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Rubisco, Rubisco activase, and global climate change

Rowan F. Sage[1,*], Danielle A. Way[1] and David S. Kubien[2]

[1] *Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada M5S 3B2*

[2] *Department of Biology, University of New Brunswick, Fredericton, NB, Canada E3B 5A3*

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Abstract

Global warming and the rise in atmospheric CO_2 will increase the operating temperature of leaves in coming decades, often well above the thermal optimum for photosynthesis. Presently, there is controversy over the limiting processes controlling photosynthesis at elevated temperature. Leading models propose that the reduction in photosynthesis at elevated temperature is a function of either declining capacity of electron transport to regenerate RuBP, or reductions in the capacity of Rubisco activase to maintain Rubisco in an active configuration. Identifying which of these processes is the principal limitation at elevated temperature is complicated because each may be regulated in response to a limitation in the other. Biochemical and gas exchange assessments can disentangle these photosynthetic limitations; however, comprehensive assessments are often difficult and, for many species, virtually impossible. It is proposed that measurement of the initial slope of the CO_2 response of photosynthesis (the A/C_i response) can be a useful means to screen for Rubisco activase limitations. This is because a reduction in the Rubisco activation state should be most apparent at low CO_2 when Rubisco capacity is generally limiting. In sweet potato, spinach, and tobacco, the initial slope of the A/C_i response shows no evidence of activase limitations at high temperature, as the slope can be accurately modelled using the kinetic parameters of fully activated Rubisco. In black spruce (*Picea mariana*), a reduction in the initial slope above 30 °C cannot be explained by the known kinetics of fully activated Rubisco, indicating that activase may be limiting at high temperatures. Because black spruce is the dominant species in the boreal forest of North America, Rubisco activase may be an unusually important factor determining the response of the boreal biome to climate change.

Key words: Black spruce, climate change, C_3 photosynthesis, *Picea mariana*, Rubisco, Rubisco activase, temperature.

Introduction

The earth's climate is predicted to warm by an average of 2–4 °C during the next century as a result of increased greenhouse gases in the atmosphere (IPCC, 2007). Much of the climate warming associated with greenhouse gas enrichment will occur at higher latitudes, higher elevations, and during the winter. In Canada, for example, the boreal region is predicted to warm by 3–10 °C (IPCC, 2007). Also higher atmospheric CO_2 levels tend to reduce stomatal conductance and transpiration, thereby lowering latent heat loss and causing higher leaf temperatures (Kimball and Bernacchi, 2006; Bernacchi *et al*., 2007). Change in the atmospheric composition and climate will therefore increase the temperature of photosynthetic tissues, particularly in high latitude ecosystems such as the boreal forest. During periods of the year when plants normally experience optimal temperatures, it can also be expected that the new climate regimes will warm leaves well above their thermal optimum, potentially to a point where electron transport and Rubisco activase are heat labile. Such warming will reduce photosynthetic capacity, possibly offsetting gains in carbon assimilation associated with elevated CO_2.

Because climate warming will increase the frequency of supra-optimal temperatures, it is important to understand the limitations on photosynthesis above the photosynthetic thermal optimum, both in the short-term (minutes to hours), as may occur during a heat wave, and over the long term (days to years). Long-term photosynthetic

* To whom correspondence should be addressed. E-mail: r.sage@utoronto.ca
Abbreviations: A, net CO_2 assimilation rate; C_i, the intercellular partial pressure of CO_2; PSII, photosystem II; VPD, vapour pressure difference.

responses can be divided into two categories. First, for individuals, long-term exposure to new thermal regimes can lead to acclimation of the photosynthetic apparatus, allowing for stable or even increased rates of photosynthesis in the new, warmer growth regimes. Secondly, long-term exposure to elevated temperatures over multiple generations leads to natural selection and adaptation of populations to higher temperatures. A major concern for the future is that species will not be adapted to the new climate regimes, leading to widespread die-offs and range shifts (Sykes and Prentice, 1995; Jump *et al*., 2006). Dire scenarios include biome collapses, crop failure, a loss of carbon sequestration, and widespread weed invasions (Patterson *et al*., 1999; Peng *et al*., 2004). Weedy species from warmer regions are capable of rapid migration and can quickly invade ecosystems where native dominants are stressed, thereby aggravating problems arising from climate change. Acclimation could allow natives to adjust to climate warming and maintain themselves, and high genetic diversity in key traits could allow for rapid adaptation of resident populations to climate change (Bradshaw and Holzapfel, 2001; van Dijk and Hautekeete, 2007). While there is uncertainty and controversy regarding the future (e.g. Xu *et al*., 2007), there is little controversy over the need to understand the mechanisms controlling biotic responses to high temperature. Such understanding will improve predictive power while providing directions for adaptation and mitigation.

At the current time, the mechanisms controlling the response of net CO_2 assimilation rate (A) to elevated temperature remain unclear and controversial (Crafts-Brandner and Salvucci, 2004; Schrader *et al*., 2004). As a result, it is difficult to interpret patterns of acclimation and adaptation to elevated temperature and to screen for variation in the critical traits that limit high temperature carbon gain. Numerous hypotheses have been proposed to explain the nature of the photosynthetic controls at high temperature, and each has substantial experimental support. The leading hypotheses for photosynthetic limitation above the thermal optimum are heat lability of Rubisco activase on the one hand, and a limitation in electron transport on the other (Salvucci and Crafts-Brandner, 2004*a*, *b*; Cen and Sage, 2005; Sharkey, 2005; Sage and Kubien, 2007). Other possibilities may be present but have not been sufficiently evaluated; for example, the contribution of the mesophyll diffusive conductance to photosynthetic limitation remains uncertain (Yamori *et al*., 2006*b*; Flexas *et al*., 2007). This review addresses the leading mechanisms proposed to limit photosynthesis above the thermal optimum, and the difficulties in identifying a specific process as a major control over carbon gain. To overcome these difficulties, it is argued that high temperature research should emphasize a holistic approach that simultaneously assesses whole leaf gas exchange and the biochemical capacity of the major processes, and uses theoretical models of photosynthesis to explain observed gas exchange patterns in terms of the biochemical controls. Such approaches have provided clarity in the understanding of the biochemical controls over the light response to photosynthesis, and the acclimation of C_3 photosynthesis to elevated CO_2 (von Caemmerer and Farquhar, 1981; Long *et al*., 2004).

Patterns of the C_3 photosynthetic response to temperature

C_3 photosynthesis typically exhibits a thermal optimum between 20 °C and 35 °C. The optimum is broad at low CO_2, with a shallow peak usually centred in the mid-20 °C range (Fig. 1a). As CO_2 levels increase, the optimum shifts to higher temperatures, and the peak sharpens, such that at high CO_2, photosynthesis exhibits a very sharp peak centred around 30–35 °C in most species (Fig. 1e; Berry and Raison, 1981; Sage, 2002). Species acclimated and/or adapted to cooler temperatures exhibit lower thermal optima, while high temperature-adapted species can exhibit thermal optima exceeding 35 °C at elevated CO_2 (Berry and Raison, 1981; Sage and Kubien, 2007).

A change in the shape of the thermal response of photosynthesis depends upon the individual temperature responses of the diffusion and biochemical limitations controlling the rate of photosynthesis. Also, the temperature responses of photorespiration and dark respiration contribute to the thermal response of A. As temperatures increase, the relative rates of photorespiration and dark respiration increase relative to the *in vivo* capacity for Rubisco carboxylation. This pattern is demonstrated in Fig. 1, which shows a modelled temperature response of the net CO_2 assimilation rate (A), the gross CO_2 assimilation rate, and the rate of RuBP consumption at three different CO_2 levels: 180 μbar (the atmospheric CO_2 level during the last ice age 18 000 years ago), the current CO_2 level of 380 μbar, and a future, high CO_2 level of 700 μbar. The RuBP consumption rate is the sum of the carboxylation and oxygenation rates of Rubisco. The difference between gross photosynthesis and RuBP consumption reflects the inhibition associated with photorespiration (arrow 1 in Fig. 1b). The difference between A and gross CO_2 assimilation indicates the limitation on carbon gain associated with day respiration in the mitochondria (arrow 2 in Fig. 1b). At low CO_2, the rise in day respiration with temperature has a proportionally larger effect due to the relatively low photosynthetic rate (Fig. 1a, b). Also, the relative effect of photorespiration is much greater at low CO_2 due to the relative lack of CO_2 to inhibit RuBP oxygenation (compare parts b and f in Fig. 1). Inhibition of photorespiration becomes substantial at elevated CO_2 levels such as 700 μbar. Under these conditions, the effect of the underlying biochemical

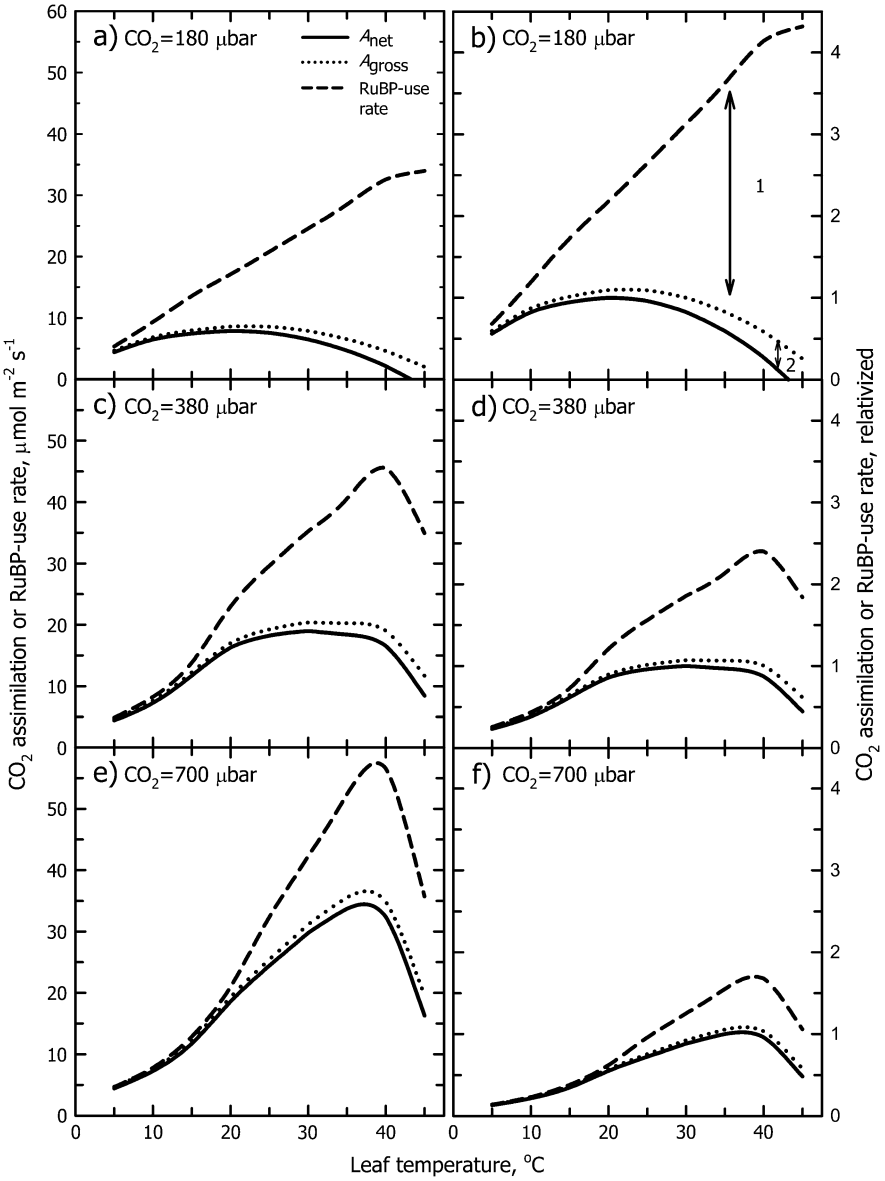

Fig. 1. The modelled temperature response of the net (A_{net}, continuous lines) and gross (A_{gross}, dotted lines) CO_2 assimilation rates, and the RuBP consumption rate at three CO_2 levels. (a), (c), and (e) are absolute responses, and (b), (d), and (f) are the same responses divided by the net CO_2 assimilation rate at the thermal optimum. At 25 °C the parameters for each curve were V_{cmax}=80 μmol m^{-2} s^{-1}, J_{max}=150 μmol m^{-2} s^{-1}, T_p=10 μmol m^{-2} s^{-1}, and the day respiration rate (R_d)=1 μmol m^{-2} s^{-1}. Temperature corrections follow Bernacchi *et al.* (2001) for Rubisco-limited *A*, Bernacchi (2003) for RuBPregeneration-limited *A*, and Hendrickson *et al.* (2004) for triose-phosphate use-limited *A*. The rate of RuBP consumption is the sum of the Rubisco carboxylation and oxygenation rates, where the oxygenation rate, V_o, was calculated as: $V_o=(2\Gamma_*/C_i)\times[\min(W_c, W_j, W_p)]$ where W_j is the electron transport-limited carboxylation rate and W_p is the triose-phosphate use-limited rate of carboxylation. Model details are described in Appendix 1.

limitations, rather than photo- and day respiration, dominates the thermal response of photosynthesis (Fig. 1f; see also Sharkey, 1988; von Caemmerer, 2000).

Diffusion limitations

Two categories of diffusion limitation on *A* are also recognized. The first is diffusion of CO_2 from the atmosphere and through the boundary layer and stomata to the intercellular spaces. The conductance to CO_2 diffusion by the stomata is the major regulatory control over this limitation, and hence it is emphasized in gas exchange analyses. Stomatal limitations tend to be greater at elevated temperature for two reasons. First, the increase in the CO_2 response slope of Rubisco-limited *A* and RuBPregeneration-limited *A* at elevated temperatures enhances the degree to which a given stomatal conductance limits *A* (Farquhar and Sharkey, 1982; Sage and Sharkey,

1987). To avoid an increase in stomatal limitation, the stomatal conductance would generally have to increase with temperature. Secondly, as temperatures increase, the vapour pressure difference (VPD) between leaf and air rises exponentially. This rise in VPD can reduce stomatal conductance, causing a reduction in intercellular CO_2 and A as temperatures increase (Berry and Bjorkman, 1980; Fredeen and Sage, 1999). Declines in A due to high VPD-induced stomatal closure are common on hot afternoons, and explain much of the phenomenon known as midday stomatal closure (Pons and Welschen, 2003; Tay *et al*., 2007). At relatively low VPD (<1.5 kPa, for example), however, stomata often open relative to photosynthesis at elevated temperatures, and hence do not appear to be a major controller of the thermal response of photosynthesis in humid environments (Berry and Bjorkman, 1980; Sage and Sharkey, 1987; Cowling and Sage, 1998; Fredeen and Sage, 1999; Hendrickson *et al*., 2004; Yamori, 2006*a*; Kubien and Sage, 2008). In species where the stomata open as temperatures increase (such as chilli pepper), the rise in intercellular CO_2 can be so large that it offsets the reduction in A due to photorespiration, producing a relatively flat temperature response curve of A with a higher thermal optimum (Sage and Sharkey, 1987).

The second category of diffusion limitation is the mesophyll transfer conductance, which reflects the limitations to CO_2 movement from the intercellular air spaces to the chloroplast stroma (Evans and Loreto, 2000). The mesophyll transfer conductance may also reflect contributions of carbonic anhydrase to CO_2 influx, and limitations on the rate at which CO_2 dissolves; hence, it is not solely a diffusion limitation. Mesophyll transfer limitations have long been assumed to vary little over the short term in a mature leaf; however, recent work indicates they may rapidly respond to changes in a number of environmental conditions, such as intercellular CO_2 (Flexas *et al*., 2007). Bernacchi *et al*. (2002) show that, in tobacco, mesophyll conductance can decline sharply above the photosynthetic thermal optimum of photosynthesis; however, no such declines were noted in oak (Warren and Dreyer, 2006). In spinach, mesophyll conductance declines slightly above the thermal optimum in cool-grown but not in warm-grown plants (Yamori *et al*., 2006*a*). No study has examined mesophyll conductance above 40 °C, but because mesophyll conductance is influenced by aquaporins and carbonic anhydrase (Evans and Loreto, 2000; Terashima *et al*., 2006; Yamori *et al*., 2006*a*), it could become a major limitation at elevated temperature should these proteins be heat labile. Given the lack of evidence for high temperature responses of mesophyll conductance, it is not discussed further, beyond noting that research is needed to delineate the contribution of mesophyll conductance to high temperature photosynthesis.

Biochemical limitations

The predominant controls over the photosynthetic thermal response are the biochemical components of the photosynthetic apparatus. For clarity, the main biochemical processes proposed to limit A at elevated temperatures are segregated into three general categories. The first category represents limitations predicted by the family of models derived from the theoretical interpretations of Farquhar *et al*. (1980) as modified by Sharkey (1985) and co-workers (Harley and Sharkey, 1991) to account for feedback effects from starch and sucrose synthesis (for various versions of the Farquhar *et al*. temperature model, see also Kirschbaum and Farquhar, 1984; von Caemmerer, 2000, Bernacchi *et al*., 2001, 2003; Medlyn *et al*., 2002; Cen and Sage, 2005; Hikosaka *et al*., 2006). These models universally assume constant and high activation states of Rubisco, and divide the limitation on A into the capacity of Rubisco to consume RuBP, the capacity of light harvesting and electron transport to regenerate RuBP, and the capacity of starch and sucrose synthesis to regenerate P_i for photophosphorylation. The second category represents control over photosynthesis by the heat lability of Rubisco activase. Heat lability of Rubisco activase has not been widely incorporated into the Farquhar *et al*. series of models, but can easily be by adjusting the fully activated carboxylation capacity (W_c) to account for the number of deactivated catalytic sites of Rubisco, as shown in equation (1) (Sage, 1990):

$$W_c' = [V_{cmax}(act)\ (C)]/[C+K_c(1 + O/K_o)] \qquad (1)$$

In equation (1), the *in vivo* carboxylation rate of Rubisco (W_c') is a function of the maximum catalytic capacity of Rubisco (V_{cmax}), the activation state of Rubisco (*act*, in ratio form, not as a percentage), the stromal CO_2 concentration (C), the Michaelis constants for carboxylation (K_c) and oxygenation (K_o), and the O_2 concentration in the stroma (O). The value of W_c' is then used to model A as shown by Farquhar *et al*. (1980) in place of the fully activated carboxylation capacity. The activation state of Rubisco is easily determined by rapidly extracting Rubisco and measuring its initial activity relative to the activity of the same extract after incubation with saturating levels of CO_2 and magnesium (Sage *et al*., 1993). There is as yet no means to model the relationship between the activation state of Rubisco and the capacity of Rubisco activase.

Temperature and the limitations arising from the capacities of Rubisco versus RuBP regeneration

The family of models derived from the two-limitation model of Farquhar *et al*. (1980) has provided a comprehensive ability to explain the biochemical controls over the photosynthetic temperature response—assuming the underlying assumptions are met. As such, they need to be

considered first in order to provide context for deviations that may occur should Rubisco activase become limiting.

At low CO_2, the Rubisco capacity to consume RuBP is the predominant limitation on photosynthesis across a wide range of temperatures (Kirschbaum and Farquhar, 1984; von Caemmerer, 2000; Hikosaka *et al*., 2006). The Rubisco limitation generally corresponds to CO_2 levels that are below the Rubisco K_m for CO_2. When an enzyme experiences substrate levels below the K_m, it typically has a low thermal dependence because the K_m and V_{cmax} have similar temperature responses (Berry and Raison, 1981). The Q_{10} values for the K_m and V_{cmax} of Rubisco are near 2, except at low temperature (<17 °C) where it can increase to over 3 in some species (Sage, 2002). The similar Q_{10} of the Rubisco K_m and V_{cmax} causes the initial slope of the CO_2 response of A to vary little with temperature between 15 °C and 35 °C (Berry and Raison, 1981). As a result, the temperature response of A at low CO_2 (<300 μbar) has a shallow slope and broad thermal optimum (Fig. 1a). As CO_2 levels rise, photosynthesis can become limited by other processes in the chloroplast, notably the ability of starch and sucrose synthesis to regenerate P_i for photophosphorylation, and the ability of electron transport to support RuBP regeneration. Both P_i regeneration and electron transport capacity have high thermal dependencies. Electron transport is typically observed to have a Q_{10} near 2 below its thermal optimum, while starch and sucrose synthesis can have Q_{10} values between 2 and 2.8 (Stitt and Grosse, 1988; Leegood and Edwards, 1996). The imposition of these limitations following CO_2 enrichment is associated with the dramatic rise in the thermal dependency of photosynthesis (Fig. 1e). P_i regeneration limitations are commonly associated with the thermal response in photosynthesis at low temperature and elevated CO_2; however, P_i regeneration capacity rapidly acclimates to low temperature in many species and may be more of a short-term phenomenon (Sage and Sharkey, 1987; Leegood and Edwards, 1996; Savitch *et al*., 2001; Hendrickson *et al*., 2004). Electron transport limitations putatively control the thermal response of photosynthesis at high CO_2 around the region of the thermal optimum and above (Cen and Sage, 2005; Sharkey and Schrader, 2006). The thermal response of A in intact leaves matches the thermal response of electron transport at elevated CO_2, and acclimation shifts in electron transport often match the acclimation shifts in high CO_2 photosynthesis (Mawson and Cummins, 1989; Yamasaki *et al*., 2002; Wise *et al*., 2004; Cen and Sage, 2005; Sage and Kubien, 2007)

At current levels of atmospheric CO_2, the control of the thermal response of photosynthesis is typically a mixture of Rubisco, electron transport, and P_i regeneration limitations (Sage and Kubien, 2007). Figure 2 illustrates this in tobacco, showing that at low temperature the modelled P_i regeneration capacity explains observed rates

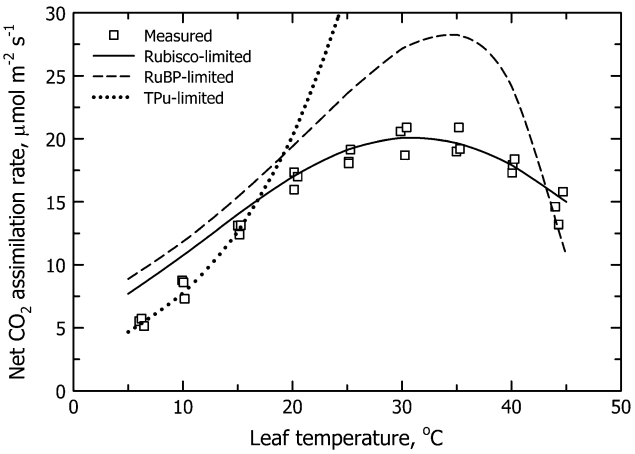

Fig. 2. The temperature response of the net CO_2 assimilation rate in tobacco. Measurements (squares) were made at 370 μbar CO_2 and 210 mbar O_2, with a light intensity of 1400 μmol m^{-2} s^{-1}. Modelled responses (lines) were determined as described in Appendix 1. The modelled response of Rubisco-limited A (solid line) was based on a V_{cmax} of 80 μmol m^{-2} s^{-1}, determined from assays of Rubisco activity *in vitro* (Kubien and Sage, 2008). The RuBP-limited estimate of A (dashed line) was based on a J_{max} of 175 μmol m^{-2} s^{-1} at 25 °C which was determined from the A versus C_i response. The thermal response of A limited by the triose-phosphate use capacity (dotted line) was based on a triose-phosphate use (TPu) rate of 11 μmol m^{-2} s^{-1} at 25 °C; this value was chosen to fit these data. Model estimates were based on an R_d of 1 μmol m^{-2} s^{-1} at 25 °C, and C_i was set to 300 μbar, the average measured value.

of A, while the modelled Rubisco-limited A explains observed A at the thermal optimum. When Rubisco capacity is limiting photosynthesis, the decline in A above the thermal optimum reflects a rise in photo- and day respiration; however, the reduction in electron transport capacity with rising temperature above the thermal optimum is large enough to allow RuBP regeneration capacity to become limiting above 40 °C (Fig. 2). When electron transport becomes limiting above the thermal optimum, the rate of A in intact leaves typically shows a steep decline as temperatures increase, reflecting the high temperature dependence of the electron transport rate (Sage *et al*., 1995; Yamasaki *et al*., 2002; June *et al*., 2004; Cen and Sage, 2005; Yamori *et al*., 2005).

The reason for the decline in the electron transport capacity above the thermal optimum remains uncertain. Direct injury to the thylakoid protein complexes is unlikely to be the main cause, because direct heat injury occurs at higher temperatures (>40 °C) than those where electron transport and A are inhibited by rising temperature (>35 °C; Berry and Bkorkman, 1980; Yamasaki *et al*., 2002; Sharkey and Schrader, 2006). Instead, the mechanisms are proposed to arise from increased proton leakiness across the thylakoid membrane, or altered interactions between membranes and the thylakoid protein complexes (Bukhov *et al*., 1999; Sharkey and Schrader, 2006). In response to these immediate effects, cyclic electron flow is proposed to be up-regulated at the

expense of linear electron transport. This maintains ATP synthesis and the thylakoid pH gradient, but leads to a loss of linear electron transport because inter-chain electron carriers and photosystem I complexes are diverted from linear electron flow to support cyclic electron flow (Bukhov *et al*., 1999). The high *trans*-thylakoid proton gradient maintained by cyclic electron flow at elevated temperature down-regulates photosystem II (PSII) by enhanced photochemical quenching and aggregation of PSII complexes (Havaux, 1998; Sharkey and Schrader, 2006; Tang *et al*., 2007).

Limitations by Rubisco activase at elevated temperature

To begin this discussion, it is necessary to provide a clear definition of an activase limitation versus a Rubisco limitation, as interpretations of the concept vary. A Rubisco limitation is the limitation on photosynthesis arising from a limited capacity of fully activated Rubisco to consume RuBP. Fully activated Rubisco in practice refers to an activation state above 90%, because a small fraction of sites are not carbamylated and are thus inactive at any given time (Sage *et al*., 1990, 1993). An activase limitation occurs when the capacity of Rubisco to consume RuBP is reduced by deactivation of catalytic sites to such an extent that RuBP consumption capacity falls below RuBP regeneration capacity. An electron transport limitation, by contrast, occurs when *A* is limited by the capacity of electron transport to regenerate RuBP for the carboxylation reaction.

A universal observation made in plants exposed to current CO_2 levels or above is that increasing temperatures above the thermal optimum of *A* reduce the activation state of Rubisco. This occurs in parallel with declines in photosynthetic capacity at elevated temperature, leading to conclusions that the decline in photosynthesis above the thermal optimum is a result of Rubisco deactivation (Law and Crafts-Brandner, 1999; Salvucci and Crafts-Brandner, 2004*a*, *b*; Yamori *et al*., 2005). Rubisco deactivation above the thermal optimum has been observed in *Arabidopsis* (Kim and Portis, 2005; Salvucci *et al*., 2006), spinach (Weis, 1981; Yamori *et al*., 2006*b*), wheat (Kobza and Edwards, 1987; Law and Crafts-Brandner, 1999), cotton (Feller *et al*., 1998; Crafts-Brandner and Law, 2000), tobacco (Crafts-Brandner and Salvucci, 2000; Sharkey *et al*., 2001), oak (Haldimann and Feller, 2004), pea (Haldimann and Feller, 2005), sweet potato (Cen and Sage, 2005), and rice (Makino and Sage, 2007). This phenomenon is not due to the heat lability of Rubisco, which is heat stable to at least 50 °C (Crafts-Brandner and Savlucci, 2000). Instead, a decline in the activity of Rubisco activase allows for deactivation of Rubisco at high temperatures (Salvucci and Crafts-Brandner, 2004*a*, *b*). At all temperatures, Rubisco activase maintains Rubisco in its active configuration by removing metabolites, such as RuBP, carboxyarabinitol 1-phosphate, and xylulose-1,5-bisphosphate, thereby promoting carbamylation and full activity of the catalytic sites (Portis, 1995, 2003). At moderately high temperatures, Rubisco deactivation is proposed to occur because the production of inhibitory compounds (e.g. xylulose-1,5-bisphosphate) exceeds the capacity of activase to remove them from the catalytic sites of Rubisco (Salvucci and Crafts-Brandner, 2004*a*, *b*). Above a species-specific temperature, Rubisco activase itself denatures, forming insoluble aggregates that are incapable of removing inhibitors and enhancing Rubisco activation (Feller *et al*., 1998; Salvucci *et al*., 2001). Consequently, a high activation state of Rubisco cannot be maintained.

Regulatory interactions between electron transport capacity, Rubisco activase, and photosynthesis: which leads and which follows?

In environmental physiology, there has often been a tendency to assign a mechanistic limitation when the rate of a biochemical process is well correlated with the rate of photosynthesis. For example, the correlation between the decline in the activation state of Rubisco and *A* is used as an argument that Rubisco activase limits *A*. Because photosynthesis is a highly regulated system, correlation alone cannot be used as a definitive indication of limitation. Rubisco activase is well known to be heavily regulated in response to energy supply from the thylakoids, while the reactions of electron transport are regulated in response to the capacity of carbon metabolism to consume ATP and reductant (Sharkey *et al*., 1988; Portis, 2003; Avenson *et al*., 2005). To ascertain whether Rubisco capacity, Rubisco activase, P_i regeneration, or the electron transport rate is limiting, it is necessary to estimate the capacity of each and the activation state of Rubisco, and to model the temperature response of *A* using input parameters based on these biochemical measurements. While easily stated, comprehensively measuring and modelling the necessary parameters across a range of temperatures has proven elusive, and only a few comprehensive attempts are presented in the literature (e.g. Wise *et al*., 2004; Cen and Sage, 2005; Yamori *et al*., 2005, 2006*a*). Tobacco temperature responses have been the most heavily studied (von Caemmerer and Quick, 2000; Bernacchi *et al*., 2001, 2002, 2003), and, as a result, parameters determined for this species are widely used in recent efforts to model the temperature response of *A*. (e.g. Medlyn *et al*., 2002; Hikosaka *et al*., 2006). A major limitation to modelling the thermal response of *A* in natural populations is that the kinetic constants of a given species Rubisco are likely to be unknown and may be different from what exists in the

few species where the thermal dependence of the Rubisco kinetics has been determined (namely spinach, *Atriplex*, bean, and tobacco; von Caemmerer and Quick, 2000; Bernacchi *et al*., 2001). The thermal response of the Rubisco kinetic coefficients varies enough between species to cause substantial variation in the modelled response of Rubisco-limited *A* to temperature (von Caemmerer and Quick, 2000); hence, accurate kinetic values are needed.

Measurements of electron transport capacity as a function of temperature are often compromised because they are commonly generated using gas exchange measurements under non-photorespiratory conditions (high CO_2 and low O_2), or via pulse-modulated fluorescence (Bernacchi *et al*., 2003; June *et al*., 2004; Hikosaka *et al*., 2006). In both procedures, a key assumption is that electron transport is limiting photosynthesis under the measurement conditions. If it is not, then the capacity of electron transport will be underestimated. At cooler temperatures, P_i regeneration is commonly limiting and hence electron transport estimates cannot be trusted unless it is specifically demonstrated that P_i regeneration is not a limiting factor (Sage and Sharkey, 1987; Sun *et al*., 1999; Savitch *et al*., 2001; Hendrickson *et al*., 2004). At elevated temperature, a limitation by Rubisco activase could result in an over-energization of the thylakoid apparatus and promote the down-regulation of PSII and electron transport (Law and Crafts-Brandner, 1999). For these reasons, direct assessment of electron transport capacity *in vitro* may be frequently required to assess electron transport limitations at moderately high and low temperatures. Similarly, estimations of Rubisco activase limitations, either directly or through the assessment of the activation state of Rubisco, can be compromised if Rubisco activase is being down-regulated in response to a limitation in electron transport capacity. For high activity, activase requires a high ATP/ADP ratio and, in many species, a supply of reductant (Portis, 2003). At the thermal optimum, a reduction in electron transport capacity by shading reduces activase activity, and the activation state of Rubisco declines as a result (von Caemmerer and Quick, 2000; Portis, 2003). Presumably, these regulatory interactions are present above the thermal optimum, raising the possibility that the heat sensitivity of activase is a regulatory interaction mediated by temperature effects on the thylakoid reactions and ATP supply. Consistent with this possibility, Salvucci *et al.* (2001) showed that adding an ATP analogue to purified tobacco activase increased the thermal stability of activase by over 5 °C *in vitro*. Alternatively, if activase is heat labile regardless of the energy supply, then there would be a strong reduction in the use of reductant and ATP by carbon metabolism, and this would feedback onto electron transport, creating high photochemical quenching that would slow electron flow through PSII (Havaux, 1998; Bukhov *et al*., 1999). Even with good models and measurements, untangling this regulatory interplay to identify the ultimate source of limitation at high temperature is likely to be a major challenge.

One approach to untangling regulated versus limiting factors is to heat leaves rapidly to over 40 °C and examine the time course of the resulting changes in activation states and metabolite pools during the transition to a new steady state. Time course approaches have been successfully used to identify RuBP regeneration limitations at low light and elevated CO_2 at the thermal optimum. Following a rapid change from high to low light, or low to high CO_2, there is a rapid decline in RuBP pool size, which recovers while Rubisco deactivates (Perchorowicz *et al*., 1981; Mott *et al*., 1984; Kobza and Edwards, 1987; Sage *et al*., 1988; Woodrow and Berry, 1988). During the period where the activation state of Rubisco declines, the CO_2 assimilation rate and the estimated electron transport rate are unchanged (Mott *et al*., 1984; Sage *et al*., 1988; Sharkey *et al*., 1988). Time course studies of photosynthetic responses to sudden heat transients are limited and contradictory, and further efforts are needed to exploit the approach fully. In Pima cotton, Schrader *et al.* (2004) observed a decline in RuBP pools, but not the activation state of Rubisco, following a rapid (7 s) increase in leaf temperature from 30 °C to 42 °C; upon Rubisco deactivation, the metabolite levels recovered. By contrast, in cotton, Crafts-Brandner and Law (2000) and Crafts-Brandner and Salvucci (2004) observed a very rapid decline in the activation state within seconds of heat stress at 42 °C.

A second approach is to force the system to be limited by Rubisco, Rubisco activase, or RuBP regeneration by using antisense plants deficient in one of these components. Numerous antisense constructs are available, but very few have been utilized in temperature-related work (Raines, 2006). Recently, Rubisco antisense constructs to the Rubisco small subunit in tobacco and rice have been used to explore limitations on photosynthesis at elevated temperature (Makino and Sage, 2007, Kubien and Sage, 2008).

Compared with wild-type lines, small-subunit antisense genotypes impose a strong Rubisco limitation on *A* (von Caemmerer and Quick, 2000), and hence should favour the reactivation of Rubisco at elevated temperature if the activation state in the wild type were reduced by a limitation in the RuBP regeneration capacity at high temperature. Antisense rice and tobacco lines with 30–35% of the wild-type Rubisco content were able to maintain significantly higher activation states of Rubisco at 40–42 °C than wild-type lines, which is consistent with the hypothesis that Rubisco is a regulated response to an electron transport limitation at elevated temperature (Makino and Sage, 2007; Kubien and Sage, 2008). In rice, Makino and Sage (2007) observed that the activation

state of Rubisco declined with increasing Rubisco content, where the content was varied with antisense and supersense constructs to the Rubisco small subunit gene. Increasing temperature from 25 °C to 41 °C reduced the activation state at each Rubisco content level, but did not change the response slope between activation state and content (Fig. 3). Makino and Sage (2007) interpreted these results to indicate that heat lability of Rubisco activase was not the primary control over Rubisco activation state at 41 °C, because the response slope between activation state and temperature should be steeper at 41 °C than 25 °C. Also, the flux control coefficient for Rubisco declined in concert with the decline in the activation state, indicating that the decline in the activation state of Rubisco was a regulated response to a limitation elsewhere in the photosynthetic apparatus. In tobacco, supporting evidence for the interpretation that the decline in activation state is a regulated response is apparent in a comparison of measured and modelled CO_2 responses of A in wild-type and antisense lines (Fig. 4). The A/C_i responses of the wild-type and antisense lines in tobacco were modelled according to Bernacchi *et al*. and input parameters measured for the tobacco lines (Bernacchi *et al*., 2001, 2003; see Appendix 1). At both 32 °C and 42 °C and lower C_i values, measured A/C_i responses were similar to modelled responses that assumed that the capacity of fully activated Rubisco was limiting (Fig. 4). At higher C_i values, measured responses were similar to modelled responses assuming electron transport capacity was limiting. No deactivation was needed to explain the observed A/C_i responses in tobacco in either the wild-type or Rubisco antisense lines.

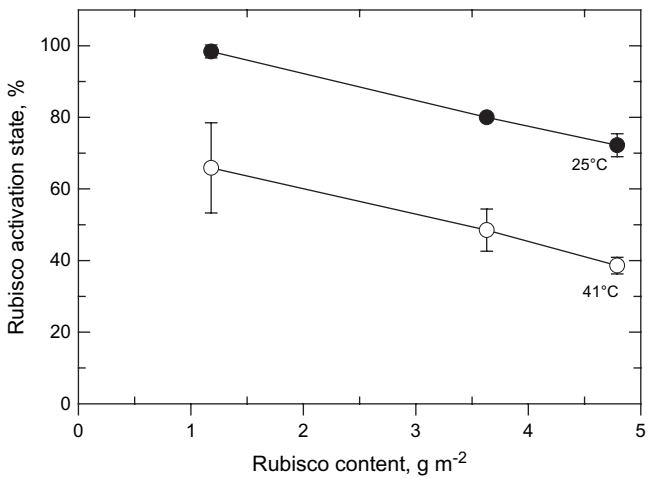

Fig. 3. The activation state of Rubisco as a function of Rubisco content at 25 °C and 41 °C in rice (*Oryza sativa*) grown in a greenhouse during summer at ~30 °C. Rubisco content was varied by antisense and supersense constructs to the Rubisco small subunit. Means ±SE, n=3. Activation state is measured as the ratio of initial activity to total Rubisco activity in crude leaf extracts. (From figure 7 of Makino and Sage (2007) with permission from Oxford University Press.)

A third approach to studying high temperature limitations is to force the system into a Rubisco limitation by lowering the atmospheric CO_2 content to such a degree that the RuBP consumption capacity falls below the RuBP regeneration capacity. If Rubisco activase is down-regulated in response to a limitation in electron transport capacity at elevated temperature, this approach would alleviate the electron transport limitation, and allow the activation state of Rubisco to recover to maximum levels at low CO_2 (Sage, 1990; Sage *et al*., 1990). If activase is limiting, then it should not be possible to reactivate

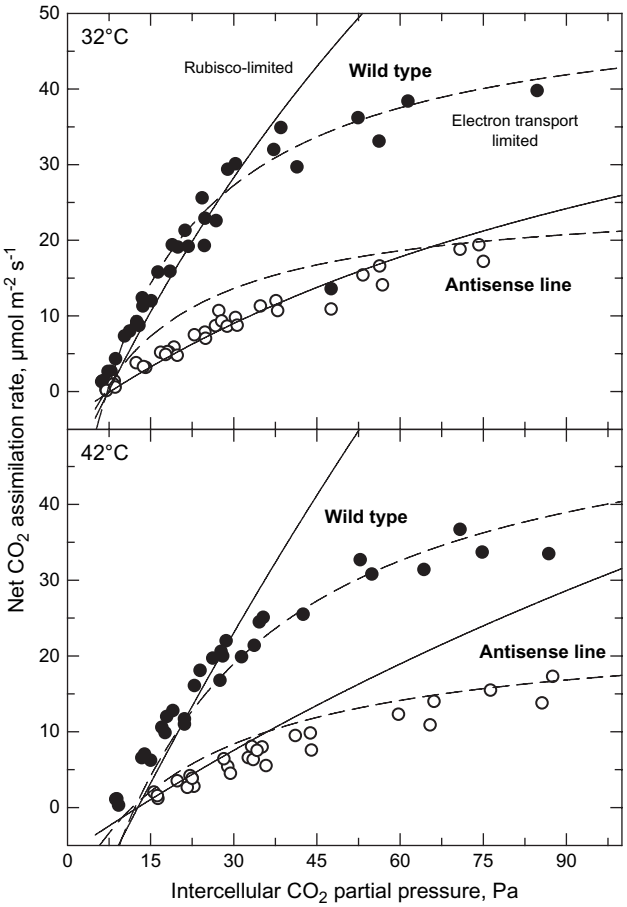

Fig. 4. The response of net CO_2 assimilation rate (A) to intercellular CO_2 in WT (filled circles) and anti-*rbc*S (open circles) tobacco at 32 °C (top) and 42 °C (bottom). Measurements were made at 210 mbar O_2. Continuous lines indicate the modelled Rubisco-limited A assuming fully activated Rubisco, based on *in vitro* V_{cmax} estimates of 110 µmol m^{-2} s^{-1} (wild type) and 31 µmol m^{-2} s^{-1} (anti-*rbc*S), which were obtained by the incorporation of $^{14}CO_2$ into acid-stable products at 25 °C. The temperature dependency of V_{cmax} is described in Appendix 1. Dashed lines indicate the RuBPregeneration-limited rate of A. For the wild-type line, J_{max} at 25 °C was determined to be 175 µmol m^{-2} s^{-1} by gas-exchange measurements; this value was adjusted to different temperatures as described in Appendix 1. For the anti-*rbc*S line, the electron transport rate (J) was calculated from chlorophyll fluorescence measurements made between 15 °C and 45 °C at 76 Pa CO_2 (Kubien and Sage, 2008), following June *et al*. (2004). At 25 °C, J_{max} in the antisense line was 98 µmol m^{-2} s^{-1}. Day respiration (R_d) was assumed to be 1% of V_{cmax} (von Caemmerer, 2000).

Rubisco; if anything, the CO_2 reduction should allow for further deactivation due to lower levels of carbamylating CO_2 and higher RuBP pool sizes (Mate *et al*., 1996). Consistent with the hypothesis that the activation state of Rubisco is down-regulated at high temperature in response to limitations in electron transport, Cen and Sage (2005) showed that CO_2 reduction fully reactivated Rubisco in sweet potato leaves at 40 °C. Also, in cotton and tobacco at 37 °C, Crafts-Brandner and Salvucci (2000) observed a higher activation state of Rubisco at the lower measurement CO_2 values than at high CO_2. In sweet potato (Cen and Sage, 2005) and tobacco (Kubien and Sage, 2008), modelled temperature responses of electron transport-limited A were able to predict observed A at high temperature and, in both species, the observed deactivation of Rubisco corresponded to temperatures where the modelled RuBP regeneration capacity exhibited large declines.

The effects of lowering CO_2 can also be evaluated by comparing measured and modelled initial slopes of the photosynthetic A/C_i response. At light saturation, the initial slope of the A/Ci response generally corresponds to the range of CO_2 levels where Rubisco capacity is limiting for A. Consequently, Rubisco capacity is directly proportional to the magnitude of the initial slope of the A/C_i response at light saturation, a feature which has been exploited to estimate Rubisco V_{cmax} from initial slope determinations (von Caemmerer and Farquhar, 1981; Wullschleger *et al*., 1993; Medlyn *et al*., 2002). When Rubisco is limiting, the initial slope is described as follows (von Caemmerer, 2000):

$$\text{initial slope} = dA/dC = V_{cmax}/[\Gamma* + K_c(1 + O/K_o)] \quad (2)$$

The terms are as given for equation (1) except for Γ_* which is the CO_2 compensation point in the absence of day respiration. For accurate modelling, good estimates of the thermal dependencies of Γ_* and the kinetic constants of Rubisco (K_c, K_o, and V_{cmax}) are required. The models also require constant mesophyll conductance, and the high activation state of Rubisco at low intercellular CO_2. While both assumptions require validation, there are few indications in the literature that the assumptions are invalid (but see Flexas *et al*., 2007).

V_{cmax} can be easily obtained with *in vitro* assays; alternatively, one can estimate V_{cmax} at 25 °C from the initial slope and then predict the value at different temperatures using published thermal dependencies of Rubisco (van Caemmerer and Quick, 2000). As long as the species of interest has similar kinetic constants to one of the published species, the modelled temperature response of the initial slope should approximate the measured temperature response over much of the thermal range. If activase becomes limiting for A, however, then it should lead to an immediate reduction in the initial slope because of reductions in RuBP consumption capacity as Rubisco deactivates. The initial slope response that is modelled assuming fully activated Rubisco would then exceed the measured response at elevated temperature; correcting the V_{cmax} term by multiplying it by the activation state of Rubisco should restore the predictive power of the model.

This technique to assess activase limitations was first used in sweet potato (Cen and Sage, 2005). In sweet potato, modelled temperature responses of the initial slope based on *in vitro* V_{cmax} measurements matched the measured temperature response between 10 °C and 41 °C (Fig. 5A). Figure 5B presents the temperature response of the initial slope of the A/C_i response in tobacco and spinach. The tobacco data were measured by Kubien and Sage (2008) using the Li-Cor 6400, *in vitro* Rubisco V_{cmax} measurements, and the published kinetic and Γ_* data for tobacco used by Bernacchi *et al*. (2001, 2003). Spinach data were measured by Yamori *et al*. (2005, 2006*a*), and modelled using their published V_{cmax} and Γ_* responses to temperature for cold-grown spinach. In both species, there was close agreement up to 42 °C between measured responses and modelled responses assuming that Rubisco was fully activated (Fig. 5B). No limitation arising from activase is apparent in these species. Notably, there were substantial differences in the initial slope response of sweet potato, tobacco, and spinach, with the cool-adapted spinach having a lower thermal optimum of the initial slope, and a more pronounced reduction at elevated temperature than the warm-adapted tobacco and sweet potato (Fig. 5). These results highlight the substantial effect that different Rubisco enzymes can have on the photosynthetic temperature response at low CO_2.

The initial slope versus temperature in black spruce [*Picea mariana* (Mill.) B.S.P.]

The concern that global warming will have large negative impacts on natural species, particularly those at high latitude, leads us to evaluate the temperature response of photosynthesis in black spruce, the predominant primary producer in the boreal forest of North America. In addition to being the major dominant species, black spruce is also the most important timber species to the Canadian economy, and serves as a major sink for carbon given its widespread distribution and ability to form dense, long-lived stands (Fig. 6). In Eurasia, related spruce species also form extensive stands that have importance for the regional economies and ecosystems (Wenhua and Pichuen, 1984). The boreal biome is the single largest biome in the northern hemisphere, and is considered to be at substantial risk because of the enhanced degree of climate warming predicted for boreal latitudes (ACIA,

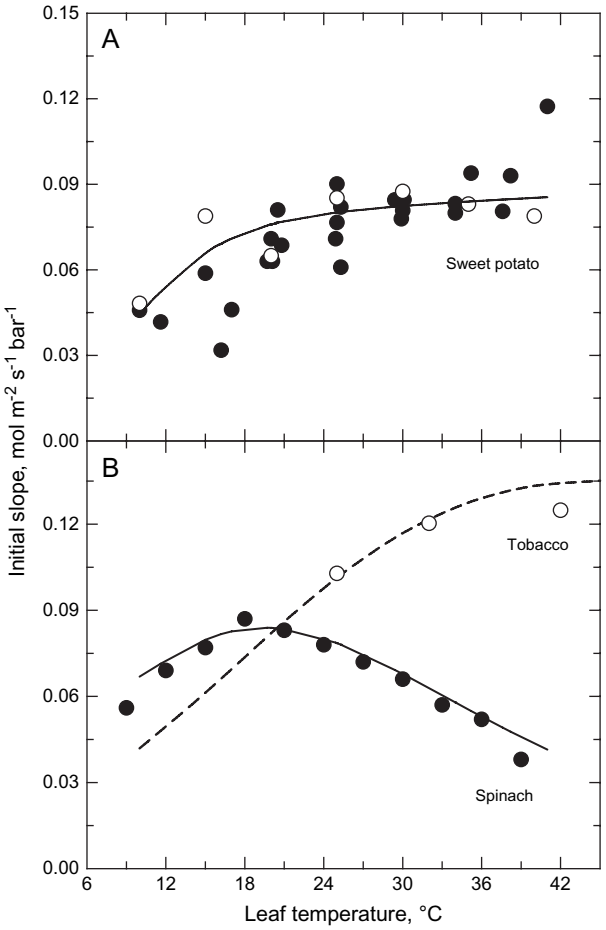

Fig. 5. The temperature response of the initial slope of the CO_2 response of net CO_2 assimilation rate in (A) sweet potato (*Ipomea batatas*) and (B) spinach and tobacco. Sweet potato data are from Cen and Sage (2005), where the filled symbols are measured data, the open symbols are modelled data from *in vitro* Rubisco activity assays, and the curved line is modelled data using the activation energies of the Rubisco V_{cmax}. The spinach data (filled symbols) are from Yamori *et al.* (2006*a*), and the modelled spinach response (continuous curve) was generated with the measured temperature response of Γ_* and V_{cmax} of cold-grown spinach (Yamori *et al.*, 2005, 2006*a*), and the spinach kinetic constants published in von Caemmerer and Quick (2000). Tobacco data were measured with a Li-Cor 6400 and modelled according to Bernacchi (2000), using Rubisco V_{cmax} measured *in vitro* between 0 °C and 40 °C (Kubien and Sage, 2008).

2005; IPCC, 2007). Also, black spruce has a high thermal sensitivity, as indicated by studies showing that annual ring growth is inversely correlated with growth season temperature (Brooks *et al.*, 1998; ACIA, 2005). When fitted to models of future climate, the annual ring width of black spruce is predicted to decline in coming decades, falling to zero by 2050 (ACIA, 2005). If true, then the predicted level of climate warming could lead to a collapse of the spruce-dominated boreal forest. Because of its ecological importance and thermal sensitivity of growth, black spruce is an ideal species in which to assess whether the reduction in carbon gain at elevated temperature is associated with the heat lability of Rubisco activase.

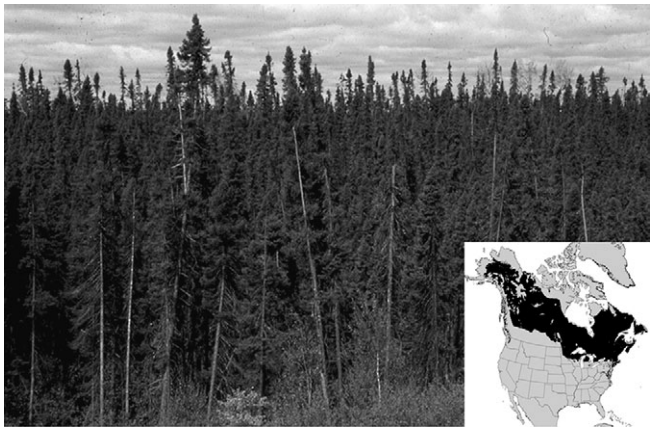

Fig. 6. A photograph of a mature black spruce stand in the boreal forest region of Canada, near Kirkland Lake, Ontario (photo by RF Sage). The inset shows the range distribution of black spruce in North America (from Little, 1971).

However, black spruce is a difficult species in which to extract enzymes for *in vitro* studies, due to tough tissue structure and a high concentration of resins. For this reason, a non-invasive technique such as assessment of the A/C_i initial slope may be the best means of screening for activase limitations in black spruce.

To investigate the mechanisms for the sensitivity of black spruce to climate change, the temperature response of photosynthesis and respiration in potted black spruce seedlings grown in a greenhouse was measured with a Li-Cor 6400 system. The photosynthetic thermal optimum of black spruce is near 25 °C. The net CO_2 assimilation rate declines 50% at 35 °C, and is below zero just above 40 °C (Fig. 7). The respiration rate doubles from 25 °C to 40 °C and, given that the shoot respiration rate is a substantial fraction of the gross photosynthesis rate, the rise in respiration aggravates the decline in photosynthesis above the thermal optimum. This supports the hypothesis that the failure of black spruce in warm climates may be related to deterioration of its carbon balance (Way and Sage, 2008).

The temperature response of the A/C_i initial slope measured in black spruce was then compared with the modelled initial slope assuming Rubisco is fully activated and limiting for A. The initial slope of the A/C_i response was also modelled assuming electron transport capacity was limiting (using equation 6 of Sage *et al.*, 2002). While not normally limiting in the initial slope region, RuBP regeneration capacity can become limiting at low CO_2 if there is a large, disproportional reduction in the electron transport capacity (Sage, 1990). This happens at low light, and causes a reduction in the initial slope of the A/C_i response (Sage *et al.*, 1990). In black spruce, the measured initial slope falls well below the modelled, Rubisco-limited initial slope above 35 °C, as would be the case if the heat lability of Rubisco activase began to assert control over A (Fig. 8). The modelled initial slope

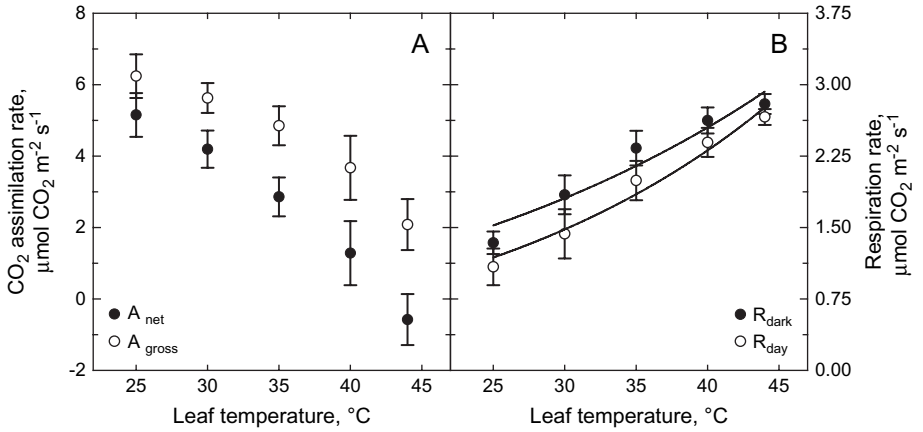

Fig. 7. The temperature response of CO_2 assimilation in black spruce seedlings. (A) Changes in net branch (filled circles) and gross needle (open circles) CO_2 assimilation rates with temperature. (B) Changes in dark (filled circles) and day (open circles) branch respiration rates with temperature. Means ±SE, n=3. One-year-old seedlings were obtained from a commercial nursery and grown in a greenhouse in Toronto for their second year at 25/19 °C day/night temperatures, 50% RH, 16 h photoperiod, and natural light intensities, supplemented with high-pressure sodium lamps if levels fell below 1000 µmol photons m^{-2} s^{-1}. Measurements were made with a Li-Cor 6400 and 6400-05 conifer cuvette between 22 and 29 August 2007. Day and dark respiration were measured using the Kok method where the net CO_2 assimilation rate was measured at 400 µmol mol^{-1} CO_2, 50–60% RH, and a light intensity between 0 µmol and 150 µmol photons m^{-2} s^{-1} at each temperature. Dark respiration was measured at 0 µmol photons m^{-2} s^{-1}, while day respiration was estimated from the y-intercept of the line drawn through 30–100 µmol photons m^{-2} s^{-1}. The CO_2 response of A was measured at 1200 µmol photons m^{-2} s^{-1} and 50–60% RH at each temperature. Net CO_2 assimilation and day respiration rates at a common set of measurement conditions were added to give the gross CO_2 assimilation rate temperature response.

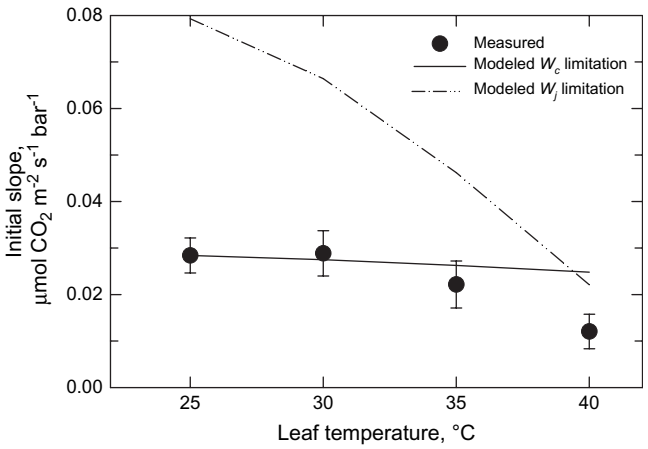

Fig. 8. The temperature response of the measured initial slopes of the CO_2 response curves (filled circles) and the modelled initial slope assuming a limitation on either A in the capacity of fully activated Rubisco (continous line) or RuBP regeneration capacity (dashed line). Means ±SE, n=3. Initial slopes of the CO_2 response curves were measured for each curve. V_{cmax} was estimated from the initial slope at 25 °C according to Farquhar and von Cammerer (1982). The temperature response of V_{cmax} from 25 °C to 44 °C was estimated using the methods and kinetic constants developed by Bernacchi *et al.* (2001) and used by Medlyn *et al.* (2002) for conifers. The estimated V_{cmax} values were then converted back to initial slopes to generate the modelled temperature response of the initial slope. J_{max}-limited initial slopes were estimated using the equation in Sage (1990), and J_{max} values calculated at each temperature from A measured at high CO_2 (von Caemmerer, 2000; Medlyn *et al.*, 2002).

assuming an electron transport limitation remained well above the measured initial slope, indicating there was sufficient RuBP regeneration capacity for A in this species at low CO_2.

While the results in Fig. 8 are consistent with an activase limitation, additional supporting data are needed to verify that the initial slope decline is indeed due to a reduction in activase capacity. This will require four important assessments. First, it will be necessary to determine the kinetic constants of black spruce Rubisco, in order to ensure the modelled responses are accurate for this species. The temperature response of Rubisco in black spruce was modelled here using activation energies from tobacco, as shown by Medlyn *et al.* (2002) for a range of conifers. The differences in the temperature response of the A/C_i initial slope between cool-adapted spinach and warm-adapted tobacco in Fig. 5 indicate this approach is problematic since black spruce is a cold-climate species. Secondly, it will be necessary to have direct estimates of J_{max} in black spruce, in order to ensure its activity does not become limiting in the initial slope region. Thirdly, the mesophyll conductance will have to be assessed to examine its contribution to the initial slope response, as mesophyll conductance is known to influence estimates of carboxylation efficiency (Evans and Loreto, 2000; Flexas *et al.*, 2007). Finally, and most importantly, the activation state of Rubisco will have to be determined and used to correct the initial slope model. If activase is limiting, it should be possible to match measured and modelled data by correcting the V_{cmax} term in equation (2) to account for the observed reduction in the Rubisco activation state.

Implications of an activase limitation in black spruce

If the decline in the initial slope of black spruce is a result of heat lability of Rubisco activase, it would implicate

a single enzyme as being the principal control over the future success of one of the world's most common species. Natural selection would thus be expected to act on any genetic variation in activase lability that may exist in black spruce populations. Alternatively, if the decline in the initial slope is due to a cooler-adapted Rubisco in black spruce, selection would instead act on any variation in Rubisco characteristics. If genetic variation is lacking in either Rubisco or Rubisco activase, then black spruce may be unable to adapt to climate change and could experience a reduction in vigour and geographic range in coming decades. It is therefore important for biologists to identify the control over the temperature response of the initial slope in order to develop strategies to guide future forest management. In the event that black spruce lacks the necessary genetic variation in Rubisco or Rubisco activase, humans could alter the genes for these proteins in order to mitigate some of the negative effects of climate change. In *Arabidopsis*, shuffling of Rubisco activase genes improves heat tolerance of Rubisco activase, carbon assimilation, and plant growth (Kurek *et al*., 2007). These results indicate human-assisted evolution of heat tolerance could be a promising approach to mitigating negative effects of climate change on critically important species in natural and managed landscapes.

Directions for the future

Natural populations are likely to be the groups of organisms most vulnerable to anthropogenic climate change because they are often highly specialized for specific habitats, rarely benefit from human interference such as breeding, and will have increasingly restricted migration options due to habitat fragmentation and land-use change. A widespread collapse in the carbon gain capacity of natural populations would be catastrophic, if for no other reason then this would reduce the ecosystem services required to sustain human health and well-being. While research emphasizing model organisms and crops species has obvious merit, it is important to also emphasize screening of natural populations for heat lability of photosynthesis, growth, and fitness. Failure to do so could limit the options for mitigating climate change effects on natural ecosystems. In this paper, analysis of the initial slope of the A/C_i curve by non-invasive gas exchange is proposed to be a useful screen for potential limitations arising from heat lability of Rubisco activase. Theoretically, the technique should work, although numerous assumptions still require verification. If Rubisco activase were found to be a widespread limitation on A in natural populations exposed to warm conditions, this would provide a relatively easy means for improving heat tolerance in natural and managed populations. If not, then research could focus on identifying the critical limitations so that heat-resistant genes could be selected for sooner, rather than later. For forest managers, sooner is especially important, because reforestation programmes have to plan for climate conditions that will predominate in coming decades and centuries, rather than in the next few years as is the case for row-crop agriculture.

Appendix 1

In Figs 1, 2, 4, and 8 the equations and constants of Bernacchi *et al*. (2001) were used for the temperature corrections of V_{cmax}, V_{omax}, K_c, K_o, Γ_*, and R_d:

$$\text{parameter}_T = \text{parameter}_{25\,^\circ C}\ \exp(c - \Delta H_a/RT_K)$$

where c is a scaling constant and ΔH_a is the activation energy (table 1 in Bernacchi *et al*., 2001). In all cases A was modelled assuming that Rubisco was fully activated. V_{cmax} and J_{max} values used are indicated in the respective figure legends. V_{omax} was modelled as $V_{cmax}/4$ at 25 °C (von Caemmerer 2000, p. 45). The Michaelis–Menten constants for CO_2 (K_c) and O_2 (K_o) were 406 μbar and 277 mbar at 25 °C, respectively. Γ_* was 43.5 μbar at 25 °C. The rate of Rubisco-limited carboxylation (W_c) was calculated as:

$$W_c = (V_{cmaxT}C_i)/[C_i + K_c(1 + O/K_o)]$$

The temperature dependency of RuBP regeneration-limited photosynthesis was estimated as described by Bernacchi *et al*. (2003). To correct J_{max} for temperature variation, eqn 10 in Bernacchi *et al*. (2003) was used:

$$J_{max} = J_{max25^\circ C}\frac{\exp(c - \Delta H_a/RT_K)}{1 + \exp[\Delta ST - \Delta H_d)/(RT_K)]}$$

where ΔH_a (43.9 kJ mol^{-1}) is the activation energy, ΔS (1.39 kJ K^{-1} mol^{-1}) accounts for entropy, ΔH_d (439.8 kJ mol^{-1}) is the energy of deactivation that accounts for a thermal optimum, R is the gas constant, T_k is temperature in Kelvin, and c is a scaling constant (17.7, dimensionless) (Bernacchi *et al*., 2003). The rate of RuBP regeneration-limited carboxylation (W_j) was modelled with the assumption that ATP production is limiting (Bernacchi *et al*., 2003):

$$W_j = JC_i/(4.5C_i + 10.5\Gamma*)$$

The temperature dependency of triose-phosphate utilization (T_{pt}) was modelled as shown by Hendrickson *et al*. (2004):

$$T_{pt} = T_{p\ (25^\circ C)}e^{\frac{(T-25)\Delta Ha}{298R(T+273)}}$$

where T is leaf temperature (°C), R is the gas constant, and ΔH_a is the activation energy (64 kJ mol^{-1}). The triose phosphate-limited rate of carboxylation (W_p) is $W_p = 3T_p$

and the rate of net CO_2 assimilation at any temperature is then:

$$A = (1-\Gamma*/C_i)\min\{W_c, W_j, W_p\} - R_{dT}$$

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