiance substantially reduces respiratory CO$_2$ release.

Our results demonstrate that the temperature sensitivity of R is greatest in darkness, decreasing as irradiance increased (Fig. 5). Leaf respiration was almost completely insensitive to temperature at high irradiance. What is the cause of this irradiance-dependent difference in temperature sensitivity? In darkness, low temperatures reduced R, probably as a result of reduced rates of carbon input into the mitochondria and/or increased adenylate control of mitochondrial electron transport (due to reduced demand for ATP at low temperatures). The activity of key enzymes that control substrate input into the mitochondria, such as the pyruvate dehydrogenase complex (PDC) and NADH-malate enzyme (ME), is likely to be reduced at low temperatures. Reductions in the activity of PDC and ME may also explain why R is inhibited by low irradiance values at all temperatures (e.g. Fig. 3), as both are rapidly inactivated by light (Budde and Randall, 1990; Hill and Bryce, 1992). The timing of inactivation of ME (Hill and Bryce, 1992) and PDC (Budde and Randall, 1987) closely mirrors the time taken for light to inhibit R (Atkin et al.,...
It is likely that the light inhibition of \( R \) is due to the rapid light inactivation of PDC and ME (Atkin et al., 1998a, 1998b; Padmasree and Raghavendra, 1998). Exposure to low temperatures may accentuate the inhibitory effect of light on PDC and ME activity and explain why the degree of light inhibition of \( R \) at low irradiances is greater at low than at high temperatures (Fig. 3C).

The suggested mechanism by which \( R \) is initially inhibited by light may also explain why the degree of inhibition remains relatively constant over a range of high irradiances when measured at moderate temperatures (i.e., the degree of inactivation of PDC and ME remains constant over a range of irradiances). However, if \( R \) did actually increase with increasing irradiance at low temperatures (as suggested when \( \Gamma \) at 6°C was assumed to be 1.73 or 2.04 Pa; Fig. 4), then the above mechanism would not provide a complete explanation for our results. Irradiance-dependent increases in \( R \) at low temperatures could occur if photosynthetic redox equivalents were exported from the chloroplast and subsequently oxidized in the mitochondria with concomitant CO\(_2\) release.

While it is easy to see how the export of photosynthetic redox equivalents could be coupled to increased mitochondrial O\(_2\) consumption in the light (Saradadevi and Raghavendra, 1992; Raghavendra et al., 1994; Hurry et al., 1995; Xue et al., 1996), it is less clear how they could be coupled to increased non-photorespiratory CO\(_2\) release (\( R \)). For the export of excess photosynthetic redox equivalents to be coupled to increased rates of CO\(_2\) release (\( R \)) in the light (and thus lower degrees of light inhibition of \( R \)), two things would need to occur. First, flux through glycolysis would need to increase to replace the carbon lost during decarboxylation of compounds used to export the excess photosynthetic redox equivalents. This seems possible, as initial exposure to low temperatures often results in the accumulation of soluble carbohydrates (Stuiver et al., 1995; Strand et al., 1997). Second, the light inhibition of PDC would have to be overcome. The light-dependent inactivation of PDC can be overcome if concentrations of pyruvate or other positive effectors are sufficiently high. Thus, while we cannot be certain that respiration actually increased with increasing irradiance at low temperatures (due to our reliance on Eq. 3 to predict \( \Gamma \) at low temperatures), increases could theoretically occur if chloroplasts exported excess redox equivalents to the mitochondria as described above.

Was our reliance on Equation 3 to predict the temperature dependence of \( \Gamma \) at both high and low temperatures justified? Jordan and Ogren (1984) calculated the temperature dependence of \( \Gamma \) from CO\(_2\)/O\(_2\) specificity values obtained from spinach enzyme extracts using the solubilities of CO\(_2\) and O\(_2\) in solution at each temperature over the 5°C to 40°C range. Our estimates of \( \Gamma \) using the Laisk (1977) method were almost identical to that predicted by Jordan and Ogren (1984) over the 20°C to 30°C range (Fig. 2), so long as our value of \( \Gamma \) at 25°C (\( \Gamma_{25} \)) was used in Equation 3. However, we were not able to estimate \( \Gamma \) below 15°C due to the negative respiration values occurring at the regression intercept (e.g., Fig. 1A). In the absence of sub-15°C estimates of \( \Gamma \), using the Laisk (1977) method, we felt that the combined use of \( \Gamma_{25} \) and Equation 3 was the most suitable way to provide estimates of \( \Gamma \) at both high and low temperatures. When combined with an analysis of what effect errors in \( \Gamma \) have on estimates of \( R_d \) (Fig. 4), this approach provides some insight into the potential impact of temperature and irradiance on \( R \) at low temperatures.

To determine the impact of irradiance on \( R \) using measurements of gas exchange at \( \Gamma \), the Laisk (1977) method assumes that \( \Gamma \) does not vary with irradiance. \( \Gamma \) reflects the specificity of Rubisco for CO\(_2\) relative to O\(_2\) and is the CO\(_2\) partial pressure where CO\(_2\) uptake by carboxylation is matched by photorespiratory CO\(_2\) release. Changes in irradiance, and thus ATP and NADPH production by photosynthetic electron transport, will have the same absolute impact on carboxylation as photorespiration; \( \Gamma \) is therefore irradiance independent. \( \Gamma \) also appears to be invariant among species, with woody species (Villar et al., 1994; Balaguer et al., 1996) exhibiting similar \( \Gamma \) values as broad-leaved, non-woody species (Brooks and Farquhar, 1985; von Caemmerer et al., 1994). Moreover, Westbeek et al. (1999) reported that there was no systematic difference in \( \Gamma \) among seven \( P_{oa} \) species.

The use of low CO\(_2\) partial pressures to estimate \( R \) in the light raises two additional issues. First, \( R \) might be underestimated at \( \Gamma \) if mitochondrial substrate supply is limiting. To assess whether this was the case, Atkin et al. (1998a) used a fast-response gas exchange system to rapidly expose illuminated leaves to \( \Gamma \), following a period of photosynthesis at ambient CO\(_2\) partial pressure. If carbon supply limited \( R \) at \( \Gamma \), then \( R \) should be initially high when first exposed to \( \Gamma \) and decrease with time as the substrate supply becomes limiting. This did not happen; rather, steady-state values of \( R \) were maintained over 10 min (Atkin et al., 1998a). Thus, as long as measurements of \( R \) are conducted during this time period, it seems likely that carbon supply does not limit \( R \) at \( \Gamma \).

A second concern about the use of low CO\(_2\) partial pressures is that \( R \) may be substantially greater at \( \Gamma \) than at ambient CO\(_2\) concentrations. \( R_w \) is inhibited by high CO\(_2\) concentrations in short-term experiments (Bunce, 1990, 1995; Amthor, 1994; Ziska and Bunce, 1994; González-Meler et al., 1996). Conversely, \( R_w \) might be stimulated at low CO\(_2\) concentrations. If correct, then \( R_d \) may also be overestimated when measured at \( \Gamma \). Although we did not determine the impact of CO\(_2\) concentration on \( R_d \), we did determine the effect of “normal” (atmospheric partial pressure of 37 Pa) and low CO\(_2\) partial pressure (near \( \Gamma \)) on \( R_w \) at several temperatures (Table I). \( R_w \) was significantly higher at \( \Gamma \). However, the fact that the absolute differences between the \( R_w \) at 37 Pa and \( \Gamma \) were small (Table I) suggests that \( R_d \) is unlikely to be substantially overestimated at \( \Gamma \). Moreover, it seems likely that the magnitude of any overestimate will be irradiance independent.

In conclusion, our measurements demonstrate that leaf respiration in the light is highly variable, being dependent on irradiance and temperature. Our results also demonstrate that variations in the degree of light inhibition of \( R \) have a substantial impact on the temperature sensitivity.
of leaf respiration. The high degree of light inhibition of R at high temperatures and high irradiance substantially reduces the proportion of photosynthetic CO₂ release that is respired.

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