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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 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 Pa CO_2) were made at several irradiances (0–2,000 μmol photons m^{-2} s^{-1}) and temperatures (6°C–30°C). At 15°C to 30°C, substantial inhibition of *R* occurred at 12 μmol photons m^{-2} s^{-1}, with maximum inhibition occurring at 100 to 200 μmol photons m^{-2} 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 CO_2 uptake (v_c), whereas photorespiratory CO_2 release was approximately 20% of v_c. If light had not inhibited leaf respiration at 30°C and high irradiance, *R* would have represented 11% of v_c. 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 D_1 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 CO_2 release in plants. Up to 35% of the 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 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_d or day respiration) is less than that in darkness (R_n 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 μmol photons m^{-2} 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 mitochondria 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 (*Eu-*

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calyptus 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 CO_2 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 CO_2 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 CO_2 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 (Atkin et al., 1997; Poot et al., 1997). Three leaf cuvettes were connected to a data acquisition 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 mol 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 CO_2 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 CO_2 response was measured. The first measurements of R_n 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 CO_2 exchange rates were measured at four to eight decreasing internal CO_2 partial pressure (p_i) values (in the range of approximately 10–2.5 Pa CO_2).

Leaves were then exposed to an atmospheric CO_2 partial pressure of 37 Pa and the rate of net CO_2 exchange determined. A linear regression of net CO_2 exchange versus p_i for the low CO_2 partial pressure range (10–2.5 Pa) was then calculated for each irradiance. The point at which three regressions intersect was used to determine Γ_* whenever possible. Γ_* is the p_i where CO_2 uptake by carboxylation is matched by photorespiratory CO_2 release, and where the rate of CO_2 release is R_d (Laisk, 1977). In our study, the three linear regressions that were used to calculate the Γ_* 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. CO_2 uptake. Γ_* 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 CO_2 exchange versus p_i over the low CO_2 partial pressure range (see above). R_d was taken as the rate of CO_2 efflux at Γ_*. Rates of carboxylatory CO_2 uptake (v_c) and photorespiratory CO_2 release (i.e. $0.5v_o$) were calculated according to the method of Farquhar and von Caemmerer (1982):

$$v_c = (A_{net} + R_d)/[1 - (\Gamma_*/p_i)] \quad (1)$$

and

$$0.5v_o = 0.5^* [v_c^*(2\Gamma_*/p_i)] \quad (2)$$

where A_{net} is the rate of net photosynthetic CO_2 uptake in the presence of an atmospheric CO_2 partial pressure of 37 Pa (von Caemmerer and Farquhar, 1981). Data from the CO_2-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 CO_2 and O_2 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 CO_2 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 CO_2 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

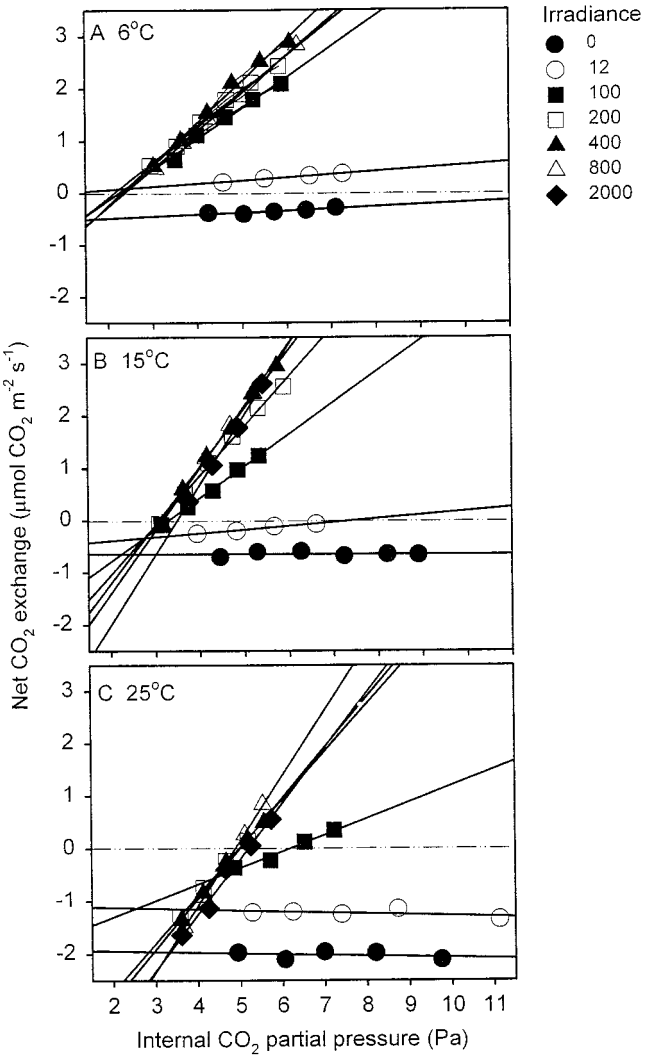

Figure 1. Example of the effect of irradiance on net CO_2 exchange (μmol CO_2 m^{-2} s^{-1}) versus p_i of a single leaf at three temperatures: 6°C (A), 15°C (B), and 25°C (C). Measurements were also conducted at 10°C, 20°C, and 30°C (not shown). The symbols represent the irradiances under which each set of measurements was made (in μmol photons m^{-2} s^{-1}). Lines represent the linear regressions at each irradiance.

Table I. *Effect of temperature on maximum carboxylation rates (V_{cmax}) and R_n measured at ambient atmospheric CO_2 partial pressure (p_a) of 37 Pa and at a low (4–5 Pa) p_i*

The V_{cmax} values were estimated from fitted CO_2-response curves similar to those shown in Figure 1 for measurements done at 2,000 μmol photons m^{-2} s^{-1}. V_{cmax} values for each temperature were calculated according to the method of Von Caemmerer and Farquhar (1981), using data from the CO_2-response curves (e.g. Fig. 1) and the Michaelis-Menten constants for CO_2 and O_2 according to the method of Von Caemmerer et al. (1994). The Γ_{*25} 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).

Temperature	V_{cmax}	R_n	
		Ambient CO_2	Low CO_2
°C		μmol CO_2 m^{-2} s^{-1}	
6	21.1 ± 3.5	0.24 ± 0.03	0.44 ± 0.04
10	27.5 ± 4.6	0.37 ± 0.03	0.48 ± 0.03
15	47.6 ± 11.3	0.64 ± 0.07	0.74 ± 0.08
20	62.7 ± 13.6	1.06 ± 0.03	1.19 ± 0.02
25	72.6 ± 2.0	1.61 ± 0.11	1.72 ± 0.13
30	105.6 ± 2.04	2.48 ± 0.21	2.67 ± 0.04

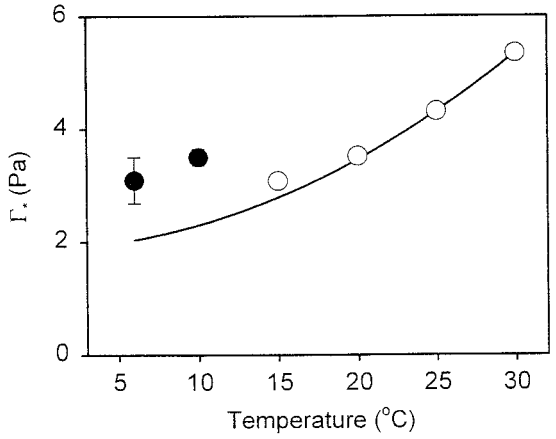

Figure 2. Effect of temperature on Γ_*. ○, Γ_* values calculated using the intercept of three linear regressions of net CO_2 exchange data versus p_i (e.g. Fig. 1) for leaves of 20°C-grown plants exposed to 15°C, 20°C, 25°C, and 30°C (e.g. Fig. 1, B and C). The three linear regressions used to calculate Γ_* were for 100, 200, and 400 μmol photons m^{-2} s^{-1} for all temperatures except 30°C, where 200, 400, and 800 μmol photons m^{-2} 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 Γ_* values for leaves exposed to 6°C and 10°C are shown for comparison (●); it was not possible to accurately calculate the Γ_* 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 Γ_* of spinach calculated from the data of Jordan and Ogren (1984) using our estimate of Γ_* at 25°C (4.31 ± 0.04 Pa).

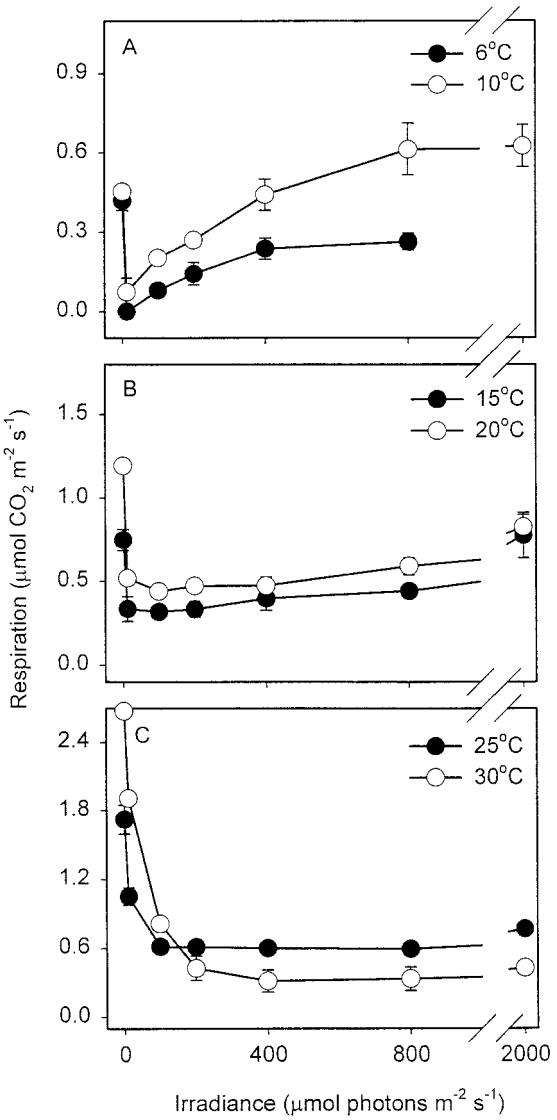

Figure 3. Relationship between *R* and irradiance at various temperatures. Values are ±SE; $n = 3$. Values of *R* were calculated using the linear regressions of net CO_2 exchange versus P_i at each irradiance (e.g. Fig. 1), our estimate of Γ_{*25} (4.31 Pa), and the temperature dependence of Γ_* given in Equation 3.

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 μmol 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 μmol 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 ($F_{1, 36} = 35.9$; $P < 0.01$; Table I).

Figure 2 shows the temperature dependence of our experimentally derived Γ_* 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 Γ_* values at 6°C and 10°C are shown for comparison. The solid line shows the temperature dependence of Γ_* calculated from data of Jordan and Ogren (1984) by Brooks and Farquhar (1985):

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

where Γ_{*T} is the Γ_* value at a set temperature (T) and Γ_{*25} is Γ_* at 25°C. With the exception of 15°C, our Γ_* values were almost identical to those predicted by Jordan and Ogren (1984) as long as we used our experimentally derived Γ_{*25} value (i.e. 4.31 ± 0.04 Pa; $n = 5$; ±SE). Given this match, and the erroneous nature of our Γ_* values at 6°C and 10°C (Fig. 2), which yielded negative respiration values, we decided to estimate *R* values for all temperatures using Γ_* values predicted by Equation 3 and our experimentally derived Γ_{*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 μmol photons m^{-2} s^{-1} at 15°C [Fig. 3B] and 400 μmol 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 Γ_*? If the Γ_* 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

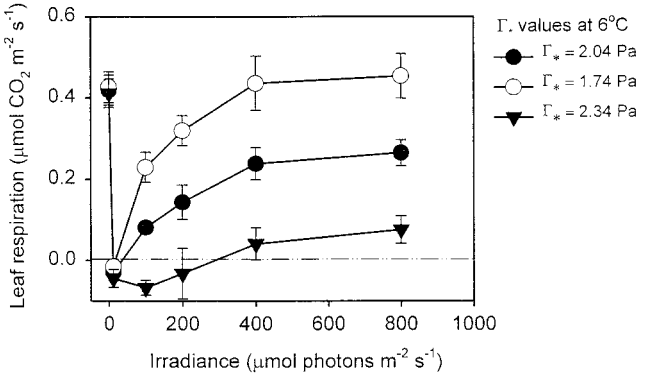

Figure 4. Determining the effect of different Γ_* values on the relationship between *R* and irradiance at 6°C using the temperature dependence of Γ_* given in Equation 3 (±SE $n = 3$). Three different estimates of Γ_* at 6°C were used in the calculations.

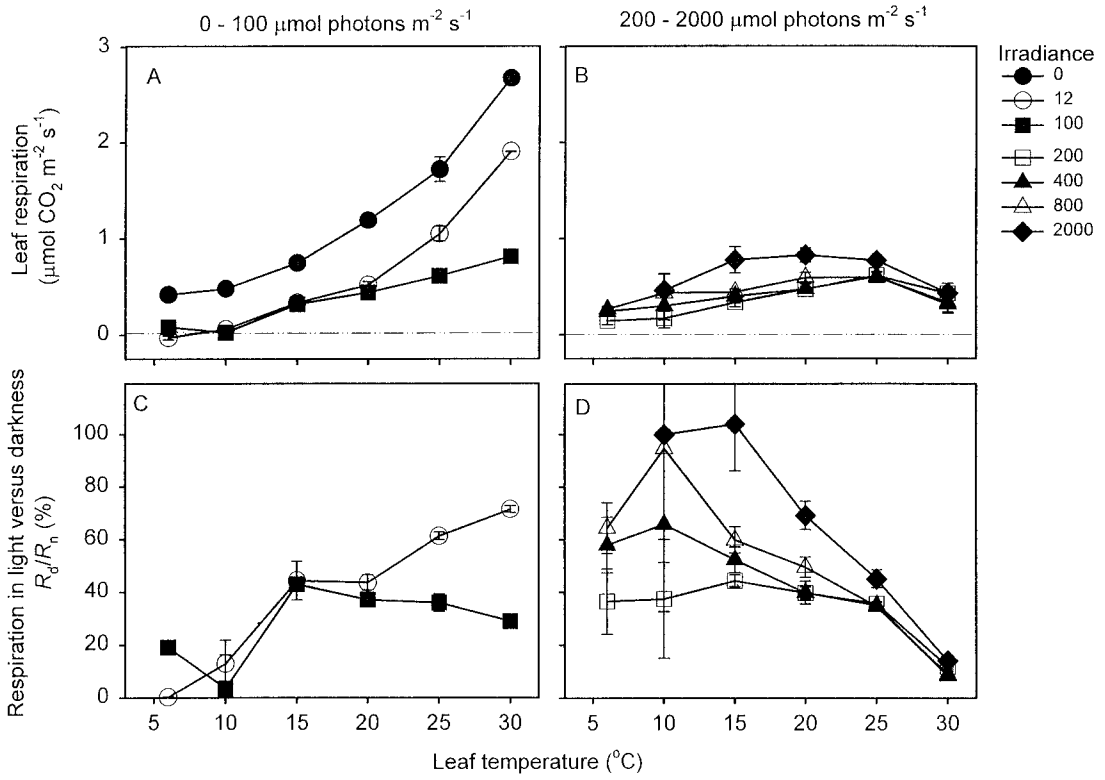

Figure 5. Effect of irradiance on the relationship between temperature and *R*. Values for 0 to 100 μmol photons m^{-2} s^{-1} are shown in A and C, whereas B and D show values for 200 to 2,000 μmol photons m^{-2} s^{-1}. Values of *R* were calculated using the linear regressions of net CO_2 exchange versus P_i at each irradiance (e.g. Fig. 1), our estimate of Γ_{*25} (4.31 Pa), and the temperature dependence of Γ_* given in Equation 3. A and B show the absolute rates of leaf respiration, while C and D show rates in the light as a percentage of those in darkness.

actual *R* value at each irradiance. To assess the impact of errors in Γ_* on our estimates of *R*, we determined the impact of Γ_* 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 Γ_* at 6°C was assumed to be 2.04 or 1.74 Pa. When Γ_* was assumed to be 2.34 Pa (i.e. Γ_{*25} = 4.61), little increase in *R* occurred until 400 μmol photons m^{-2} s^{-1}; the Γ_* 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 each irradiance varied substantially with temperature (Fig. 5, C and D). In leaves exposed to low irradiances (e.g. 12 and 100 μmol photons m^{-2} s^{-1}; Fig. 5C), maximum inhibition of *R* occurred in the cold (i.e. 6°C and 10°C). In contrast, little or no inhibition occurred in the cold in leaves exposed to high irradiance (e.g. 800 and 2,000 μmol photons m^{-2} s^{-1}; Fig. 5D). The degree of light 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. v_c) or the percentage of v_c that is respired at each temperature and irradiance. In leaves exposed to ≥200 μmol photons m^{-2} s^{-1}, increasing the temperature increased v_c (Fig. 6A) but had little effect on the percentage of v_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 v_c that was

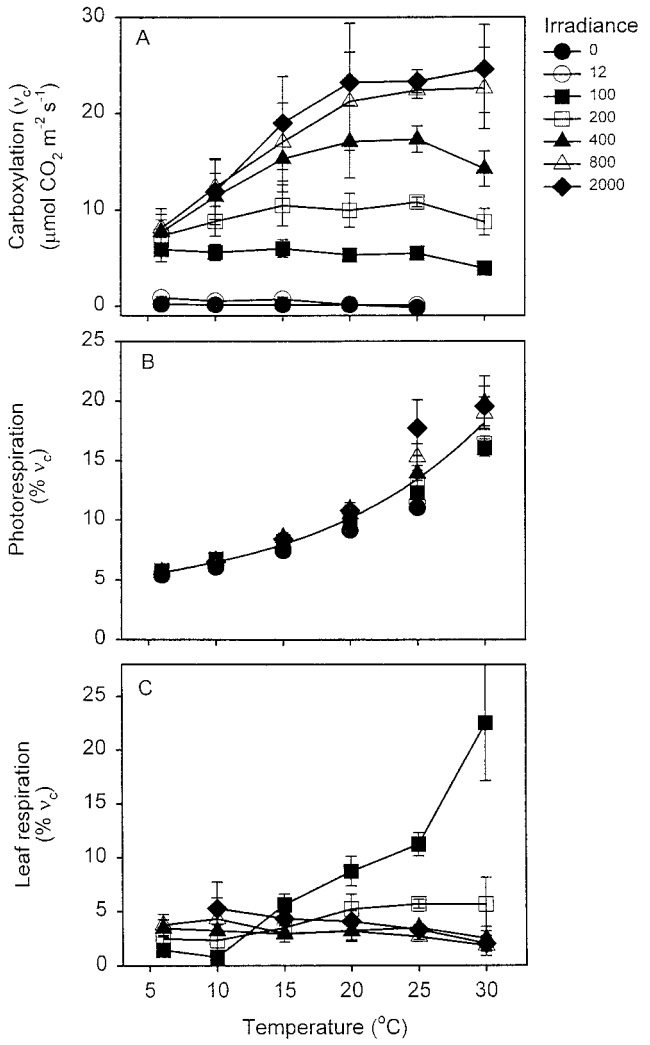

Figure 6. Relationship between temperature and the Rubisco carboxylation rate (ν_c) (A), the ratio of photorespiratory CO_2 release to Rubisco carboxylation (B), and the ratio of non-photorespiratory respiration to Rubisco carboxylation (R/ν_c) (C). Rates of ν_c, photorespiration, and R at each temperature and irradiance were calculated as described in the "Materials and Methods." The line in B is fitted to all of the data; variations in photorespiration at a particular temperature were due to variations in p_i.

released by photorespiration (i.e. 0.5 ν_o) (Fig. 6B). However, the percentage of CO_2 fixed by ν_c that was subsequently released by R_d did increase with temperature in leaves exposed to 100 μmol photons m^{-2} s^{-1}: at this low irradiance, R_d increased with temperature (Fig. 5A), whereas ν_c did not (Fig. 6A). Up to 23% of the CO_2 fixed was respired by R_d at 30°C in leaves exposed to 100 μmol photons m^{-2} s^{-1} (Fig. 6C).

DISCUSSION

Our study has demonstrated that leaf respiration rates in the light are highly variable, being dependent on irradiance and temperature. The degree to which light inhibited R 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? Photorespiratory CO_2 release can represent a large percentage of ν_c, particularly at high temperatures (Fig. 6B; Sage, 1995). In contrast, R_d represents a minor proportion of ν_c at all temperatures in leaves exposed to high irradiance values (e.g. only 2% at 30°C and 2,000 μmol photons m^{-2} s^{-1}; Fig. 6C). A substantially greater proportion of ν_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 ν_c at 2,000 μmol 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 ν_c. Incomplete inhibition of R by light contributed to the high percentage of ν_c that was respired (23%) in leaves exposed to 30°C and 100 μmol 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 NAD^+-malic 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.,

1998a, 1998b). 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, 1999b; 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 irradiance (e.g. 12–100 μmol photons m^{-2} s^{-1}) was 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 Γ_* 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 Γ_* 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 Γ_* at both high and low temperatures justified? Jordan and Ogren (1984) calculated the temperature dependence of Γ_* 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 Γ_* 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 Γ_* at 25°C (Γ_{*25}) was used in Equation 3. However, we were not able to estimate Γ_* 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 Γ_* using the Laisk (1977) method, we felt that the combined use of Γ_{*25} and Equation 3 was the most suitable way to provide estimates of Γ_* at both high and low temperatures. When combined with an analysis of what effect errors in Γ_* 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 Γ_*, the Laisk (1977) method assumes that Γ_* does not vary with irradiance. Γ_* 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; Γ_* is therefore irradiance independent. Γ_* also appears to be invariant among species, with woody species (Villar et al., 1994; Balaguer et al., 1996) exhibiting similar Γ_* 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 Γ_* among seven *Poa* 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 Γ_* 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 Γ_* following a period of photosynthesis at ambient CO_2 partial pressure. If carbon supply limited *R* at Γ_*, then *R* should be initially high when first exposed to Γ_* 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 Γ_*.

A second concern about the use of low CO_2 partial pressures is that *R* may be substantially greater at Γ_* than at ambient CO_2 concentrations. R_n 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_n might be stimulated at low CO_2 concentrations. If correct, then R_d may also be overestimated when measured at Γ_*. 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 Γ_*) on R_n at several temperatures (Table I). R_n was significantly higher at Γ_*. However, the fact that the absolute differences between the R_n at 37 Pa and Γ_* were small (Table I) suggests that R_d is unlikely to be substantially overestimated at Γ_*. 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 CO_2 release that is respired.

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