Leaf Respiration in Light and Darkness

Owen K. Atkin*2, Milka H.M. Westbeek, Marion L. Cambridge, Hans Lambers, and Thijs L. Pons

Department of Plant Ecology and Evolutionary Biology, Utrecht University, P.O. Box 800.84, 3508 TB Utrecht, The Netherlands

We investigated whether leaf dark respiration (nonphotorespiratory mitochondrial CO$_2$ release) is inhibited by light in several Poa species, and whether differences in leaf 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 CO$_2$ release in the light ($R_d$) was estimated according to the Laisk method. Photosynthesis was measured at ambient CO$_2$ 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 $R_d$ and respiration in darkness were also substantially lower in the alpine species. Nonphotorespiratory CO$_2$ release in darkness was higher than $R_d$ 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 ($R_d$) as in darkness ($R_n$). 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 $R_d$ equals $R_n$ (e.g. Poorter et al., 1990, 1992; Atkin et al., 1996b). Similarly, $R_d$ has been assumed to equal $R_n$ 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; Krómer, 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 $R_d$, 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 $R_d$ for a fast-growing, deciduous species that exhibits high rates of net $P_{Si}$ than for a slow-growing, evergreen species that exhibits a low $P_{Si}$. 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 $P_{Si}$ 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 $P_{Si}$ values may exhibit greater light inhibition of $R$ than slow-growing, low-$P_{Si}$ 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 $P_{Si}$) and congenic, slow-growing alpine species (which exhibit a low $P_{Si}$; Atkin et al., 1996b). We used the Laisk (1977) method (as extended by Brooks and Farquhar, 1985) to obtain estimates of $R_d$ 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).

---

1 This work was supported by a European Union Postdoctoral Fellowship (Human Capital and Mobility Programme) to O.K.A.

2Present address: Environmental Biology Group, Research School of Biological Sciences, The Australian National University, Canberra, A.C.T., 0200, Australia.

* Corresponding author; e-mail atkin@rsbs-central.anu.edu.au; fax 61-6-249-4919.

Abbreviations: NR, nitrate reductase; $p_l$, low internal partial pressures of CO$_2$, $P_{Si}$, photosynthesis per unit leaf dry mass; $R$, nonphotorespiratory mitochondrial CO$_2$ release; $R_d$, $R$ in the light; $R_n$, $R$ in darkness; RGR, relative growth rate; $v_c$, rate of carboxylation; $v_o$, rate of oxygenation.
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*, 123; and *P. p foetida*, 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 dehumidifying the inlet air, then subsequently dehumidifying the air leaving the cuvette was monitored with a humid-temperature-controlled and humidity-controlled. Leaf temperatures, kept constant at 20°C, were measured using two 0.08-mm type K thermocouples per cuvette, which were inserted into the leaf sections (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.

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

$$PSₘ = v_c - 0.5v_o = R_d$$

where *v_c* and *v_o* are the rates of carboxylation and oxygenation per unit dry mass, respectively. *R_d* is the rate of CO₂ release per unit mass at a *p_i* at which *v_c* and 0.5*v_o* are equal (Γ*). 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 *p_i* values (in the range of approximately 10–3 Pa). A linear regression of *PSₘ* versus *p_i* values was then calculated for each of the three light intensities. The point at which the three regressions intersected was then used to determine Γ* and *R_d* (the rate of CO₂ release at Γ*). The final values of *R_d* for each species represent the mean of six to eight separate plants.

Measurements of *Rₚₐ* (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 *p_i* 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 *p_i* 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 *Rₚₐ* immediately after the light was switched off at time 0 (*I*ₚₐ).
Leaf Respiration in Light and Darkness in Poa Species

Figure 1. Net assimilation (nmol CO₂ [g dry mass]⁻¹ s⁻¹) versus CO₂ internal partial pressure (pi; 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. RD and R₂ are indicated.

All other species showed similar temporal changes in Rn. Maximum Rn values occurred following 5 to 10 min in darkness, with stable Rn values not being established until after 25 to 30 min in darkness. These stable values usually represented 75 to 80% of the maximum Rn values. We elected to use the stable Rn values after 30 min in darkness for comparison with the Rd measurements, as was the case in previous comparisons of Rn and Rd (e.g. Villar et al., 1995).

Figure 4 shows the Rd and R₂ values plotted against the PSn values (measured at 500 μmol m⁻² s⁻¹) for each species. Rd and R₂ were both positively correlated with PSn (Fig. 4). A significant positive correlation also occurred between R (in light and darkness) and RGR (data not shown). Rn and R₂, therefore, appear to be closely associated in the seven Poa species.

Given that Rd was lower than R₂ in all species, it is clear that light does inhibit leaf R in all species when Rd is determined according to the Laisk method (Fig. 4). However, no correlation was observed between the level of inhibition and PSn, regardless of whether the level of inhibition was expressed on an absolute (Fig. 5A) or a relative basis.

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 35 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). Lowland species, P. annua (O), P. trivialis (O), P. compressa ( ), and P. pratensis ( ); subalpine species, P. costiulana ( ) and P. fawcettiae (O). Akin et al. (1996b) describes each species' natural habitat and origin of the seeds.

Figure 3. Rd (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.
the Figure 2 legend for identification of each species.

CO₂ to respiratory mitochondrial respiration by light is positively correlated with PSₚ, as suggested by Villar et al. (1995). Villar et al. (1995) based their hypothesis on the fact that light inhibits leaf Rᵩ in a species (Lepechinita fragans Greene) than in a low-PSₚ 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 PSₚₙ (Fig. 5, A and B). Therefore, we reject the hypothesis that PSₚₙ per se is responsible for the inhibition of R by light.

The conclusion that light inhibits R in our Poa species depends on whether accurate estimates of Rᵩ are obtained using the Laisk (1977) method. The effect of low CO₂ partial pressures on Rᵩ is, however, not known. Nevertheless, several studies have demonstrated that low CO₂ partial pressures stimulate Rᵩ (see Villar et al., 1994, and refs. cited therein) rather than inhibit it. This raises the possibility that Rᵩ may be overestimated at Γ, rather than underestimated.

The conclusion that light inhibits R in our Poa species is also partly dependent on whether Rᵩ is compared with Rₚ measured at 35 Pa CO₂ partial pressure or to Rₚ measured at Γ. We measured Rₚ at 35 Pa rather than at ɣ₂. Measurements of Rₚ at ɣ₂ would likely have resulted in higher estimates of Rₚ than shown in Figure 4, because of the stimulatory effect that low CO₂ partial pressures have on Rₚ (Villar et al., 1994). Thus, the degree of inhibition of R by light is likely to have been even greater than shown in Figure 5 if Rᵩ had been measured at ɣ₂.

Independent confirmation that light inhibits R comes from recent work using a ¹⁴C-labeling, pulse-chase technique in which R was measured at ambient CO₂ partial pressure (Pärnink and Keerberg, 1995). In their study, Pärnink and Keerberg found that R is lower in the light than in the dark in wheat (0.55 and 0.64 μmol CO₂ m⁻² s⁻¹ in light and darkness, respectively), tobacco (0.68 and 1.27 μmol CO₂ m⁻² s⁻¹), and barley (0.27 and 0.60 μmol CO₂ m⁻² s⁻¹). R is not, however, inhibited by light in all species. For example, Hurry et al. (1996) recently reported that R is higher in the light (0.59 μmol CO₂ m⁻² s⁻¹) than in the dark (0.45 μmol CO₂ m⁻² s⁻¹) in rye when measured with the ¹⁴C-labeling technique.

Our results demonstrate that Rᵩ and Rₚₙ are closely associated in the leaves of the selected Poa species (Fig. 4). 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 Rᵩ 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 Rᵩ and Rₚₙ 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 Rᵩ 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.

**ACKNOWLEDGMENTS**

We thank Drs. V.M. Hurry and R. Villar for their comments on previous versions of this manuscript.

**DISCUSSION**

Our study addressed whether the inhibition of nonphotosynthetic mitochondrial respiration by light is positively correlated with PSₚₙ, 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-PSₚₙ species (Lepechinita fragans Greene) than in a low-PSₚₙ 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 PSₚₙ (Fig. 5, A and B). Therefore, we reject the hypothesis that PSₚₙ per se is responsible for the inhibition of R by light.

The conclusion that light inhibits R in our Poa species depends on whether accurate estimates of Rᵩ are obtained using the Laisk (1977) method (i.e. using low CO₂ partial pressures). If low CO₂ partial pressures lead to underestimates of Rᵩ, then the degree of light inhibition might be overestimated by the Laisk (1977) method. The effect of low CO₂ partial pressures on Rᵩ is, however, not known. Nevertheless, several studies have demonstrated that low CO₂ partial pressures stimulate Rᵩ (see Villar et al., 1994, and refs. cited therein) rather than inhibit it. This raises the possibility that Rᵩ may be overestimated at Γ, rather than underestimated.

The conclusion that light inhibits R in our Poa species is also partly dependent on whether Rᵩ is compared with Rₚ measured at 35 Pa CO₂ partial pressure or to Rₚ measured at Γ. We measured Rₚ at 35 Pa rather than at ɣ₂. Measurements of Rₚ at ɣ₂ would likely have resulted in higher estimates of Rₚ than shown in Figure 4, because of the stimulatory effect that low CO₂ partial pressures have on Rₚ (Villar et al., 1994). Thus, the degree of inhibition of R by light is likely to have been even greater than shown in Figure 5 if Rᵩ had been measured at ɣ₂.

Independent confirmation that light inhibits R comes from recent work using a ¹⁴C-labeling, pulse-chase technique in which R was measured at ambient CO₂ partial pressure (Pärnink and Keerberg, 1995). In their study, Pärnink and Keerberg found that R is lower in the light than in the dark in wheat (0.55 and 0.64 μmol CO₂ m⁻² s⁻¹ in light and darkness, respectively), tobacco (0.68 and 1.27 μmol CO₂ m⁻² s⁻¹), and barley (0.27 and 0.60 μmol CO₂ m⁻² s⁻¹). R is not, however, inhibited by light in all species. For example, Hurry et al. (1996) recently reported that R is higher in the light (0.59 μmol CO₂ m⁻² s⁻¹) than in the dark (0.45 μmol CO₂ m⁻² s⁻¹) in rye when measured with the ¹⁴C-labeling technique.

Our results demonstrate that Rᵩ and Rₚₙ are closely associated in the leaves of the selected Poa species (Fig. 4). 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 Rᵩ 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 Rᵩ and Rₚₙ 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 Rᵩ 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.

**ACKNOWLEDGMENTS**

We thank Drs. V.M. Hurry and R. Villar for their comments on previous versions of this manuscript.

![Figure 5](image-url)
Leaf Respiration in Light and Darkness in Poa Species

Received August 13, 1996; accepted November 16, 1996.
Copyright Clearance Center: 0032-0889/97/113/0961/05.

LITERATURE CITED

Laisk AK (1977) Kinetics of Photosynthesis and Photorespiration in $\text{C}_4$-Plants. Nauka, Moscow