The response of the high altitude C₄ grass
*Muhlenbergia montana* (Nutt.) A.S. Hitchc. to
long- and short-term chilling

Jarmila Pittermann² and Rowan F. Sage¹

*Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario, Canada M5S3B2*

Received 22 June 2000; Accepted 8 November 2000

Abstract

The acclimation of C₄ photosynthesis to low temperature was studied in the montane grass *Muhlenbergia montana* in order to evaluate inherent limitations in the C₄ photosynthetic pathway following chilling. Plants were grown in growth cabinets at 26 °C days, but at night temperatures of either 16 °C (the control treatment), 4 °C for at least 28 nights (the cold-acclimated treatment), or 1 night (the cold-stress treatment). Below a measurement temperature of 25 °C, little difference in the thermal response of the net CO₂ assimilation rate (A) was observed between the control and cold-acclimated treatment. By contrast, above 30 °C, A in the cold-acclimated treatment was 10% greater than in the control treatment. The temperature responses of Rubisco activity and net CO₂ assimilation rate were similar below 22 °C, indicating high metabolic control of Rubisco over the rate of photosynthesis at cool temperatures. Analysis of the response of A to intercellular CO₂ level further supported a major limiting role for Rubisco below 20 °C. As temperature declined, the CO₂ saturated plateau of A exhibited large reductions, while the initial slope of the CO₂ response was little affected. This type of response is consistent with a Rubisco limitation, rather than limitations in PEP carboxylase capacity. Stomatal limitations at low temperature were not apparent because photosynthesis was CO₂ saturated below 23 °C at air levels of CO₂. In contrast to the response of photosynthesis to temperature and CO₂ in plants acclimated for 4 weeks to low night temperature, plants exposed to 4 °C for one night showed substantial reduction in photosynthetic capacity at temperatures above 20 °C. Because these reductions were at both high and low CO₂, enzymes associated with the C₄ carbon cycle were implicated as the major mechanisms for the chilling inhibition. These results demonstrate that C₄ plants from climates with low temperature during the growing season can fully acclimate to cold stress given sufficient time. This acclimation appears to involve reversal of injury to the C₄ cycle following initial exposure to low temperature. By contrast, carbon gain at low temperatures generally appears to be constrained by the carboxylation capacity of Rubisco, regardless of acclimation time. The inability to overcome the Rubisco limitation at low temperature may be an inherent limitation restricting C₄ photosynthetic performance in cooler climates.

Key words: Chilling, C₄ plants, *Muhlenbergia*, photosynthesis, temperature response, Rubisco.

Introduction

C₄ plants often dominate high light habitats from warm climates, but are uncommon above 50° latitude and 2000 m elevations where minimum growing season temperatures average less than 8 °C (Long, 1983). Numerous explanations have been proposed for failure of most C₄ species to occur in cold climates. On the one hand, the C₄ pathway may be inherently limiting at low temperature because chilling damages one of the steps of the C₄ pathway. In particular, phosphoenolpyruvate (PEP) regeneration by PEP carboxykinase or pyruvate

---

¹ To whom correspondence should be addressed; Fax: +1 416 978 5878. E-mail: rsage@botany.utoronto.ca
² Current address: Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA.

Abbreviations: A, the rate of net CO₂ assimilation; C, intercellular partial pressure of CO₂; PEPCase, phosphoenolpyruvate carboxylase; PPK, pyruvate-phosphate-dikinase.

© Society for Experimental Biology 2001
Pi-dikinase (PPDK) has been implicated as the leading cold-sensitive step that curtails C₄ photosynthesis in colder climates (Long, 1983; Leegood and Edwards, 1996). Long-term chilling experiments with *Echinochloa crus-galli*, for example, show a reduction in the activity of PPDK following a 1–3 d exposure to chilling temperature that is correlated with reduced photosynthetic performance (Potvin *et al*., 1986; Simon and Hatch, 1994). Similarly, 14 d chilling of the warm-adapted grass *Zea japonica* caused a loss of PEP carboxykinase capacity that was correlated with a 75% decrease in CO₂ assimilation rate (Matsuba *et al*., 1997). Alternatively, photosynthetic pathway may be of relatively little importance in cooler settings, with prior adaptation to tropical conditions minimizing the success of most C₄ species in cold climates (Ehleringer and Monson, 1993; Long, 1999). Because the C₄ syndrome is putatively of recent evolutionary origin in tropical climates (Ehleringer *et al*., 1997), there may have been insufficient time for more than a few C₄ species to evolve chilling tolerance and invade low temperature habitats. As with tropical C₃ species, multiple lesions may follow chilling exposure in most C₄ plants, both within the photosynthetic apparatus and throughout the whole plant.

To avoid complications associated with adaptation to warm climates, the question of whether there are inherent weaknesses of the C₄ pathway at low temperature should be addressed using C₄ species originating from the cold-extremes of the C₄ geographic range. Numerous C₄ species from cold climates have been identified, most often in the genera *Bouteloua,Miscanthus, Muhlenbergia*, and *Spartina* (Long, 1999; Sage *et al*., 1999). These species are noted for stable photosynthesis rates at low temperatures, and do not show chilling injury in the C₄ cycle enzymes as observed in species originating in low latitudes. For example, in *Spartina anglica*, a PCK monocot from cool temperate salt marshes, C₄ cycle enzymes are not inhibited and A shows a 43% increase following chilling treatment (Matsuba *et al*., 1997). Similarly, the montane genus *Miscanthus* from east Asia has numerous species that exhibit stable photosynthetic capacity at low temperature and are able to produce high yields despite chilling (Long, 1999). From these studies, it is apparent that the photosynthetic apparatus of C₄ plants is not necessarily prone to failure in cold conditions, and thus chilling injury may not be the ultimate explanation for the general absence of the C₄ syndrome from cold climates. These observations, however, do not address whether overall performance of C₄ photosynthesis is poor relative to C₃ photosynthesis at low temperature. While the C₄ photosynthetic apparatus may be stable at low temperature, it remains possible that there is an inherent limitation preventing C₄ plants from matching the performance of C₃ species. Inferior quantum yield of C₄ relative to C₃ plants is an example of a performance limitation that may restrict C₄ photosynthesis in cool, shady habitats (Ehleringer *et al*., 1997). Lower quantum yield, however, should not prevent C₄ occurrence in high light habitats of low temperature.

In a recent report, the temperature response of net CO₂ fixation was evaluated in *Bouteloua gracilis* from the Rocky Mountains, USA (Pittermann and Sage, 2000). No evidence was observed for dissociation of C₄-cycle enzymes at low temperature, and photoinhibition was minor under the experimental conditions used. Rubisco has been implicated as an important control over C₄ photosynthesis at moderate to low temperature (Björkman and Pearcy, 1971; Pearcy, 1977; Caldwell *et al*., 1977). Consistently, the photosynthetic response to temperature in *Bouteloua gracilis* was equivalent to the temperature response of the Rubisco $V_{\text{max}}$ below 17 °C (Pittermann and Sage, 2000). These results indicate the low Rubisco content of C₄ relative to C₃ plants may be an important impediment to the success of C₄ species in cold climates. Unlike C₃ species that have an abundance of Rubisco in all photosynthetic cells, Rubisco in C₄ species is restricted to the bundle sheath. On a leaf area basis, Rubisco quantity in a C₄ leaf is typically one-quarter to one-third that of a C₃ plant with an equivalent photosynthesis rate at 25 °C to 30 °C (Sage and Pearcy, 2000). Because of the high thermal dependence of the Rubisco $V_{\text{max}}$, the in vivo capacity of Rubisco below 10–15 °C is low relative to its value above 25 °C. Thus, because C₄ species have low quantities of Rubisco, they are prone to also have lower photosynthetic potential at cool temperatures than C₃ species (Leegood and Edwards, 1996; Pittermann and Sage, 2000). Also, by directly limiting carbon gain at low temperature, low Rubisco capacity could predispose C₄ plants to photoinhibition.

In addition to the genus *Bouteloua*, the other leading C₄ group from high altitude regions of North America is the grass genus *Muhlenbergia*. A number of *Muhlenbergia* species occur above 3300 m, making it North America’s highest C₄ genus. The Rocky Mountain species *Muhlenbergia montana* (NAD-malic enzyme subtype; Hattersley and Watson, 1992) occurs to at least 3100 m in the sub-alpine zone. To complement the prior work with *B. gracilis*, the research reported here examined the temperature response of photosynthesis and Rubisco activity in a high elevation ecotype of *Muhlenbergia montana*. In addition to short-term temperature responses of net CO₂ assimilation and Rubisco activity, the acclimation responses of photosynthesis and the PSII redox state to chilling conditions were investigated.

**Materials and methods**

**Plant material**

*Muhlenbergia montana* tillers were obtained in the vicinity of Kenosha Pass, Colorado (39°25’ N, 105°45’ W; 3100 m above
sea level) and planted into 4.0 l pots of 60% Pro-mix (Premier Brands, Redhill, Pennsylvania), 20% perlite and 20% sand. All grasses were initially grown outdoors during August of 1996. They were subsequently transferred to a growth cabinet (Convivon E-15, Convivon Ltd. Winnipeg, Manitoba) where light intensity and temperature were controlled to gradually increase from a 16 °C, 8 h dark period to a 26 °C, 16 h light period at a maximum PPFD of 700 μmol m⁻² s⁻¹. All plants were fertilized weekly with a half-strength Hoagland’s solution.

Assessments of leaf gas exchange, fluorescence and Rubisco activity

In alpine environments, plants may be exposed to high temperatures during the day, but may be subject to overnight lows near freezing (Jordan and Smith, 1995). The acclimation potential of *M. montana* was examined by subjecting a subset of the 26/16 °C-grown tillers to 4 °C overnight (8–10 h) conditions for 4 weeks, at which time gas exchange comparisons of photosynthetic responses to temperature and intercellular partial pressure of CO₂ commenced. Leaf gas exchange was measured as described earlier (Pittermann and Sage, 2000), using a null balance, thermally-controlled gas exchange system modelled after that described previously (Sharkey, 1985; Field *et al.*, 1989). In all gas exchange measurements, photosynthetic photon flux density was controlled to be slightly above the photosynthetic light saturation point in order to allow for improved thermal control at lower measurement temperatures. Because the light saturation point declined with temperature (Fig. 1), this meant that the measurement PPFD was reduced from above 1800 μmol photons m⁻² s⁻¹ at the warmer temperatures to near 800 μmol m⁻² s⁻¹ at the cooler temperatures. Vapour pressure difference between leaf and air was set between 8–12 mbar except above 30 °C where it was allowed to rise to near 20 mbar.

The response of A to temperature was determined by first equilibrating leaves at the daytime growth temperature of 26 °C and saturating light levels for 30–60 min. After this, temperature was increased to near 40 °C and then decreased in steps of approximately 4 °C, with measurements at each step. The exposure to temperature near 40 °C was not observed to depress A subsequently measured at 26 °C, indicating the measurement procedure was sound. The response of A to intercellular CO₂ was determined by first equilibrating leaves at 26 °C, light saturation, and air levels of CO₂, and then stepping the measurement temperature up to 33 ± 1 °C. After equilibration, the ambient partial pressure of CO₂ was increased to approximately 450 mbar, and then reduced in a series of steps to near the CO₂ compensation point. Gas exchange parameters were determined at each step after a 15–30 min equilibration period. After completion of the response, the ambient CO₂ level was returned to approximately 360 mbar, allowed to stabilize, and A was determined again. If A was within 10% of the original value, the temperature was reduced to 26 °C and the A versus Cᵢ response was again measured as done at 33 °C. After completion of this curve, the procedure was repeated at 13 °C.

During measurements of the temperature response of leaf gas exchange, fluorescence parameters were assessed with an OptiScience OS-500 (Haverhill, MA) modulated fluorimeter. Coefficients of photochemical and non-photochemical quenching (q₀ and qᵥ, respectively), the electron-transport rate, and photochemical yield were measured for the 26 °C treatment only. Photochemical quenching equalled \((Fₘₐₛ – Fₕ) / Fₘₐₛ\) and \(qᵥ\) equalled \(Fₘₐₛ – Fₘᵢₐₜ – Fₕ\), where \(Fₘₐₛ\) is maximum fluorescence in the presence of actinic light, \(Fₕ\) is steady-state fluorescence in actinic light, \(Fₘᵢₐₜ\) is the maximum fluorescence of dark-acclimated leaves, and \(Fₙₐₜ\) is the minimal fluorescence of darkened leaves in the presence of post-actinic far red illumination (Schreiber *et al.*, 1995, as modified in the OS-500 manual).

To determine the effect of short-term chilling on photosynthetic parameters, plants grown at 26/16 °C were transferred to 4 °C for 1 night. The following morning, the grasses were placed in a growth cabinet at 23 °C and 150 μmol photons m⁻² s⁻¹ for 1–2 h. The temperature and CO₂ responses of A were then determined as described above. To evaluate photoinhibition following the start of night chilling, dark-adjusted \(Fₘᵢₐₜ\) was measured over a 12 h period after transfer of *M. montana* plants from 16 °C to 4 °C night values. \(Fₘᵢₐₜ\) was measured 30 min after dark exposure on five tillers that were sampled at 4 °C just before morning (time 0 measurement), and 4, 6 and 8 h into the 26 °C day cycle.

Rubisco activity was measured as described previously (Sage *et al.*, 1993; Pittermann and Sage, 2000). Briefly, multiple leaves from at least three fully illuminated plants grown at 26/16 °C were sampled by clipping and rapidly immersing in liquid N₂. Samples were then stored in liquid N₂ until assay. Activity assays consisted of determining the rate of ¹⁴C-CO₂ incorporation into acid stable products following a 5–20 min incubation in 15 mM MgCl₂ and 10 mM NaHCO₃ to activate the extracted Rubisco fully. This procedure produced \(kₗₐₜ\) values of 38 mol CO₂ mol⁻¹ Rubisco s⁻¹ at 28 °C for *M. montana* Rubisco. These are similar to the high \(kₗₐₜ\) values reported for a range of C₄ species (Seemann *et al.*, 1984; Sage and Seemann, 1993), indicating our assay procedures were sound.

**Results**

**Photosynthetic acclimation of *M. montana* to low night temperature**

In *Muhlenbergia montana* plants grown at 26/16 °C, the saturating light intensity was approximately 1700 μmol m⁻² s⁻¹ at 23 °C and 33 °C, but declined at lower temperature such that at 13 °C, it was near 1000 μmol m⁻² s⁻¹ (Fig. 1). Subsequent gas exchange analysis was conducted at 1800 μmol photons m⁻² s⁻¹ above 20 °C, and 800–1500 μmol m⁻² s⁻¹ below 20 °C.
Initially, the gas exchange and fluorescence responses of plants grown at 26/16 °C day/night temperature (the control treatment) were compared with responses of plants grown at 26/4 °C for over 28 d (the chilled treatment). The temperature response of CO₂ assimilation in control and chilled plants was unaffected by growth temperature below a measurement temperature of 25 °C, but diverged above a measurement temperature of 30 °C (P < 0.05; Fig. 2). Grasses grown at 26/4 °C reached a maximum photosynthetic rate of 36 μmol m⁻² s⁻¹ at a thermal optimum of 36 °C, which is approximately 10% higher than Aobserved in the 26/16 °C plants. In both chilled and control plants, A and Rubisco activity respond similarly to temperature below 22 °C. Above 25 °C, the temperature response of Rubisco activity diverged from the photosynthetic response, with Rubisco exhibiting a maximum activity that was nearly three times the rate of net CO₂ assimilation at 39 °C (Fig. 2).

Growth at either 26/16 °C or 26/4 °C had no obvious effect on stomatal responses to temperature below 30 °C (Fig. 3). Stomatal conductance in both cold-hardened and control grasses increased from approximately 0.1 mol m⁻² s⁻¹ at 7 °C to 0.3 mol m⁻² s⁻¹ at 30 °C. Relative to the warm-grown plants, the conductance was higher in the 26/4 °C tillers at 33–40 °C (Fig. 3A). The ratio of intercellular to ambient CO₂ remained constant across a range of temperatures in both chilled and non-chilled *M. montana* tillers, despite an increase of VPD above 28 °C (Fig. 3B). As a result, C₁ corresponding to air levels of CO₂ was over 150 μbar for grasses from both growth regimes, a level that was above the CO₂ saturation point of A (Fig. 4).

A reduction in temperature from 33 °C to 13 °C had little effect on the initial slope of the CO₂ response in either treatment; in contrast, the CO₂-saturated plateau of A declined dramatically with temperature reduction from 33 °C to 13 °C (Fig. 4). Consequently, the CO₂ saturation point of A declined with temperature reduction in plants from each growth regime, from approximately 100 μbar at 33 °C to 75 μbar at 23 °C, and then to below 50 μbar at 13 °C.

**The short-term fluorescence response to temperature in *M. montana***

The fluorescence response to decreasing temperature in *M. montana* grown at 26/16 °C showed that photochemical quenching (q₁) declined from 0.93 at 30 °C to 0.22 at 7 °C (Fig. 5B). By contrast, non-photochemical
Fig. 4. The response of the rate of net CO₂ assimilation to variation in intercellular CO₂ partial pressure (Cₖ) in M. montana grown at 26/4 °C and 26/16 °C. Arrows indicate the Cₖ that corresponds to an ambient CO₂ level of 360 µbar (mean ± SE, N = 3–4).

Fig. 5. A representative response of (A) net CO₂ assimilation rate and (B) fluorescence parameters to temperature variation in M. montana grown at 26/16 °C. (C) Relative responses of CO₂ assimilation rate (from A), Rubisco activity (from Fig. 2) and electron transport rate (ETR).

quenching (qₑ) varied little over the thermal spectrum, so that the change in fluorescence yield with temperature largely reflected the change in photochemical quenching. The low variation observed in qₑ reflects the modulation of PPFD to maintain it near the light saturation point of A. Using the yield value, an assumed leaf absorbance of 0.84 and the prevailing PPFD during a measurement, the in vivo temperature response of electron transport rate was expressed relative to the rate at 11 °C to facilitate comparison with A and Rubisco activity (Fig. 5C). Electron transport rate showed a similar relative response to temperature as Rubisco and A below 20 °C, but diverged from the two above this temperature.

Exposure of non-hardened M. montana plants to low temperature

To test the short-term effect of a reduction in temperature on M. montana plants acclimated to 26/16 °C, several tillers were placed in a 4 °C chamber overnight and the temperature and CO₂ response of A was determined the following day. Below 20 °C, there was little difference in the response of A to temperature between the tillers exposed to cold for 1 night versus 28 (Fig. 6A). Above 20 °C, however, the maximum CO₂-assimilation rate was 35% less in the non-acclimated, stressed plants relative to the cold-acclimated plants, and the thermal optimum of A was reduced 7 °C after the first night at 4 °C. The stress treatment also reduced both the initial slope and the CO₂-saturated rate of A by a large amount (Fig. 6B). For example, after the initial 4 °C exposure, A in the non-acclimated plants was reduced by over 60% at a Cₖ of 50 µbar compared to the cold-acclimated grasses. After the first night at 4 °C, dark-adapted Fᵥ/Fₘ declined from 0.77 in the early morning to 0.70 8 h later (Fig. 7). The Fᵥ/Fₘ ratios were near 0.78 after 7 and 12 d of 4 °C night treatment, indicating full recovery. Chlorosis and other leaf discoloration were not observed in any tillers during the chilling exposure.

Discussion

The temperature response of A in plants grown at 26/16 °C and 26/4 °C

Muhlenbergia montana is tolerant of low temperatures given sufficient time to acclimate. Although there was
Fig. 6. (A) The CO$_2$ assimilation rate as a function of leaf temperature in *M. montana* grown at 26°C and subjected to a one-time 4°C overnight temperature the night before the measurements (non-acclimated curves). Dashed lines represent data from Fig. 2 for the cold-acclimated treatment. (B) Net CO$_2$ assimilation rate as a function of intercellular CO$_2$ at the indicated measurement temperatures for *M. montana* exposed to 4°C the night before the measurements. Dashed lines indicate responses from the cold-acclimated plants in Fig. 4 while arrows indicate the intercellular CO$_2$ level corresponding to an ambient CO$_2$ level of 360 μbar (mean ± SE, N = 2).

Fig. 7. Dark adjusted F$_{v}$/F$_{m}$ ratios plotted as a function of time into the daylight period in *M. montana* following a 4°C overnight treatment for the number of days indicated beside each curve. Samples were collected at 4°C (0 h) and subsequently at 23–26°C at 2–8 h after the onset of illumination (N = 5, SE range is ±0.03–0.08).

In C$_4$ plants, Rubisco operates near CO$_2$ saturation, in contrast to C$_3$ species where it operates well below CO$_2$ saturation except at low temperature (Leegood and Edwards, 1996; Pittermann and Sage, 2000). As a result, *in vitro* Rubisco capacity should reflect photosynthetic capacity in C$_4$ plants under conditions where Rubisco is limiting A (von Caemmerer and Furbank, 1999). In *M. montana* (Fig. 2) and *Bouteloua gracilis* (Pittermann and Sage, 2000), Rubisco capacity is very similar to photosynthetic capacity below 18°C, indicating high Rubisco control of A. Above 20°C, Rubisco capacity rises well above A, indicating little metabolic control. This interpretation is also supported by analysis of the CO$_2$ response of A. In *M. montana*, the occurrence of the operational C$_1$ above the CO$_2$-saturation point at all measurement temperatures demonstrates that stomatal limitations have no effect on the temperature response of A below the thermal optimum. Instead, the temperature response of A depends upon the biochemical processes controlling the CO$_2$-saturated rate of A. In C$_4$ species, the CO$_2$-saturated rate of A primarily reflects either the capacity of Rubisco, the capacity of electron transport to regenerate RuBP, or pyruvate-P$_i$ dikinase activity (von Caemmerer and Furbank, 1999). Reductions in Rubisco content by antisense procedures reduce the CO$_2$-saturated plateau of A, but have little effect on the initial slope of the CO$_2$ response of A (Furbank et al., 1996, 1997; von Caemmerer et al., 1997). Consistent with a Rubisco limitation, low temperature had a large effect on the CO$_2$-saturated rate of A, but had little effect on the initial slope of the A versus C$_1$ response.

In C$_4$ plants, PEPCase is a major limitation in the initial slope region of the A/C$_1$ response at low CO$_2$ (von Caemmerer and Furbank, 1999). For example, in *Amaranthus edulis*, PEPCase accounts for nearly 70%...
of the metabolic control of $A$ at a $C_i$ of 30 μbar, but only 20–30% of the control in air at 25°C (Dever et al., 1997). When $A$ is reduced by reductions in PEPCase activity, the initial slope is reduced, as shown by transformation using PEPCase-deficient mutants (Dever et al., 1997) and PEPCase inhibitors (Brown and Byrd, 1993). In *M. montana*, the initial slope of the $A/C_i$ response is little affected by low measurement temperature, indicating little controlling role of PEPCase at air levels of CO$_2$ and cooler temperatures.

The response of $A$ versus $C_i$ in C$_4$ species cannot readily be used to differentiate between limitations in electron transport versus Rubisco capacity, as reductions in each can have similar qualitative effects on the $A/C_i$ response (von Caemmerer and Furbank, 1999). The fluorescence data, however, does evaluate whether electron transport capacity is a principal control at low measurement temperature. When the relative response of Rubisco capacity, $A$ and the electron transport rate are compared, similar relative responses below 20°C were observed, indicating the low temperature response of electron transport rate in *M. montana* is largely a function of the temperature response of photochemical quenching. Changes in photochemical quenching often reflect limitations in carbon metabolism enzymes, for example, limitations in Rubisco capacity (Labate et al., 1990; Furbank et al., 1996). Because the $V_{max}$ of Rubisco appears to establish a maximum ceiling for CO$_2$ fixation, it may also determine the rate of photochemical quenching. The rate of electron transport in vivo could, in turn, be regulated to match this limitation.

The leading mechanism proposed for failure of C$_4$ species to occur in cold climates has centred around cold-lability of PPDK (Long, 1983; Simon and Hatch, 1994). PPDK from warm-adapted species dissociates from its tetramer state upon exposure to temperatures less than 11°C, although PPDK forms from cold-adapted species appear more stable at low temperature (Edwards et al., 1985; Leegood and Edwards, 1996). Modelled responses of $A$ to $C_i$ in C$_4$ plants indicate that a limitation in PEP regeneration via PPDK decreases the CO$_2$-saturated plateau of $A$ in a manner similar to that observed in *M. montana* at lower temperature (von Caemmerer and Furbank, 1999; Fig. 4). Because of this, the results presented here do not rule out a PPDK limitation at low temperature. In cold-acclimated *M. montana* plants, however, there is little evidence that PPDK dominates metabolic control over $A$ at low temperature. Should cold-lability cause PPDK activity to become limiting at low temperature, $A$ in the grasses grown at 26.4°C would be less between 7°C and 36°C relative its value in the grasses grown at 26.16°C. This response was not observed—the CO$_2$ assimilation rate was equal in both chilled and warm-acclimated *M. montana* below 20°C (Fig. 1A). Furthermore, an enhancement of photosynthesis occurred at the thermal optimum of the cold-hardened plants, indicating acclimation to chilling increases the capacity of the step that controls $A$ at the thermal optimum. Should PPDK share in the metabolic control of $A$ at low temperature, its level of control would at most be close to that of Rubisco, given the similarity between the Rubisco $V_{max}$ response and $A$ below 20°C.

While results with *M. montana* show no evidence for a photosynthetic lesion in cold-acclimated plants, there is significant inhibition in the rate of photosynthesis in non-acclimated plants the day after initial exposure to 4°C nights. This inhibition is associated with marked reduction in both the initial slope of the CO$_2$-response of $A$ and the CO$_2$-saturated rate of $A$ above 20°C. Notably, there is little change in $A$ below 18°C where Rubisco control is hypothesized to be high. Photo-inhibition is slight as indicated by a modest reduction in $F_r/F_m$ in the dark. These results indicate that the cause of the reduction in $A$ the day after initial night chilling is not Rubisco capacity nor a photoinhibitory lesion, but may be more likely a problem with electron transport capacity or one of the C$_4$ cycle reactions. Electron transport probably does not become limiting in these conditions, based on gas exchange responses of C$_4$ plants in which electron transport capacity has been selectively reduced. In C$_4$ species, reduction in light intensity disproportionately reduces the rate of electron transport, and this in turn reduces $A$ (von Caemmerer and Furbank, 1999). Light reduction affects only the CO$_2$-saturated plateau of the $A/C_i$ response in C$_4$ plants, not the initial slope (Sage, 1986; Leegood and von Caemmerer, 1989; Peisker and Diez, 1990; Furbank et al., 1996), thus indicating that a disproportionate reduction in the rate of electron transport would have little effect on the initial slope, contrary to what was observed in the cold-stressed plants. The reduction in $A$ at both high and low $C_i$ is consistent with a marked reduction in either the capacity of PEPCase and PPDK (von Caemmerer and Furbank, 1999). The observation that the reduction in $A$ occurs at warmer rather than cool temperatures is also consistent with a reduction in one of the C$_4$ cycle enzymes. At warmer temperatures, the $k_{in}$ of Rubisco for CO$_2$ rises substantially, while the specificity for CO$_2$ declines (Leegood and Edwards, 1996). Because of these changes in Rubisco kinetics, any lesion in the ability of the C$_4$ cycle to concentrate CO$_2$ in the bundle sheath would have a greater effect at warmer, rather than cooler temperatures.

The possible lesion in the C$_4$ cycle of *M. montana* the day after initiating cold treatment is consistent with numerous studies observing cold-induced injury to PEPCase or PPDK in chilled C$_4$ plants. In *Echinochloa crus-galli*, a reduction in both the initial slope and the CO$_2$-saturated portion of the $A/C_i$ curve occurs following transfer of plants to 14/8°C (Potvin et al., 1986).
reduction is associated with a concurrent decrease in PPDK activity (Potvin et al., 1986; Simon, 1987). Loss of PEPCase and PEP carboxykinase activity accounted for a severe reduction in photosynthesis at 25 °C in Zoysia japonica following growth at 10.7 °C (Matsuba et al., 1997). Numerous other reports for PEPCase and PPDK decline in response to cold have been reported in a number of C₄ species such as Panicum maximum and Digitaria sanguinalis (Leegood and Edwards, 1996). Notably, most of the species where cold-induced injury to C₄ cycle enzymes occurred either originated in warm climates, or were associated with short-term responses where little time for acclimation was allowed (Leegood and Edwards, 1996; Pittermann and Sage, 2000).

In any case, during the acclimation process, the lesion that causes the photosynthetic depression in M. montana the day after first chilling is repaired and A recovers, eventually exceeding the rate of photosynthesis of control plants at the thermal optimum. This pattern is consistent with acclimation responses observed in C₄ plants from colder climates such as Spartina anglica (Matsuba et al., 1997), Miscanthus species (Long, 1999), and Bouteloua gracilis from high (but not low) elevation (Bowman and Turner, 1993; Pittermann and Sage, 2000). In contrast to these cold-hardy C₄ grasses, C₃ species from warm habitats such as Zea mays and Zoysia japonica respond to prolonged chilling with increasing reduction of A (Matsuba et al., 1997; Long, 1999).

Temperature effects on the in vivo fluorescence response in Muhlenbergia montana

Photoinhibition is a characteristic symptom of low temperature stress in moderate to high light conditions (Huner et al., 1993). In warm-adapted grasses such as maize, the length of chilling exposure determines the degree of photodamage (Long, 1983, 1999), indicating that thermophilic species cannot easily acclimate to low temperature/high light conditions. By contrast, in M. montana and other cold-adapted C₄ species such as Cyperus longus and Bouteloua gracilis, prolonged exposure to sub-optimal temperatures caused little observable damage to the PSII reaction centres during chilling treatments (Blowers and Baker, 1995; Long, 1999; Pittermann and Sage, 2000). Similarly, in cold-adapted C₃ species, leaves are capable of full recovery of PSII quantum yield following exposure to 4 °C (Somersalo and Krause, 1990; Huner et al., 1993). This is because of increased rates of electron transport and carbon assimilation and the prolonged accumulation of carotenoids, notably zeaxanthin (Adams and Demmig-Adams, 1995). In M. montana, the high level of non-photochemical quenching across a range of temperature indicates that zeaxanthin formation may be a major means of dealing with excess excitation energy. Non-photochemical quenching primarily involves antenna-level dissipation of light energy by zeaxanthin (Demmig-Adams et al., 1996).

Conclusions

In a companion study, photosynthetic responses to low temperature of the Rocky Mountain species Bouteloua gracilis were examined using similar techniques as here (Pittermann and Sage, 2000). Consistent with results from M. montana, the key constraint in B. gracilis below 20 °C appeared to be Rubisco carboxylation, as its thermal response matched closely that of net CO₂ assimilation. Little evidence was observed in either study for high control over A by C₄ cycle enzymes of plants either acclimated to low temperature, or adapted to low temperature and grown in warmer conditions. Together, these results indicate that the failure of the C₄ syndrome in cold environments is not because of a cold-induced lesion, but may be more the result of inferior performance at low temperature that is related to how C₄ plants use Rubisco. The ceiling on A that Rubisco establishes below 18 °C could prevent C₃ species from matching the photosynthetic performance of C₃ competitors in cold climates. To overcome the Rubisco limitation, C₄ plants would need to maintain much higher levels of Rubisco, potentially at considerable ecological cost. Some cold-adapted C₄ grasses such as Spartina and Miscanthus have more Rubisco relative to C₄ plants from warmer environments (Long, 1999), but whether this influences low temperature performance remains to be determined. To evaluate fully whether Rubisco content is a major inherent limitation over C₄ performance in the cold, it will be important to examine the pattern of Rubisco use across a range of temperatures using C₄ genotypes of varying Rubisco content.

Acknowledgements

We are grateful to David Kubien for critical reading of the manuscript, and the technical assistance of Sam Puvendran. This study was supported by an NSERC grant No. OGP0154273 to RFS and a Strong-Hull Scholarship to JP.

References


Acclimation of C_4 plants to chilling temperatures

837

of photosynthetic carbon assimilation in leaves of Zea mays. Planta 178, 258–266.


Leegood RC, von Caemmerer S. 1989. Some relationships between contents of photosynthetic metabolites and the rate
Somersalo S, Krause GH. 1990. Reversible photoinhibition of unhardened and cold-acclimated spinach leaves at chilling temperatures. Plant 
