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 faewcettiae Vick.) Poa species differing in whole plant relative growth rates were grown under identical controlled conditions. Nonphotorespiratory mitochondrial CO₂ release in the light (R₄) 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 R₄ and respiration in darkness were also substantially lower in the alpine species. Nonphotorespiratory CO₂ release in darkness was higher than R₄ 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 (R₄) as in darkness (R₅). 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 R₄ equals R₅ (e.g. Poorter et al., 1990, 1992; Atkin et al., 1996b). Similarly, R₅ has been assumed to equal R₄ 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 R₄, 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 R₄ for a fast-growing, deciduous species that exhibits high rates of net PS₅ than for a slow-growing, evergreen species that exhibits a low PS₅. 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 PS₅ 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 PS₅ values may exhibit greater light inhibition of R than slow-growing, low-PS₅ 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 PS₅) and congenic, slow-growing alpine species (which exhibit a low PS₅; Atkin et al., 1996b). We used the Laisk (1977) method (as extended by Brooks and Farquhar, 1985) to obtain estimates of R₄ 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 faewcettiae 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).

Abbreviations: NR, nitrate reductase; pₖ, low internal partial pressures of CO₂, PS₅, photosynthesis per unit leaf dry mass; R, nonphotorespiratory mitochondrial CO₂ release; R₄, R in the light; R₅, R in darkness; RGR, relative growth rate; vₚ, rate of carboxylation; vₒ, rate of oxygenation.

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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. faconti- tina*, 111 mg dry mass [g dry mass]^{-1} d^{-1}. The RGR value for *P. annua* was 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 520 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).

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Water vapor pressure was controlled by initially humidifying the inlet air, then subsequently dehumidifying the air in a temperature-controlled glass column. The water vapor pressure was measured with a dew point hygrometer (General Eastern, Watertown, MA). The vapor pressure of the air leaving the cuvette was monitored with a humidity sensor (HMP 35A, Vaisala, Helsinki, Finland) that was calibrated against the dew point hygrometer at regular intervals during measurements.

CO₂ partial pressures were controlled by mixing CO₂-free air with CO₂ using flow controllers (Brooks Instruments, Veenendaal, The Netherlands). The ratios of the flow controller signals were calibrated for estimation of the CO₂ partial pressures against gas mixing pumps (Wöstoff, Bochum, Germany) using the differential IR gas analyzer. Particular care was taken to calibrate the flow-controller signals in the low-CO₂ partial pressure range used in our experiments. Gas-exchange parameters were calculated according to Von Caemmerer and Farquhar (1981).

Gas-exchange measurements were conducted when the leaves 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.

Rₐ 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 Rₐ than the Kok (1948) method. The Laisk method analyzes the rate of net CO₂ gas exchange (PSₘ) at pₛ and varying light intensities. PSₘ is related to Rₐ according to:

\[
PSₘ = vₐ - 0.5vᵦRₐ
\]

where \(vₐ\) and \(vᵦ\) are the rates of carboxylation and oxygenation per unit dry mass, respectively. Rₐ is the rate of CO₂ release per unit mass at a \(pₛ\), at which \(vₐ\) and 0.5\(vᵦ\) are equal (\(Γᵦ\)).

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, PSₘ rates were measured at five decreasing \(pₛ\) values (in the range of approximately 10–3 Pa). A linear regression of PSₘ versus \(pₛ\) values was then calculated for each of the three light intensities. The point at which the three regressions intersected was then used to determine \(Γᵦ\) and Rₐ (the rate of CO₂ release at \(Γᵦ\)). The final values of Rₐ for each species represent the mean of six to eight separate plants.

Measurements of Rₐ (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 \(pₛ\) values for *P. compressa* at three light intensities. Similar results were observed for the other six *Poa* species (data not shown). Over the range of low \(pₛ\) 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 PSₘ at 500 μmol m⁻² s⁻¹ and 35 Pa CO₂ partial pressure for the seven *Poa* species. At this light intensity and CO₂ partial pressure, PSₘ of the near-fully expanded leaves was positively correlated with the whole plant RGR. PSₘ 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 *Poa* species exhibit lower rates of PSₘ 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 Rₐ immediately after the light was switched off at time 0 (\(Pₐ\).
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Figure 1. Net assimilation (nmol CO₂ [g dry mass]⁻¹ s⁻¹) versus CO₂ internal partial pressure (p; Pa) for one set of P. compressa leaves. Numbers indicate the incident PPFD 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_r are indicated.

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. R_r (nmol CO₂ [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 CO₂ partial pressure of 35 Pa.

Figure 2. Leaf net photosynthesis rates (nmol CO₂ [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 CO₂ partial pressure of 15 Pa. The line represents the linear regression between photosynthesis and RGR and is statistically significant (P = 0.024, r = 0.821). 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).

Figure 4. Leaf R_d (open symbols) and R_r (closed symbols) plotted against leaf net photosynthesis (nmol CO₂ [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_r were measured at a CO₂ 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_r, P = 0.044, r = 0.767). See the Figure 2 legend for identification of each species.
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to respiratory mitochondrial respiration by light is positively correlated with PSNm, as suggested by Villar et al. (1995). Villar et al. (1995) based their hypothesis on the fact that light inhibits leaf R to a greater extent in a high-PSNm species (Lepechinia fragans Greene) than in a low-PSNm species (Heteromeles arbutifolia Ait.). Other studies have also suggested that photosynthetic processes are responsible for the inhibition of R by light (Graham, 1980; Villar et al., 1995). However, our results demonstrate that despite variability in the degree to which light inhibits leaf R in the selected Poa species, no relationship was observed between the degree of inhibition and PSNm (Fig. 5, A and B). Therefore, we reject the hypothesis that PSNm per se is responsible for the inhibition of R by light.

Figure 5. Inhibition of leaf Rg plotted against photosynthesis (nmol CO2 [g dry mass] \(^{-1}\) s\(^{-1}\)), measured at a light intensity of 500 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) for seven Poa species. A, Inhibition expressed on an absolute basis (Rg - Rg, nmol CO2 [g dry mass] \(^{-1}\) s\(^{-1}\)). B, Inhibition expressed on a percentage of Rg. Values represent the mean of 7 to 11 replicate measurements (±SE). The dotted lines in both A and B represent the linear regression between photosynthesis and the inhibition values. Neither is statistically significant. See the Figure 2 legend for identification of each species.

Our study addressed whether the inhibition of nonphotosynthetic respiratory mitochondrial respiration by light is positively correlated with PSNm, as suggested by Villar et al. (1995). Brooks and Farquhar (1985) and Villar et al. (1995) also demonstrated that leaf dark respiration in light and in darkness are closely associated. Variation in Rg between species and/or treatments is often attributed to a variation in the demand for respiratory products for growth and/or maintenance processes (Amthor, 1989; Lambers et al., 1996). Following this reasoning, then the higher values of Rg and Rn exhibited by the fast-growing Poa species (Fig. 4) may have been due to higher rates of growth (whereas we used near-fully expanded leaves, mature leaves of the fast-growing plants presumably export more sugars and amino acids to other developing tissues) and hence greater demands for growth respiration (i.e. ATP, NAD(P)\(^{+}\)H, and/or the tricarboxylic acid cycle intermediates). If this were the case, then the greater demand for respiratory products in the fast-growing species occurred both in the dark and the light.

In conclusion, our measurements of Rg obtained with the Laisk (1977) method demonstrate that light inhibits leaf R in several Poa species. However, the degree of inhibition is not correlated with the rate of photosynthesis per se.

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