

**Photoinhibition in C<sub>4</sub> and C<sub>3</sub> grasses native to high latitudes**

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**Introduction**

C<sub>4</sub> plants can dominate sites in warm, dry climates if that they have access to at least moderate light intensities (Sage *et al.* 1999). However, C<sub>4</sub> species represent a relatively small portion of local floras in high latitude or high elevation sites (e.g. Terri and Stowe 1976; Tieszen *et al.* 1979). The occurrence of C<sub>4</sub> grasses is best correlated with the minimum temperature during the growing season in North America; C<sub>4</sub> grasses are generally absent when the lowest July temperature is less than 8°C (Terri and Stowe 1976). The reasons for this are uncertain. One possibility is that enzymes of the C<sub>4</sub>-cycle are impaired at low temperatures, but this is an insufficient explanation *in vivo* (Edwards *et al.* 1985, Yamazaki and Sugiyama 1984). Alternatively, C<sub>4</sub> plants might be more susceptible to photoinhibition at chilling temperatures because of the extremely low rates of photorespiration and the slower induction of photosynthesis (Long 1983). Further, at low temperatures C<sub>4</sub> photosynthesis may be limited by Rubisco capacity (Pittermann and Sage 2000); the reduced potential to consume reductant could lead to increased excitation pressure. The possibility of greater susceptibility to photoinhibition has received relatively little subsequent attention, except in cultivated species such as *Zea mays*.

Most studies of C<sub>4</sub> photosynthesis at low temperatures have utilised plants of tropical origin, which might render it difficult to separate the combined effects of chilling and high light on the photosynthetic apparatus from some form of overall low temperature impairment. We tested the hypothesis that C<sub>4</sub> plants are more susceptible to photoinhibition at chilling temperatures than their C<sub>3</sub> competitors using *Muhlenbergia glomerata* (C<sub>4</sub>) and *Calamagrostis canadensis* (C<sub>3</sub>). These grasses are native to northern Canada and regularly experience chilling temperatures during the growing season. We used chlorophyll fluorescence techniques to assess any differences in the ability of these species to tolerate exposure to chilling temperatures at high irradiance.

**Methods and Materials**

*C. canadensis* (C<sub>3</sub>) and *M. glomerata* (C<sub>4</sub>) were collected from a fen near Plevna ON (44°59N 76°53W). Plants were grown in 6-l pots containing a 4:1:1 (v:v:v) mixture of pro-mix:sand:compost, watered daily and fed weekly with 1/6x Hoagland's solution and 1mM NH<sub>4</sub>NO<sub>3</sub>. Plants were grown in controlled environment chambers (PG-15, Conviron, Canada) under 16 hour photoperiods at a maximum photosynthetic photon flux density (PPFD) of 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The day/night temperature regime was either 14/10°C (cool-grown) or 26/22°C (warm-grown). Fully expanded leaves were used in all experiments.

Photoinhibition can be detected as a reduction in the ratio of variable to maximal fluorescence (F<sub>v</sub>/F<sub>m</sub>), which is closely correlated to the quantum yield of photosynthesis.

Chlorophyll fluorescence was measured with a pulse-modulated fluorometer (OS-500, Opti-Science, MA, USA) connected to a chart-recorder. All fluorescence nomenclature follows van Kooten and Snel (1990). Maximal fluorescence ( $F_m$  or  $F_m'$ ) was measured by applying a 0.8s pulse of white light (approx. 4000  $\mu\text{E}$ ). To measure the minimal fluorescence of illuminated leaves ( $F_o'$ ), the leaf was rapidly darkened in the presence of far-red light.

Excised leaves were photoinhibited by exposing the adaxial surface to a PPFD of 1900  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at leaf temperatures of 8°C for two hours. Illumination was provided by two cold-light sources (KL2500 and KL1500, Schott, Germany). Leaves were mounted in a fixed position in a water bath to maintain a constant temperature. The adaxial surface was exposed to air throughout the treatment and recovery phases. Pre-treatment  $F_v/F_m$  measurements were made after leaves had been kept in the dark for 30 minutes at the measurement temperature. Measurements of  $F_v'/F_m'$  were made at 30 minute intervals during the photoinhibitory treatment. Initial recovery conditions (temperature 8°C or 20°C, PPFD 0 or 10  $\mu\text{E}$ ) were applied in a factorial experiment.  $F_v/F_m$  (or  $F_v'/F_m'$  in the low light recovery) was measured every 30 minutes during the recovery phase. After a 2-hour recovery, the leaves were darkened, and a post-treatment  $F_v/F_m$  measurement was obtained after 30 minutes. For half of the replicates,  $F_v/F_m$  was also measured after a further 24 hours of recovery in the dark at room temperature. Xanthophyll pigments were assayed by HPLC on leaf material that had been frozen in liquid  $\text{N}_2$  directly after harvest from the growth chambers. The data were analysed using a repeated-measures ANOVA.

## Results and Discussion

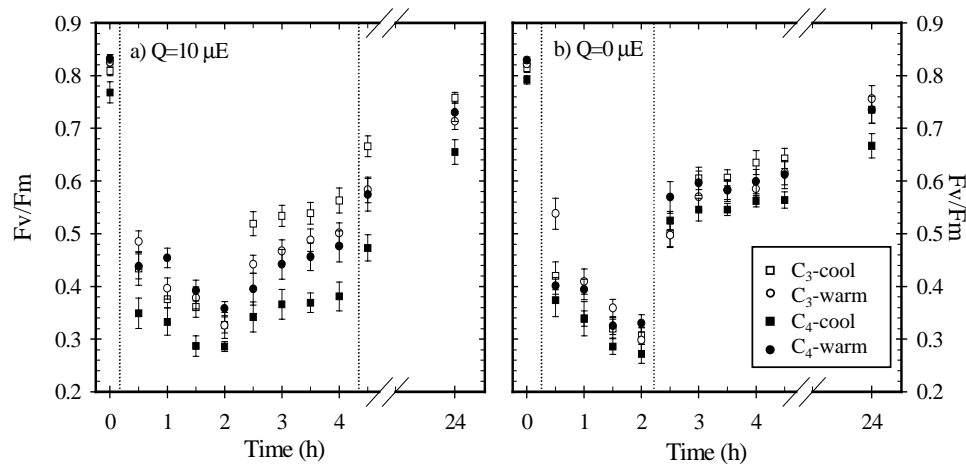
No differences in the pre-treatment  $F_v/F_m$  were observed between the  $\text{C}_3$  or warm-grown  $\text{C}_4$  (Table 1). However, the  $F_v/F_m$  of the cool-grown  $\text{C}_4$  averaged 0.783, which was significantly ( $p < 0.01$ ) lower than the other species/growth temperature combinations. Growth temperature did not affect changes in the total pool of the xanthophyll-cycle pigments in *M. glomerata* (Table 1). However, the cool-grown  $\text{C}_4$  had an epoxidation state of 0.501 under the growth conditions, which was significantly lower than the other combinations. This indicates that these plants maintain a greater capability for non-photochemical quenching even under the relatively low illumination of the growth chamber. Other authors have noted a reduction in  $F_v/F_m$  when maize is grown at low temperatures (Leipner et al. 1997, Massacci et al. 1995), while in other cases growth temperature had no effect on maize (e.g. Verheul et al. 1995).

**Table 1.** The fluorescence ( $F_v/F_m$ ) and xanthophyll characteristics of *Calamagrostis canadensis* ( $\text{C}_3$ ) and *Muhlenbergia glomerata* ( $\text{C}_4$ ). Fluorescence measurements were made on excised leaves following 30 minutes of darkness at 8°C.  $F_v/F_m$  values represent the mean ( $\pm$  s.d.) of 40 measurements. Xanthophylls were measured on leaf material collected directly from the growth conditions described above. The epoxidation state (EPS) was calculated as  $(V+0.5A)/(V+A+Z)$ . Xanthophyll pool size and EPS measurements represent the mean (s.e.) of 5 measurements. Values with different superscripts are significantly different ( $p < 0.05$ ).

Species	Growth Temperature (°C)	$F_v/F_m$ (s.d.)	$V+A+Z$ ( $\mu\text{mol m}^{-2}$ )	EPS
<i>Calamagrostis canadensis</i> ( $\text{C}_3$ )	14/10	0.821 <sup>a</sup> (0.024)	30.7 <sup>a</sup> (1.78)	0.754 <sup>a</sup> (0.019)
	26/22	0.830 <sup>ab</sup> (0.016)	22.3 <sup>b</sup> (0.68)	0.909 <sup>a</sup> (0.029)
<i>Muhlenbergia glomerata</i> ( $\text{C}_4$ )	14/10	0.783 <sup>c</sup> (0.048)	37.6 <sup>c</sup> (1.76)	0.501 <sup>b</sup> (0.036)
	26/22	0.839 <sup>b</sup> (0.023)	33.6 <sup>c</sup> (1.55)	0.840 <sup>a</sup> (0.026)

After the two-hour photoinhibitory treatment,  $F_v/F_m'$  was reduced to roughly 35% of  $F_v/F_m$  in all cases (Fig. 1). The cool-grown  $C_4$  recovered significantly more slowly ( $p < 0.05$ ) than the other combinations during the initial 2-hour recovery phase under low light ( $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) but not in darkness, indicating that a fluorescence quenching mechanism remains active under even low irradiance (Fig. 1). Recovery during the initial two-hour period was more rapid at  $20^\circ\text{C}$  versus  $8^\circ\text{C}$  in all cases (data not shown), consistent with enhanced rates of protein synthesis or zeaxanthin de-epoxidation. No differences were observed during the recovery in darkness.

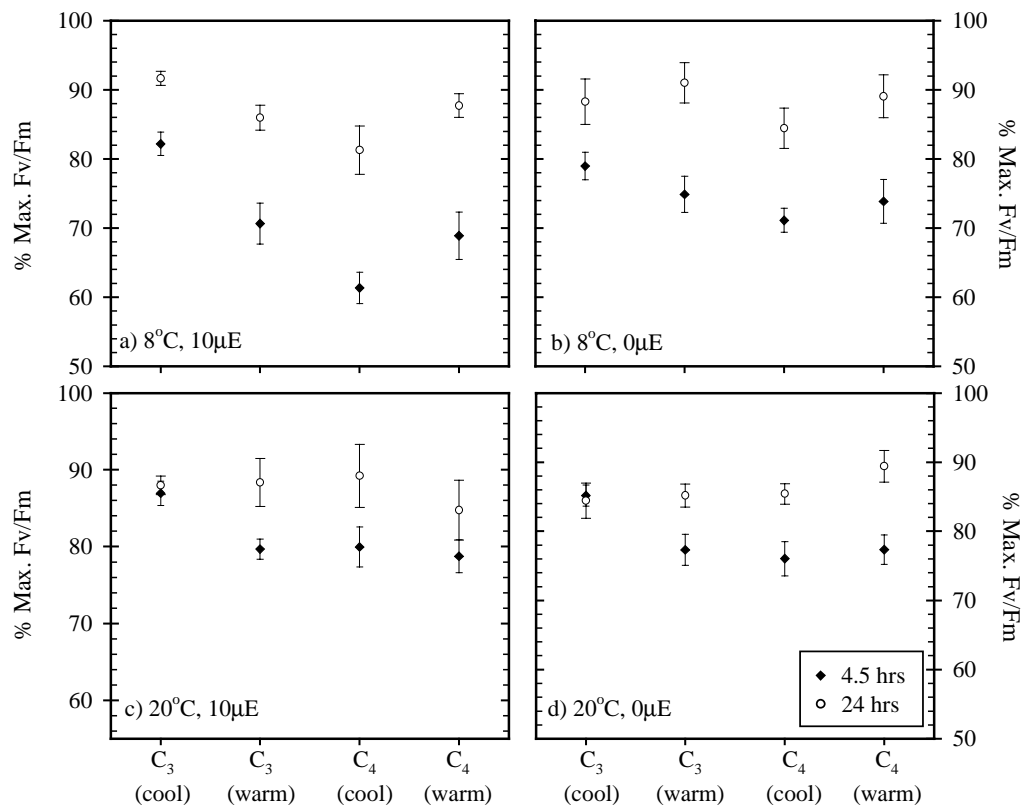
Following the 30-minute dark period after the initial recovery phase,  $F_v/F_m$  was greater in plants that had recovered at  $20^\circ\text{C}$  (Fig. 2). The  $F_v/F_m$  of the cool-grown  $C_4$  was significantly less than the other groups when the initial recovery occurred under low light at  $8^\circ\text{C}$ , but not under the other recovery conditions. For all species/growth temperature combinations,  $F_v/F_m$  after 24 hours of recovery was between 85 and 90% of the pre-treatment value (Fig. 2), regardless of the initial recovery conditions. The lack of complete recovery indicates at least some degree of chronic photoinhibition (e.g. photodamage), but there were no differences between any of the groups ( $p > 0.35$ ).



**Fig 1.** The time course of photoinhibition and recovery in *M. glomerata* ( $C_4$ ) and *C. canadensis* ( $C_3$ ) grown as described in the text. Leaves were treated at  $8^\circ\text{C}$  and  $1900 \mu\text{mol m}^{-2} \text{s}^{-1}$  for two hours; the first two hours of recovery occurred at  $8^\circ\text{C}$  under a PPFD of a)  $10 \mu\text{E}$ , or b) darkness. Subsequent recovery occurred at room temperature in darkness. Each value represents the mean ( $\pm$  s.e.) of 10 measurements, except for the 24-hr values ( $n=5$ ). The values between the dashed lines on each panel indicate measurements made under actinic illumination ( $F_v/F_m'$ ).

Our data indicate that *M. glomerata* is not inherently more susceptible to photoinhibition at chilling temperatures than *C. canadensis*. Low growth temperatures affected changes in the xanthophyll pool and in the kinetics of fluorescence quenching (e.g. photoprotection) in the  $C_4$  species. Growth at  $14^\circ\text{C}$  did not enhance the tolerance of *M. glomerata* to low temperature photoinhibition relative to plants grown at  $26^\circ\text{C}$ . In contrast, Nie *et al.* (1992) found that low growth temperatures in *Zea mays* were associated with increased tolerance to chilling dependent photoinhibition and with reduced light-saturated assimilation rates, relative to warm-grown plants. They suggested that the lower capacity for assimilation that resulted from low growth temperature would constitute an important disadvantage when temperatures were above the chilling range. Consistent with this observation, the maximum assimilation

rate of cool-grown *M. glomerata* is approximately 20% lower than its warm-grown counterpart when measured at 25°C (Kubien and Sage, unpublished results). Pittermann and Sage (2000) suggested that the capacity of Rubisco might limit  $C_4$  photosynthesis at chilling temperatures, which would lead to increased excitation pressure. The higher epoxidation state of the xanthophyll cycle pigments in cool-grown *M. glomerata* likely acts as a compensatory mechanism in this regard. The competitive ability of  $C_4$  species in cool climates may be negatively influenced in this fashion, but they do not appear to be more susceptible to chronic photoinhibition than co-occurring  $C_3$  species.



**Fig. 2.** The effect of a photoinhibitory treatment on *M. glomerata* ( $C_4$ ) and *C. canadensis* ( $C_3$ ) grown at 14/10°C (cool) and 26/22°C (warm). Data are expressed as a percentage of the pre-treatment, dark-adapted  $F_v/F_m$  value. After a two-hour treatment, leaves recovered for two hours at the conditions indicated in each panel, followed 30 minutes of darkness at the same temperature (4.5 hours, ♦). Further recovery occurred in darkness at room temperature (24 hours, ○). Each value represents the mean ( $\pm$  s.e.) of 10 measurements, except for the 24 hour measurements ( $n=5$ ).

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