Leaf Respiration in Light and Darkness

A Comparison of Slow- and Fast-Growing Poa Species

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We investigated whether leaf dark respiration (nonphotorespiratory mitochondrial CO₂ release) is inhibited by light in several Poa species, and whether differences in light inhibition between the species are related to differences in the rate of leaf net photosynthesis. Four lowland (Poa annua L., Poa compressa L., Poa pratensis L., and Poa trivialis L.), one subalpine (Poa alpina L.), and two alpine (Poa costiniana Vick. and Poa fawcettiae Vick.) Poa species differing in whole plant relative growth rates were grown under identical controlled conditions. Nonphotorespiratory mitochondrial CO₂ release in the light (R₅) was estimated according to the Laisk method. Photosynthesis was measured at ambient CO₂ partial pressure (35 Pa) and 500 μmol photons m⁻² s⁻¹. The rate of photosynthesis per unit leaf mass was positively correlated with the relative growth rate, with the slow-growing alpine Poa species exhibiting the lowest photosynthetic rates. Rates of both R₅ and respiration in darkness were also substantially lower in the alpine species. Nonphotorespiratory CO₂ release in darkness was higher than R₅ in all species. However, despite some variation between the species in the level of light inhibition of respiration, no relationship was observed between the level of inhibition and the rate of photosynthesis. Similarly, the level of inhibition was not correlated with the relative growth rate. Our results support the suggestion that rates of leaf respiration in the light are closely associated with rates in darkness.

Leaf dark respiration (R) is often assumed to continue at the same rate in the light (R₅) as in darkness (R₆). For example, estimates of the proportion of daily fixed carbon that is respired in inherently fast- and slow-growing plants have been based on the assumption that R₅ equals R₆ (e.g. Poorter et al., 1990, 1992; Atkin et al., 1996b). Similarly, R₅ has been assumed to equal R₆ in studies investigating diurnal patterns of R (e.g. Collier et al., 1991). However, several studies using the Laisk (1977) and/or Kok (1948) approach have suggested that light inhibits R in photosynthetic tissues (Sharp et al., 1984; Brooks and Farquhar, 1985; Kirschbaum and Farquhar, 1987; Villar et al., 1994, 1995; Krömer, 1995).

The degree to which R in leaves is inhibited by light appears to be highly variable, with inhibition values of 16 to 77% having been reported (Sharp et al., 1984; Brooks and Farquhar, 1985; Villar et al., 1995). The variation in the degree of inhibition might result from several factors, including the methods used to estimate R₅, the environmental conditions during growth and measurement, the developmental state of the tissues, and the different species used (Villar et al., 1995). There is relatively little information available on the degree to which light inhibition of R varies between plant species. Villar et al. (1995) reported a greater inhibition of R₅ for a fast-growing, deciduous species that exhibits high rates of net PSₑ than for a slow-growing, evergreen species that exhibits a low PSₑ. Given that in some species (Brooks and Farquhar, 1985; Villar et al., 1995) the degree of inhibition is also greater at high light intensities (i.e. when PSₑ is high) than at low light intensities, the degree of inhibition of R may be partly determined by the rate of photosynthesis. If this is the case, then fast-growing species that exhibit high PSₑ values may exhibit greater light inhibition of R than slow-growing, low-PSₑ species, as was suggested by Villar et al. (1995).

This study investigates whether the inhibition of R by light differs between the leaves of fast-growing lowland grass species (which exhibit a high PSₑ) and congeneric, slow-growing alpine species (which exhibit a low PSₑ; Atkin et al., 1996b). We used the Laisk (1977) method (as extended by Brooks and Farquhar, 1985) to obtain estimates of R₅ in leaves of fast-growing lowland and slow-growing alpine Poa species.

MATERIALS AND METHODS

Seven Poa species were chosen for investigation. These included one annual lowland Poa species (Poa annua L.) and six perennial species: two alpine species (Poa costiniana J. Vickery and Poa fawcettiae J. Vickery), one subalpine species (Poa alpina L.), and three lowland species (Poa pratensis L., Poa compressa L., and Poa trivialis L.). All plants were grown hydroponically from seed under controlled environment conditions (constant 20°C temperature; 14/10 h day/night rhythm; 520 μmol photons m⁻² s⁻¹; 70% RH). Full details on the growth conditions and growth analysis are given by Atkin et al. (1996a, 1996b).
The mean whole plant RGRs of all species (with the exception of *P. annua*) during early growth are reported by Atkin et al. (1996b) and have been used in this study to assess the relationship between leaf dark respiration, leaf photosynthesis, and RGR. The RGRs of all species other than *P. annua* were as follows: *P. trivialis*, 255; *P. compressa*, 188; *P. pratensis*, 179; *P. alpina*, 166; *P. costiniana*, 125; and *P. fawcettia*, 111 mg dry mass [g dry mass]^{-1} d^{-1}. The RGR value for *P. annua* was taken as that reported by Poorter and Remkes (1990; 272 mg dry mass [g dry mass]^{-1} d^{-1} at 300 μmol m^{-2} s^{-1}). We expect that a similar RGR would have been observed for *P. annua* at 520 μmol m^{-2} s^{-1}, because other *Poa* species grown at the two light intensities have exhibited similar RGRs. For example, the RGR of *P. pratensis* grown at 300 μmol m^{-2} s^{-1} is 185 (Van Arendonk and Poorter, 1994), whereas its RGR at 520 μmol m^{-2} s^{-1} is 179 mg dry mass [g dry mass]^{-1} d^{-1} (Atkin et al., 1996b). Moreover, similar RGR values are found at 300 (J.J.C.M. Van Arendonk, personal communication) and 520 μmol m^{-2} s^{-1} (Atkin et al., 1996b) for *P. compressa* (191 and 188 mg dry mass [g dry mass]^{-1} d^{-1}, respectively), and *P. trivialis* (236 and 255 mg dry mass [g dry mass]^{-1} d^{-1}, respectively).

Measurements of CO₂ uptake and release in intact, attached leaves were conducted using an IR gas analyzer (model 225 MK3, ADC, Hoddesdon, UK) in the differential mode in an open system (Poot et al., 1996). Three leaf cuvettes were connected to a data acquisition system (Keithley 575, Cleveland, OH) and were measured simultaneously. Air in each chamber was mixed with a fan, which resulted in boundary layer conductances of approximately 10 mol m^{-1} s^{-1} calibrating the inlet air, then subsequently dehumidifying the vapor pressure was measured with a dew point hygrometer (Veenendaal, The Netherlands). The ratios of the flow controller signals were calibrated against the dew point hygrometer at regular intervals during measurements. Gas-exchange parameters were calculated according to Von Caemmerer and Farquhar (1981).

Gas-exchange measurements were conducted when the plants had produced leaves of sufficient length to be inserted into the leaf chambers (approximately 10–15 cm long). The leaf chambers were 7 cm in length. Determinations of leaf gas exchange commenced after at least 2 h of photosynthesis in the growth cabinets. The upper sections of two to four (depending on the width of individual leaves) of the most recently, near-fully expanded leaves from individual plants were inserted into each chamber.

*R*ₐ was measured using the Laisk (1977) method as extended by Brooks and Farquhar (1985). Villar et al. (1994) recently concluded that this method provides more accurate estimates of *R*ₐ than the Kok (1948) method. The Laisk method analyzes the rate of net CO₂ gas exchange (*P*ₘₚ) at *pₗ* and varying light intensities. *P*ₘₚ is related to *R*ₐ according to:

\[
P_{m} = v_{c} - 0.5v_{n} - R_{d}
\]

where *vₜ* and *vₙ* are the rates of carboxylation and oxygenation per unit dry mass, respectively. *R*ₚ is the rate of CO₂ release per unit mass at a *pₗ* at which *vₛ* and 0.5*vₚ* are equal (*Γₚ*).

Each set of leaves was then exposed to a range of PPFD values (100, 200, and 300 μmol m^{-2} s^{-1}). At each light intensity, *P*ₘₚ rates were measured at five decreasing *pₗ* values (in the range of approximately 10–3 Pa). A linear regression of *P*ₘₚ versus *pₗ* values was then calculated for each of the three light intensities. The point at which the three regressions intersected was then used to determine *Γₚ* and *R*ₚ (the rate of CO₂ release at *Γₚ*). The final values of *R*ₚ for each species represent the mean of six to eight separate plants.

Measurements of *R*ₚ (at 35 Pa CO₂ partial pressure) in darkness were conducted 30 min after leaves had been exposed to a light intensity of 300 μmol m^{-2} s^{-1}. Rates of gas exchange were measured every 2 min for 50 min after exposure to darkness; it took 25 to 30 min for respiration rates to stabilize.

Sampling of the leaf sections exposed to the chambers took place immediately after each set of measurements. The dry weights (following freeze-drying; Virtus, Unitop 600SL, Gardiner, NY) of the leaf sections were then determined.

**RESULTS**

Figure 1 shows an example of the CO₂ exchange at a range of *pₗ* values for *P. compressa* at three light intensities. Similar results were observed for the other six *Poaceae* species (data not shown). Over the range of low *pₗ* values used, the responses at each light intensity were linear for all of the species (e.g. Fig. 1).

Figure 2 shows the rates of *P*ₘₚ at 500 μmol m^{-2} s^{-1} and 35 Pa CO₂ partial pressure for the seven *Poaceae* species. At this light intensity and CO₂ partial pressure, *P*ₘₚ of the near-fully expanded leaves was positively correlated with the whole plant RGR. *P*ₘₚ was also positively correlated with RGR when measured at 35 Pa CO₂ partial pressure and light-saturating PPFD values (between 1200 and 2000 μmol m^{-2} s^{-1}; data not shown). Thus, the slower-growing alpine *Poaceae* species exhibit lower rates of *P*ₘₚ in whole shoots (Atkin et al., 1996b) and in near-fully expanded leaves (Fig. 2).

Figure 3 shows an example of the time dependence of *R*ₚ immediately after the light was switched off at time 0 (t).
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All other species showed similar temporal changes in $R_n$. Maximum $R_n$ values occurred following 5 to 10 min in darkness, with stable $R_n$ values not being established until after 25 to 30 min in darkness. These stable values usually represented 75 to 80% of the maximum $R_n$ values. We elected to use the stable $R_n$ values after 30 min in darkness for comparison with the $R_d$ measurements, as was the case in previous comparisons of $R_n$ and $R_d$ (e.g., Villar et al., 1995).

Figure 3 shows the $R_d$ and $R_n$ values plotted against the $P_{S_m}$ values (measured at 500 μmol m$^{-2}$ s$^{-1}$) for each species. $R_d$ and $R_n$ were both positively correlated with $P_{S_m}$ (Fig. 4). A significant positive correlation also occurred between $R$ (in light and darkness) and RGR (data not shown). $R_n$ and $R_d$, therefore, appear to be closely associated in the seven Poa species.

Given that $R_d$ was lower than $R_n$ in all species, it is clear that light does inhibit leaf $R$ in all species when $R_d$ is determined according to the Laisk method (Fig. 4). However, no correlation was observed between the level of inhibition and $P_{S_m}$ regardless of whether the level of inhibition was expressed on an absolute (Fig. 5A) or a

Figure 1. Net assimilation (nmol CO$_2$ [g dry mass]$^{-1}$ s$^{-1}$) versus CO$_2$ internal partial pressure ($p_i$; Pa) for one set of Poa compressa leaves. Numbers indicate the incident PPDF under which each set of measurements was made. Lines represent the linear regressions at each light intensity. The dotted line indicates zero net assimilation. $R_d$ and $R_n$ are indicated.

Figure 2. Leaf net photosynthesis rates (nmol CO$_2$ [g dry mass]$^{-1}$ s$^{-1}$) versus the average whole plant RGR (mg dry mass [g dry mass]$^{-1}$ d$^{-1}$) for seven Poa species. Measurements of net photosynthesis were made at a light intensity of 500 μmol m$^{-2}$ s$^{-1}$ and a CO$_2$ partial pressure of 35 Pa. The line represents the linear regression between photosynthesis and RGR and is statistically significant ($r = 0.821$, $P = 0.024$). A near-identical trend with respect to RGR was observed when net photosynthesis was measured at light saturation for each species (1200–2000 μmol m$^{-2}$ s$^{-1}$). Lowland species, P. annua (O), P. trivialis (O), P. compressa (O), and P. pratensis (V); subalpine species, P. costiniana (■); and alpine species, P. fawcettiae (O). Atkin et al. (1996b) describes each species’ natural habitat and origin of the seeds.

Figure 3. $R_d$ (nmol CO$_2$ [g dry mass]$^{-1}$ s$^{-1}$) versus time in darkness for P. compressa. Leaves had previously been exposed to 300 μmol m$^{-2}$ s$^{-1}$ for 30 min. Values represent the mean of three replicate measurements (±SE; three leaves per chamber per measurement). Measurements were done at a CO$_2$ partial pressure of 35 Pa.

Figure 4. $R_d$ (open symbols) and $R_n$ (closed symbols) plotted against leaf net photosynthesis (nmol CO$_2$ [g dry mass]$^{-1}$ s$^{-1}$), measured at a light intensity of 500 μmol m$^{-2}$ s$^{-1}$ and for seven Poa species. Values represent the mean rates of 7 to 11 replicate measurements (±SE). Both photosynthesis and $R_n$ were measured at a CO$_2$ partial pressure of 35 Pa. The lines represent the linear regression between the $R$ values and photosynthesis and are both statistically significant ($R_d$: $P = 0.001$, $r = 0.963$; $R_n$: $P = 0.044$, $r = 0.767$). See the Figure 2 legend for identification of each species.
the degree of inhibition and PS$_n$ (Fig. 5, A and B). There-

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