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Leaf Respiration in Light and Darkness[1]

A Comparison of Slow- and Fast-Growing *Poa* Species

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We investigated whether leaf dark respiration (nonphotorespiratory mitochondrial CO_2 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 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; 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 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 PS_m than for a slow-growing, evergreen species that exhibits a low PS_m. 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 PS_m 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 PS_m values may exhibit greater light inhibition of R than slow-growing, low-PS_m 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 PS_m) and congeneric, slow-growing alpine species (which exhibit a low PS_m; 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^{-1} s^{-1}; 70% RH). Full details on the growth conditions and growth analysis are given by Atkin et al. (1996a, 1996b).

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Abbreviations: NR, nitrate reductase; p_i, low internal partial pressures of CO_2, PS_m, 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*, 125; and *P. fawcettiae*, 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 CO_2 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^{-2} s^{-1}. Different light intensities were obtained by placing small mesh wire netting filters in front of slide projector lamps that were mounted above each cuvette, which were 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 mounted on the underside of the leaves.

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.

CO_2 partial pressures were controlled by mixing CO_2-free air with CO_2 using flow controllers (Brooks Instruments, Veenendaal, The Netherlands). The ratios of the flow controller signals were calibrated for estimation of the CO_2 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-CO_2 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 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_d 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_d than the Kok (1948) method. The Laisk method analyzes the rate of net CO_2 gas exchange (PS_m) at p_i and varying light intensities. PS_m is related to R_d according to:

$$PS_m = v_c - 0.5v_o - R_d$$

where v_c and v_o are the rates of carboxylation and oxygenation per unit dry mass, respectively. R_d is the rate of CO_2 release per unit mass at a p_i at which v_c and $0.5v_o$ 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, PS_m rates were measured at five decreasing p_i values (in the range of approximately 10–3 Pa). A linear regression of PS_m 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 CO_2 release at Γ_*). The final values of R_d for each species represent the mean of six to eight separate plants.

Measurements of R_n (at 35 Pa CO_2 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 CO_2 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 PS_m at 500 μmol m^{-2} s^{-1} and 35 Pa CO_2 partial pressure for the seven *Poa* species. At this light intensity and CO_2 partial pressure, PS_m of the near-fully expanded leaves was positively correlated with the whole plant RGR. PS_m was also positively correlated with RGR when measured at 35 Pa CO_2 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 PS_m 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_n immediately after the light was switched off at time 0 (*P.*

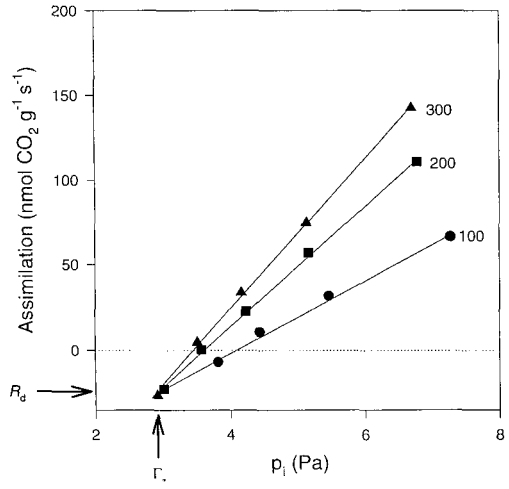

Figure 1. Net assimilation (nmol CO_2 [g dry mass]$^{-1}$ s^{-1}) versus CO_2 internal partial pressure (p_i; Pa) for one set of *P. compressa* leaves. Numbers indicate the incident PPFD under which each set of measurements was made. Lines represent the linear regressions at each light intensity. The dotted line indicates zero net assimilation. R_d and Γ are indicated.

compressa). 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

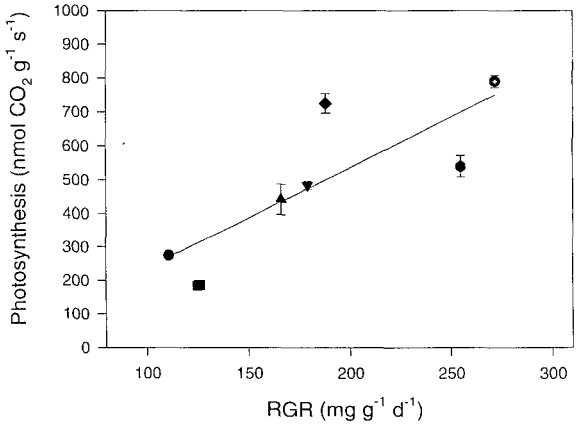

Figure 2. Leaf net photosynthesis rates (nmol CO_2 [g dry mass]$^{-1}$ s^{-1}) versus the average whole plant RGR (mg dry mass [g dry mass]$^{-1}$ d^{-1}) for seven *Poa* species. Measurements of net photosynthesis were made at a light intensity of 500 μmol m^{-2} s^{-1} and a CO_2 partial pressure of 35 Pa. The line represents the linear regression between photosynthesis and RGR and is statistically significant ($P = 0.024$, $r = 0.821$). 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* (❍), *P. trivialis* (●), *P. compressa* (◆), and *P. pratensis* (▼); subalpine species, *P. alpina* (▲); and alpine species, *P. costiniana* (■) and *P. fawcettiae* (●). Atkin et al. (1996b) describes each species' natural habitat and origin of the seeds.

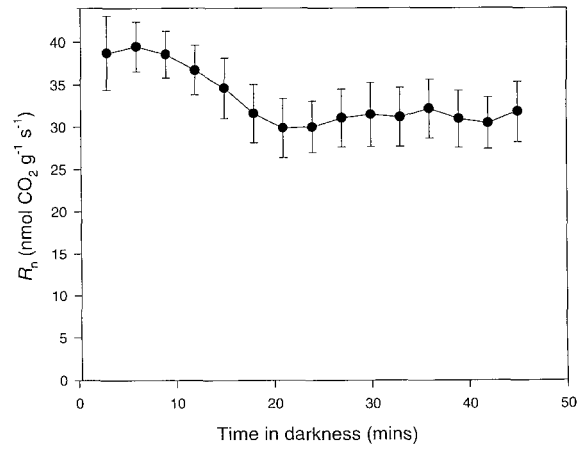

Figure 3. R_n (nmol CO_2 [g dry mass]$^{-1}$ s^{-1}) versus time in darkness for *P. compressa*. Leaves had previously been exposed to 300 μmol m^{-2} s^{-1} for 30 min. Values represent the mean of three replicate measurements (±SE; three leaves per chamber per measurement). Measurements were done at a CO_2 partial pressure of 35 Pa.

was the case in previous comparisons of R_n and R_d (e.g. Villar et al., 1995).

Figure 4 shows the R_d and R_n values plotted against the PS_m values (measured at 500 μmol m^{-2} s^{-1}) for each species. R_d and R_n were both positively correlated with PS_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 PS_m, regardless of whether the level of inhibition was expressed on an absolute (Fig. 5A) or a

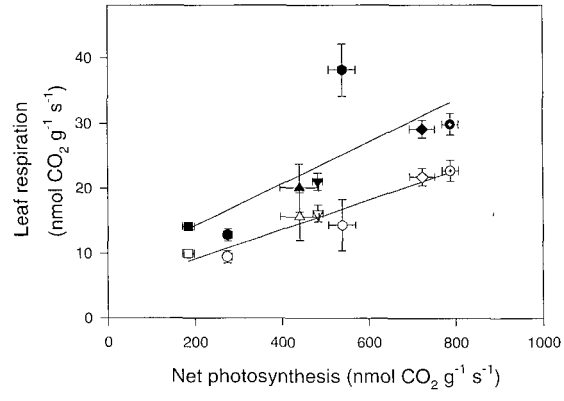

Figure 4. Leaf R_d (open symbols) and R_n (closed symbols) plotted against leaf net photosynthesis (nmol CO_2 [g dry mass]$^{-1}$ s^{-1}, measured at a light intensity of 500 μmol m^{-2} s^{-1}) and for seven *Poa* species. Values represent the mean rates of 7 to 11 replicate measurements (±SE). Both 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.

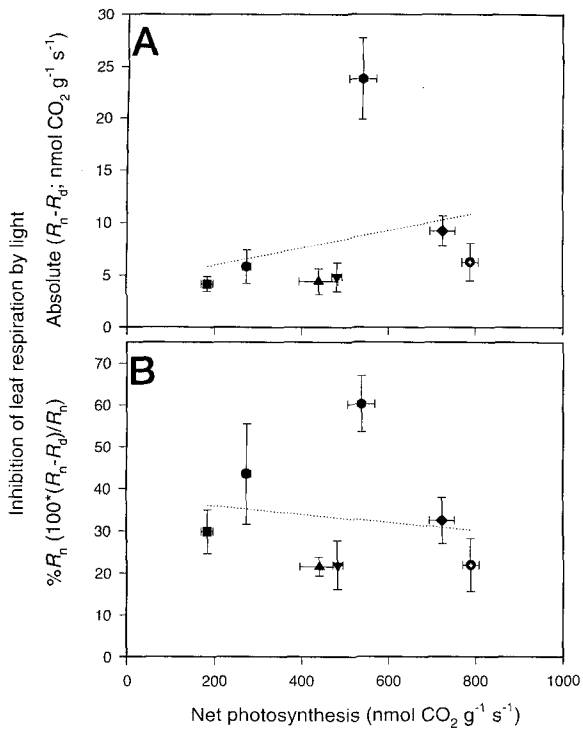

Figure 5. Inhibition of leaf R_d plotted against photosynthesis (nmol CO_2 [g dry mass]$^{-1}$ s^{-1}, measured at a light intensity of 500 μmol m^{-2} s^{-1}) for seven *Poa* species. A, Inhibition expressed on an absolute basis ($R_n - R_d$); nmol CO_2 [g dry mass]$^{-1}$ s^{-1}). B, Inhibition expressed on a percentage of R_n. Values represent the mean respiration rates 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.

percentage (Fig. 5B) basis. Similarly, the degree to which leaf *R* was inhibited by light was not correlated with RGR (data not shown).

DISCUSSION

Our study addressed whether the inhibition of nonphotorespiratory mitochondrial respiration by light is positively correlated with PS_m, 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-PS_m species (*Lepechinia fragans* Greene) than in a low-PS_m 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 PS_m (Fig. 5, A and B). Therefore, we reject the hypothesis that PS_m 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 R_d are obtained using the Laisk (1977) method (i.e. using low CO_2 partial pressures). If low CO_2 partial pressures lead to underestimates of R_d, then the degree of light inhibition might be overestimated by the Laisk (1977) method. The effect of low CO_2 partial pressures on R_d is, however, not known. Nevertheless, several studies have demonstrated that low CO_2 partial pressures stimulate R_n (see Villar et al., 1994, and refs. cited therein) rather than inhibit it. This raises the possibility that R_d may be overestimated at Γ_* rather than underestimated.

The conclusion that light inhibits *R* in our *Poa* species is also partly dependent on whether R_d is compared with R_n measured at 35 Pa CO_2 partial pressure or to R_n measured at Γ_*. We measured R_n at 35 Pa rather than at γ_*. Measurements of R_n at Γ_* would likely have resulted in higher estimates of R_n than shown in Figure 4, because of the stimulatory effect that low CO_2 partial pressures have on R_n (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 R_n had been measured at Γ_*.

Independent confirmation that light inhibits *R* comes from recent work using a ^{14}C-labeling, pulse-chase technique in which *R* was measured at ambient CO_2 partial pressure (Pärnik and Keerberg, 1995). In their study, Pärnik and Keerberg found that *R* is lower in the light than in the dark in wheat (0.55 and 0.64 μmol CO_2 m^{-2} s^{-1} in light and darkness, respectively), tobacco (0.68 and 1.27 μmol CO_2 m^{-2} s^{-1}), and barley (0.27 and 0.60 μmol CO_2 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 CO_2 m^{-2} s^{-1}) than in the dark (0.45 μmol CO_2 m^{-2} s^{-1}) in rye when measured with the ^{14}C-labeling technique.

Our results demonstrate that R_d and R_n 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 R_n 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 R_d and R_n 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 R_d 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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