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Thermal acclimation of photosynthesis in black spruce [*Picea mariana* (Mill.) B.S.P.]

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ABSTRACT

We investigated the thermal acclimation of photosynthesis and respiration in black spruce seedlings [*Picea mariana* (Mill.) B.S.P.] grown at 22/14 °C [low temperature (LT)] or 30/22 °C [high temperature (HT)] day/night temperatures. Net CO_2 assimilation rates (A_{net}) were greater in LT than in HT seedlings below 30 °C, but were greater in HT seedlings above 30 °C. Dark and day respiration rates were similar between treatments at the respective growth temperatures. When respiration was factored out of the photosynthesis response to temperature, the resulting gross CO_2 assimilation rates (A_{gross}) was lower in HT than in LT seedlings below 30 °C, but was similar above 30 °C. The reduced A_{gross} of HT seedlings was associated with lower needle nitrogen content, lower ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) maximum carboxylation rates (V_{cmax}) and lower maximum electron transport rates (J_{max}). Growth treatment did not affect V_{cmax} : J_{max}. Modelling of the CO_2 response of photosynthesis indicated that LT seedlings at 40 °C might have been limited by heat lability of Rubisco activase, but that in HT seedlings, Rubisco capacity was limiting. In sum, thermal acclimation of A_{net} was largely caused by reduced respiration and lower nitrogen investments in needles from HT seedlings. At 40 °C, photosynthesis in LT seedlings might be limited by Rubisco activase capacity, while in HT seedlings, acclimation removed this limitation.

Key-words: acclimation; boreal; climate change; Pleistocene; respiration; temperature.

INTRODUCTION

The boreal forest covers much of Alaska, Canada and the high latitudes of Eurasia. In North America, a major boreal dominant is black spruce [*Picea mariana* (Mill.) B.S.P.], which often forms dense stands that store large amounts of carbon and play an important role in vegetation–climate feedbacks because of the low albedo of the foliage. Because high latitudes will experience greater climate warming than low latitudes, the boreal forest may be extremely vulnerable. Climate models predict an increase in mean annual temperatures in the boreal region of 4–10 °C by the year 2100 (Sala *et al.* 2000; Christensen *et al.* 2007). Black spruce may be particularly sensitive to warming. Dendrochronology studies indicate a negative relationship between growing season temperatures and annual ring thickness in black spruce (Dang & Lieffers 1989; Brooks, Flanagan & Ehleringer 1998; Arctic Climate Impact Assessment 2005), leading to the predicted loss of this species from much of its range by 2100 (Arctic Climate Impact Assessment 2005). Because of its importance in the boreal forest, a collapse of black spruce populations could dramatically change the composition, carbon storage and albedo of the boreal region, with important consequences for the global climate system.

The mechanism for the predicted decline of black spruce in warmer climates is unknown. Heat-associated drought has been implicated (Angert *et al.* 2005), but an imbalance between photosynthesis and respiration may also be a factor (Way & Sage 2008). The inhibition of growth in black spruce at high temperatures is associated with reduced carbon balance because of lower photosynthesis and greater respiration (Tjoelker, Oleksyn & Reich 1999a; Way & Sage 2008). Way & Sage (2008) studied the effects of growth temperature on black spruce and found that while respiration acclimated to temperature, photosynthesis was less plastic: warm-grown seedlings had 12% lower net photosynthetic rates at their growth temperature and 58% lower biomass than cool-grown seedlings.

Thermal acclimation of photosynthesis is an important means of compensating for any deleterious effects of rising temperature. Species from cool climates appear to have a low capacity for photosynthetic acclimation to rising temperatures, which may contribute to the sensitivity of the boreal biome to climate change (Atkin, Scheurwater & Pons 2006; Ow *et al.* 2008). Alternatively, the relatively small responses of net photosynthesis to changes in growth temperature may reflect offsetting acclimation responses. Thermal acclimation can reflect three general patterns. Firstly, there can be an altered thermal sensitivity of individual components of the photosynthetic apparatus, such as ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) activase or thylakoid membrane integrity (Kim & Portis 2005; Yamori *et al.* 2006). Secondly, there can be altered ratios of photosynthetic components, such as the amount of Rubisco to electron transport enzymes [indicated by a shift in the ratio of maximum electron transport

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rate (J_{max})/maximum carboxylation rate of Rubisco (V_{cmax})] or the relative investment in orthophosphate regeneration (Makino, Nakano & Mae 1994; Hikosaka, Murakami & Hirose 1999). Thirdly, there can be altered investment in all photosynthetic components equally, producing leaves with higher or lower protein content, but no change in the thermal response of photosynthesis. The first two processes can be viewed as qualitative changes in the photosynthetic apparatus, whereas the third method involves a quantitative change in resource investment. Acclimation involving a purely quantitative change of resource investment in the photosynthetic apparatus should not by itself alter the general shape of the thermal response of gross photosynthesis. If coupled with acclimation of the respiration response to temperature, however, a proportionally uniform change in photosynthetic investment could alter the thermal optimum and shape of the net photosynthesis response to temperature; this could erroneously indicate a qualitative shift in the photosynthetic apparatus. In black spruce, warm-grown seedlings have lower leaf nitrogen than cool-grown seedlings, indicating reduced investment in photosynthetic processes; however, the shape of the net photosynthesis response to temperature also changes with growth temperature, indicating either qualitative changes in the photosynthetic apparatus or a strong respiratory interaction (Way & Sage 2008).

The ability of black spruce to resist climate change may depend to a considerable extent on the nature of the limitation on photosynthesis at elevated temperatures. This limitation is currently unknown, although a prior study from our group indicates that a common limitation may control photosynthesis above the thermal optimum in both cool- and warm-grown black spruce (Way & Sage 2008). In C_3 plants, photosynthesis is limited by the capacity of Rubisco to carboxylate ribulose 1,5-bisphosphate (RuBP) (Rubisco limitation), the ability of electron transport to provide ATP and NADPH to regenerate RuBP (RuBP regeneration limitation), and the ability of starch and sucrose synthesis to release inorganic phosphate (P_i) for ATP synthesis (P_i regeneration limitation) (Farquhar, Caemmerer & Berry 1980; Sharkey 1985). At super-optimal temperatures, there is evidence that photosynthesis in C_3 plants is limited by either electron transport capacity (Yamasaki *et al.* 2002; Wise *et al.* 2004; Cen & Sage 2005) or the capacity of Rubisco activase to maintain high activation of Rubisco (Salvucci *et al.* 2001; Haldimann & Feller 2004; Salvucci & Crafts-Brandner 2004a,b,c). In conifers, there is little, if any, work examining whether Rubisco activase becomes limiting at elevated temperatures, although in a variety of C_3 species, activase is heat labile above 38–42 °C (Salvucci & Crafts-Brandner 2004c). Because the activation state of Rubisco can decline in response to reductions in RuBP regeneration capacity, determining which process limits photosynthesis above the thermal optimum requires estimating both Rubisco and RuBP regeneration capacities (Cen & Sage 2005). This can be achieved using theoretically based models of the CO_2 response of photosynthesis (Farquhar *et al.* 1980; Medlyn *et al.* 2002; Hikosaka *et al.* 2006; Sage & Kubien 2007). If Rubisco activase becomes limiting, modelled rates assuming Rubisco or RuBP regeneration limitations would overestimate observed photosynthesis rates.

The purpose of this study was to examine the mechanism of thermal acclimation of photosynthesis in black spruce. We also wished to determine whether acclimation was driven by changes in the quantity of photosynthetic enzymes (quantitative acclimation) or by changes associated with either disproportional shifts in the capacity of individual photosynthetic components or altered thermal sensitivity of photosynthetic enzymes and membranes (qualitative acclimation). The concepts of quantitative and qualitative acclimation build upon the idea of types I and II acclimation proposed to explain thermal acclimation of respiration (Atkin & Tjoelker 2003). Type I acclimation reflects changes in the Q_{10} of a metabolic process, as would occur in qualitative acclimation where ratios of enzymes or thermal sensitivities are altered. Type II acclimation reflects changes in the rate of a process across a range of temperatures with no corresponding change in the Q_{10}; this is consistent with quantitative acclimation.

MATERIALS AND METHODS

Black spruce seeds (seed zone 36, southern Ontario) were planted in 3.8 L pots filled with peat moss and were watered as needed to maintain a moist rooting medium. One hundred pots were placed in a greenhouse at 22/14 °C day/night temperatures [low temperature (LT)] and another 100 pots in a greenhouse at 30/22 °C [high temperature (HT)]. Both greenhouses had an approximately 1.4 kPa vapour pressure deficit (*VPD*) between needles and air, maintained by a computer-controlled misting system. Seeds germinated between April 24 and 29, 2006 and grew for 3 weeks under natural photoperiods and daylight photon flux densities (PFDs) that were supplemented with high-pressure sodium lamps to maintain a minimum PFD of 500 μmol photons m^{-2} s^{-1}.

On May 23, 2006, the pots were moved into four growth chambers (Bigfoot Model #GC-20; Enconair Ecological Chambers, Inc., Winnipeg, MB, Canada) which allowed for two replicate chambers per treatment. Fifty pots of LT seedlings were put into each of two LT growth chambers (22/14 °C day/night temperatures), and 50 pots of HT seedlings were put into each of two HT growth chambers (30/22 °C day/night temperatures). All chambers had 16 h photoperiods with light levels of 400–500 μmol photons m^{-2} s^{-1} at seedling height; the seedlings were rotated within each chamber weekly to minimize intra-chamber variation. The seedlings were thinned to 1 per pot on June 14, 2006. Leaf temperatures were measured continuously on needles from three random seedlings in each chamber with copper–constantan thermocouples attached to a datalogger (Spectrum 1700; Veriteq Instruments, Richmond, BC, Canada). Seedlings were fertilized weekly with a solution of 0.1 mg g^{-1} N of conifer fertilizer (19-9-18 + 1.42% S; Plant Products, Brampton, ON, Canada). Shoot height was measured on all seedlings over the first 3 months of growth. The

absolute growth rate was estimated by fitting an exponential curve to the shoot height data.

Gas exchange

Gas exchange measurements were conducted on at least three random 5- to 7-month-old seedlings per chamber, giving a total of at least 6 seedlings per growth temperature. All measurements were made on branches using an open photosynthesis system and a conifer cuvette (Li-6400 and Li-6400-05; Li-Cor Inc., Lincoln, NB, USA). Needles in the cuvette were harvested after gas exchange and were photographed with a digital camera for projected leaf area calculation (ImageJ, v. 1.33u; National Institutes of Health, Bethesda, Maryland, USA). Leaf samples from the gas exchange measurements were dried at 65 °C for 48 h and were weighed for dry mass. Leaf nitrogen was assessed on a CN analyser (Costech Elemental Combustion System CHNS-O; Costech, Valencia, CA, USA). These leaf areas and masses were used to calculate leaf mass per area (*LMA*). All gas exchange results were expressed on a projected leaf area basis.

Respiration

The light response of net CO_2 assimilation (A_{net}) was measured at 400 μbar ambient CO_2 at 10, 20, 30 and 40 °C; the *VPD* was maintained between 0.6 and 2.0 kPa, except at 40 °C where it rose to 4 kPa. Measurements were conducted at ambient (21%) and low (2%) O_2 concentrations by attaching the air intake of the Li-Cor 6400 to mass flow controllers (model 840; Sierra Instruments, Monterey, CA, USA) that controlled N_2 and O_2 concentrations supplied by high-pressure gas cylinders (CO_2 was controlled through the Li-Cor 6400). Improved temperature control of the Li-Cor 6400 was obtained by blowing air through a heat exchanger onto the leaf cuvette, where the heat exchanger was connected to a temperature-controlled water bath. Infrared radiation was reduced by mounting a water-filled glass dish between the lights and leaf cuvette. Net CO_2 assimilation was first measured at 21% O_2 and 10 °C under saturating light (provided by 150 W cool-beam flood lamps), then at 100, 80, 60, 45, 35, 15, 5, 0, 0 μmol photons m^{-2} s^{-1}. Measurements were taken after A_{net} had stabilized, usually within 15–20 min of changing the light level. The light curve was then re-measured at 2% O_2, starting with saturating light again. This procedure was repeated at 20, 30 and 40 °C for each set of needles.

The two measurements at 0 μmol photons m^{-2} s^{-1} were averaged for dark respiration (R_{dark}) at a given O_2 concentration and temperature. Day respiration (R_{day}) was estimated using the Kok method by extending the linear portion of the light curve between 45 and 80 μmol photons m^{-2} s^{-1} (30–80 μmol photons m^{-2} s^{-1} for 10 °C) to the *y* intercept (Wang *et al.* 2001). For each O_2 level and growth temperature, day and dark respiration rates were fit to exponential curves to estimate rates every 5 °C between 10 and 40 °C. The activation energy (E_a) of dark respiration and the Q_{10} of dark and day respiration were calculated between 10 and 40 °C (Berry & Raison 1981).

Temperature response of photosynthesis

The temperature response of A_{net} was measured between 15 and 40 °C. Prior to measuring a curve, the seedling and gas exchange system were placed in a growth chamber (Bigfoot Model #GC-20, Enconair Ecological Chambers, Inc.) and were acclimated to 15 °C. The cuvette was maintained at 400 μbar CO_2 and saturating light (800–1000 μmol photons m^{-2} s^{-1}) provided by the chamber's fluorescent tubes and additional 150 W cool-beam flood lamps. Cuvette temperature was controlled by using the Li-Cor 6400 temperature control and by varying the air temperature in the growth chamber. The cuvette humidity was controlled to maintain a leaf *VPD* between 1 and 2 kPa. Gas exchange measurements were determined at 5 °C increments; samples spent a minimum of 30 min at a given temperature and had stable net CO_2 fluxes before measurements were logged. After the 40 °C point was taken, net CO_2 assimilation was re-measured at 25 °C to check for hysteresis. Gross CO_2 assimilation rates (A_{gross}) were calculated by adding day respiration rates at each temperature to A_{net}.

O_2 sensitivity of gross photosynthesis

To evaluate possible P_i regeneration limitations, we measured the response of photosynthesis to a 90% reduction in O_2 partial pressure, and then compared the measured O_2 sensitivity with the predicted O_2 sensitivity modelled according to Sage & Sharkey (1987). Where the measured sensitivity is less than modelled sensitivity, a P_i regeneration limitation is indicated (Sharkey 1985). The temperature response of net CO_2 assimilation was measured at both ambient (21%) and low (2%) O_2 concentrations, controlled as described earlier. Saturating light (800–1000 μmol photons m^{-2} s^{-1}) was provided with 150 W cool-beam flood lamps; *VPD* was maintained between 1 and 2 kPa (10–30 °C) and below 3 kPa at 40 °C. The net CO_2 assimilation rate was measured at 21% O_2 and a constant intercellular CO_2 concentration of 300 μbar CO_2; the O_2 concentration was then reduced to 2% O_2, and net CO_2 assimilation was re-measured. Reversing the order of the O_2 concentrations had no effect on the values of CO_2 flux (data not shown). Gross photosynthesis was estimated by adding day respiration rates measured at 21 and 2% O_2 to net CO_2 assimilation rates. O_2 sensitivity was calculated as $1 - A_{gross21\%}/A_{gross2\%} \times 100\%$ (Sage & Sharkey 1987). The O_2 sensitivity of Rubisco-limited and RuBP regeneration-limited photosynthesis was modelled using the temperature responses of Γ^*, K_c and K_o (CO_2 compensation point in the absence of mitochondrial respiration, Michaelis coefficients for Rubisco carboxylation and oxygenation, respectively) of Rubisco from Jordan & Ogren (1984).

CO_2 response of net photosynthesis at different temperatures

The CO_2 response of net CO_2 assimilation (the A/C_i response) was measured at 10, 20, 30 and 40 °C. Measurements were made at 21% O_2, saturating light (800–1000 μmol photons m^{-2} s^{-1}, provided by 150 W cool-beam flood lamps), and a leaf *VPD* between 0.5 and 2.0 kPa, except for 40 °C where the *VPD* was below 4 kPa. Net CO_2 assimilation was measured at cuvette CO_2 partial pressures between 50 and 1000 μbar CO_2 at 10 °C. Measurements began at current CO_2 levels, were gradually lowered to the minimum CO_2 value, re-measured at current CO_2 concentrations, and then increased to the maximum CO_2 levels. After increasing the temperature to 20 °C, the system was allowed to equilibrate for a minimum of 30 min, and the A/C_i curve was re-measured; this procedure was repeated for 30 and 40 °C. The response of A_{gross} to intercellular CO_2 partial pressure was estimated by adding day respiration rates to the net A/C_i curves.

Modelling

The measured response of gross photosynthesis to variation in the intercellular partial pressure of CO_2 at 10, 20, 30 and 40 °C was analysed using the Farquhar *et al.* (1980) model, as modified by Medlyn *et al.* (2002). Because there are no published kinetic constants for conifer Rubisco, we used Rubisco Γ^*, K_c and K_o values from spinach. Spinach and black spruce are cool-adapted species, and therefore both may have LT types of Rubisco with similar thermal responses (Sage, Way & Kubien 2008). Γ^* was estimated at all measurement temperatures using the third-order polynomial fit to the Γ^* versus temperature response given in the legend of fig. 5 of Yamori *et al.* (2006). K_c and K_o values at 10–40 °C were derived from the activation energies for spinach from Jordan & Ogren (1984). The maximum carboxylation rate of Rubisco (V_{cmax}) was estimated at 20 °C from the initial slope of the A/C_i response in black spruce (eqn 42 in Farquhar *et al.* 1980). V_{cmax} at 10, 30 and 40 °C was then estimated using an Arrhenius function and the E_a of V_{cmax} for a model plant from von Caemmerer & Quick (2000). The maximum electron transport rate (J_{max}) was estimated using A_{gross} measured at 1000 μbar CO_2 for each A/C_i curve (Medlyn *et al.* 2002). Rubisco-limited and RuBP regeneration-limited gross CO_2 assimilation were then estimated from the modelled V_{cmax} and J_{max} (Medlyn *et al.* 2002). Because we specified Γ^* in our modelling, the gross A/C_i curves were adjusted so the estimated Γ^* values corresponded to the predicted Γ^* values.

V_{cmax} and J_{max} estimated from gas exchange have to assume Rubisco capacity and electron transport are limiting; this would not be the case if Rubisco activase limits the RuBP consumption capacity of Rubisco, or if P_i regeneration capacity limits photosynthesis at high CO_2 (Sage & Kubien 2007). Therefore, we use the terms 'apparent V_{cmax}' and 'apparent J_{max}' to refer to estimates of Rubisco and electron transport capacity derived from gas exchange. Because Rubisco capacity may potentially be limited by Rubisco activase, we required a means of estimating Rubisco capacity that was not directly dependent upon the apparent V_{cmax} estimate. This was accomplished by comparing V_{cmax} estimates using the Arrhenius response of V_{cmax} (von Caemmerer & Quick 2000 as described earlier) with the apparent V_{cmax} estimated at 10–40 °C using eqn 42 from Farquhar *et al.* (1980) and estimates of Γ^*, K_c and K_o (described previously). This approach is valid because the activation state of Rubisco is near 100% at 20–30 °C in most species (Cen & Sage 2005; Yamori *et al.* 2006; Makino & Sage 2007) and thus, the Arrhenius response should estimate a fully activated V_{cmax} at the thermal extremes.

Statistics

Data were analysed using SigmaStat (v. 3.0.1, SPSS, Chicago, IL, USA). Because the growth chamber was the unit of replication, all results are presented as means ± SE of the two replicated chambers per treatment. Shoot height, absolute growth rate (*AGR*), *LMA* and leaf nitrogen were tested with two-way analyses of variance (ANOVAS) with growth temperature and replicate as factors.

The thermal optimum of each temperature response curve was estimated by fitting a second-order polynomial to each curve, with the thermal optimum taken as the temperature where predicted CO_2 assimilation was greatest. Differences between thermal optima were tested using a two-way ANOVA with growth temperature and chamber replicate as factors. A_{net} and A_{gross} at each measured temperature were tested between LT and HT seedlings with *t*-tests to determine if rates were significantly different.

Differences between the temperature responses of O_2 sensitivity, A_{gross}, day and dark respiration rates (all on a leaf area and nitrogen basis), Q_{10} of dark respiration, initial slopes of the A/C_i curves, apparent J_{max}, apparent V_{cmax} and J_{max}/V_{cmax} were tested with three-way ANOVAS, using growth temperature, leaf temperature and chamber replicate as factors, and within each measurement temperature with a two-way ANOVA using growth temperature and replicate as factors. Day and dark respiration rates from each growth temperature measured at 10, 20, 30 and 40 °C were tested with *t*-tests for differences between the two growth treatments.

RESULTS

Daytime LT needle temperatures were 22–26 °C and HT leaf temperatures were 32–36 °C (data not shown). The HT seedlings were 27% shorter than the LT seedlings by mid-August ($P < 0.001$, Table 1). Leaf mass per area (*LMA*) of the HT seedlings was 35% lower than that of the LT seedlings ($P < 0.001$, Table 1).

Leaf nitrogen content was 15% lower in HT seedlings than in LT seedlings ($P < 0.05$, Table 1). Leaf nitrogen content gradually declined as the seedlings aged, but this did not alter the relative difference in nitrogen content between treatments (Table 1). The reduction in leaf

Table 1. Seedling morphology and leaf characteristics of black spruce seedlings grown at 22/14 °C [low temperature (LT)] or 30/22 °C [high temperature (HT)] day/night temperatures

	LT	HT	% Difference
Shoot height (cm)	15.2 ± 0.2	11.1 ± 0.0**	–27
Shoot *AGR* (cm day^{-1})	0.028 ± 0.00	0.021 ± 0.00	NS
LMA (g m^{-2})	170 ± 0	110 ± 0**	–35
Leaf N content (mmol N m^{-2})			
From all measurements	168 ± 8	143 ± 7*	–15
Temperature response curves	194 ± 12	161 ± 12*	–17
O_2 sensitivity curves	164 ± 13	136 ± 12*	–17
CO_2 response curves	147 ± 12	130 ± 9*	–12

Means ± SE, asterisks indicate significant differences between treatments (*$P < 0.05$, **$P < 0.001$). Shoot height was measured 108 d after planting. Leaf nitrogen content is shown for all samples pooled together, and for samples corresponding to each set of gas exchange measurements. $n = 2$ chambers, 50 seedlings per chamber for shoot height and absolute growth rate (*AGR*), 9 seedlings per chamber for leaf mass per area (*LMA*) and pooled leaf nitrogen content, and 3 seedlings per chamber for non-pooled leaf nitrogen content.
NS, non-significant.

nitrogen was associated with a decline in maximum A_{net} over the course of the experiment. This altered the maximum photosynthetic rates in the A/C_i curves relative to the temperature response curves; however, treatment differences persisted. Respiration rates were measured towards the end of the experiment, after nitrogen content had declined 22% from the temperature response measurements of net CO_2 assimilation. If respiration is directly proportional to needle nitrogen content, a 22% decline in respiration corresponds to a decline of only 0.06–0.8 μmol m^{-2} s^{-1}. Given that this potential respiration shift is relatively small in comparison with A_{net}, we used the same respiration rates to estimate A_{gross} throughout the experiment. Factoring out respiration was necessary because much of the respiratory signal was associated with the stem fraction of the shoots measured.

Respiration

The light compensation point rose from about 20 μmol photons m^{-2} s^{-1} at 10 °C to more than 100 μmol photons m^{-2} s^{-1} at 40 °C; HT seedlings had lower light compensation points than LT seedlings at each measurement temperature (Fig. 1). HT seedlings had 30–35% lower dark respiration rates and 30–45% lower day respiration rates than LT seedlings at equivalent measurement temperatures, and there was no significant difference between dark respiration rates at the night-time growth temperature ($P > 0.1$, Fig. 2a,b). Dark respiration rates relative to needle nitrogen content were also significantly lower in HT than LT seedlings ($P < 0.05$, Fig. 2c and Table 2). There was no difference between treatments in the Q_{10} of dark respiration ($P = 0.85$); the Q_{10} declined from 2.1 (between 10 and 20 °C) to 1.3 (between 30 and 40 °C). The E_a of dark respiration was –44.8 and –46.6 kJ mol^{-1} from 10 to 30 °C, but –24.5 and –15.1 kJ mol^{-1} from 30 to 40 °C for LT and HT seedlings, respectively. Dark respiration rates were higher here than in a companion study (Way & Sage 2008), because the dark respiration here was measured shortly after darkening needles, whereas the companion study measured dark respiration towards the end of the dark period when respiration had decayed to a stable rate.

Temperature response of photosynthesis

Net CO_2 assimilation rates were 19–26% lower in HT seedlings than in LT seedlings below 25 °C, but up to 128% greater above 25 °C (Fig. 3a). Net photosynthetic rates measured at the respective daytime growth temperatures were similar in HT and LT seedlings ($P > 0.1$). The thermal optimum of A_{net} was 19 ± 1 °C in LT seedlings and 25 ± 1 °C in HT seedlings ($P < 0.001$). There was no difference between A_{net} measured at 25 °C before and after leaf temperatures reached 40 °C in either treatment, demonstrating that there was no damage from the HT exposure (Fig. 3a). When day respiration rates were accounted for, HT seedlings had 13–29% lower A_{gross} than LT seedlings below 30 °C, and similar rates to LT seedlings between 30 and 40 °C (Fig. 3b). At the growth temperature, A_{gross} was 10% lower in HT seedlings than in LT seedlings. The thermal optima of A_{gross} were 22 ± 1 °C for LT seedlings and 28 ± 1 °C for HT seedlings ($P < 0.001$). Stomatal conductance showed little response to temperature and the ratio of intercellular to ambient CO_2 (C_i/C_a) rose at elevated temperatures, indicating that stomatal limitations were not significant (data not shown).

To investigate whether higher leaf nitrogen content in LT seedlings was responsible for their greater photosynthetic capacity than HT seedlings, we plotted the thermal response of A_{gross} on a leaf nitrogen basis (Fig. 3c). There was no growth treatment effect on the temperature response of A_{gross} on a leaf nitrogen basis ($P = 0.11$, Table 2).

O_2 sensitivity of gross photosynthesis

The O_2 sensitivity of A_{gross} at a constant C_i was little affected by growth temperature ($P = 0.98$, Fig. 4). O_2 sensitivity rose from 17% at 10 °C to 37% at 30 °C, but declined to 32% at 40 °C (Fig. 4). Measured O_2 sensitivity never approached zero. The modelled responses of Rubisco-limited and RuBP

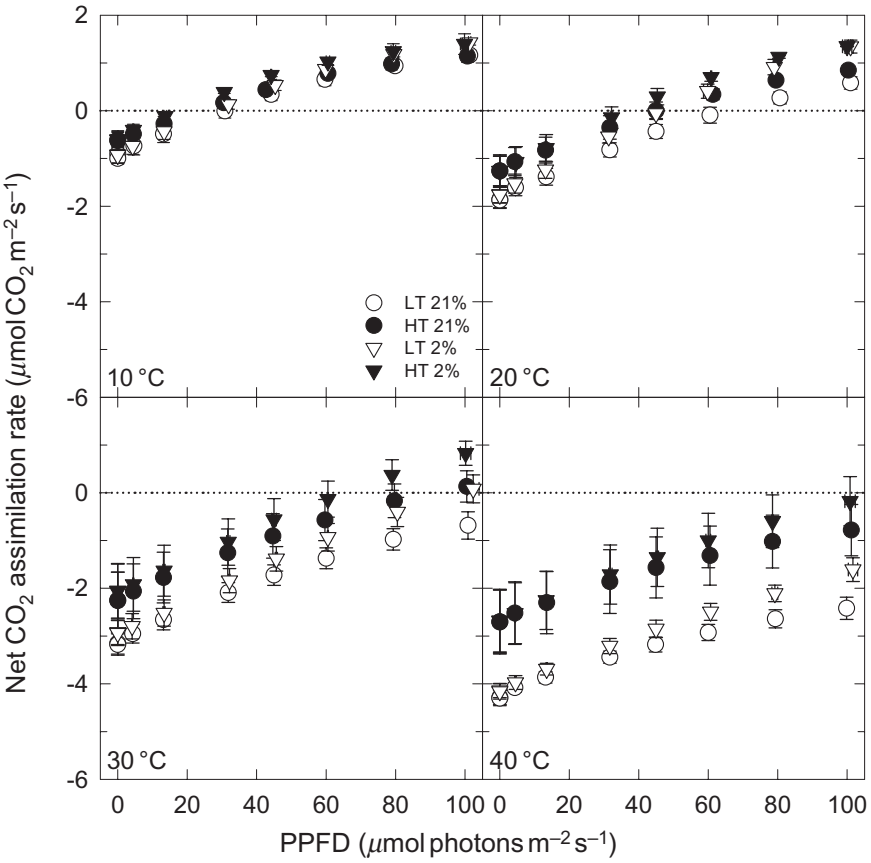

Figure 1. The light response of net CO_2 assimilation in low-temperature (LT) (22/14 °C day/night growth temperatures) and high-temperature (HT) (30/22 °C) black spruce seedlings measured at 21 and 2% O_2, and 10, 20, 30 and 40 °C. Means ± SE; $n = 2$ chambers, 3 seedlings per chamber. PPFD, Photosynthetic photon flux density.

regeneration-limited O_2 sensitivities were similar to the measured data between 10 and 35 °C. At 40 °C, the measured O_2 sensitivity at 40 °C was 10 percentage points lower than the modelled O_2 sensitivity.

CO_2 response of net photosynthesis at different temperatures

Between 10 and 30 °C, LT initial slopes were 43–66% greater than HT initial slopes ($P < 0.05$), and A_{net} was 25–33% greater in LT seedlings at 1000 μbar CO_2 than in HT seedlings ($P \leq 0.055$, Fig. 5). At 40 °C, there was no difference in the LT and HT initial slopes ($P > 0.9$), and the slopes were offset such that HT seedlings had higher A_{net} at all measurement CO_2 partial pressures.

From 10 to 30 °C, both LT and HT seedlings appear to be co-limited by Rubisco and RuBP regeneration capacities at a C_i value corresponding to a C_a of 400 μbar, as indicated by similar values between the measured A_{gross} and predicted A_{gross} assuming either Rubisco or RuBP regeneration rates were limiting (Fig. 6). At 40 °C, the modelled RuBP regeneration-limited rate of photosynthesis was similar to the measured photosynthetic rate of LT seedlings at a C_a of 400 μbar and above. However, at CO_2 values below 400 μbar, the modelled responses assuming either Rubisco or RuBP regeneration limitation overestimated observed photosynthetic rates in LT seedlings. In HT seedlings, A_{gross} at 40 °C was modelled to be Rubisco limited at low CO_2 and RuBP regeneration limited at elevated CO_2 as indicated by similar values of observed and modelled A_{gross}. At 40 °C, the modelled Rubisco-limited initial slope of the A/C_i curve agreed with the measured initial slope in HT seedlings, but the modelled Rubisco-limited initial slope overestimated the observed initial slope in LT seedlings (Fig. 6).

LT seedlings had higher apparent V_{cmax} values than HT seedlings at 10, 20 and 30 °C ($P < 0.05$, Fig. 7a), and there was no difference in the response of the apparent V_{cmax} to increasing leaf temperature between LT and HT seedlings ($P > 0.6$). The apparent V_{cmax} increased with leaf temperature between 10 and 40 °C in HT seedlings and 10 and 30 °C in LT seedlings. However, the apparent V_{cmax} at 40 °C was similar to the 30 °C values in LT seedlings ($P > 0.1$) and was below the V_{cmax} predicted using an Arrhenius function. While growth temperature affected the apparent J_{max} ($P = 0.002$), there was no significant growth temperature by leaf temperature interaction, indicating that the apparent J_{max} of LT and HT seedlings had similar responses to leaf temperature ($P > 0.35$, Fig. 7b). The ratio of apparent J_{max} to V_{cmax} declined with measurement temperature, but there was no difference in this ratio between growth temperature treatments ($P > 0.05$, Fig. 7c).

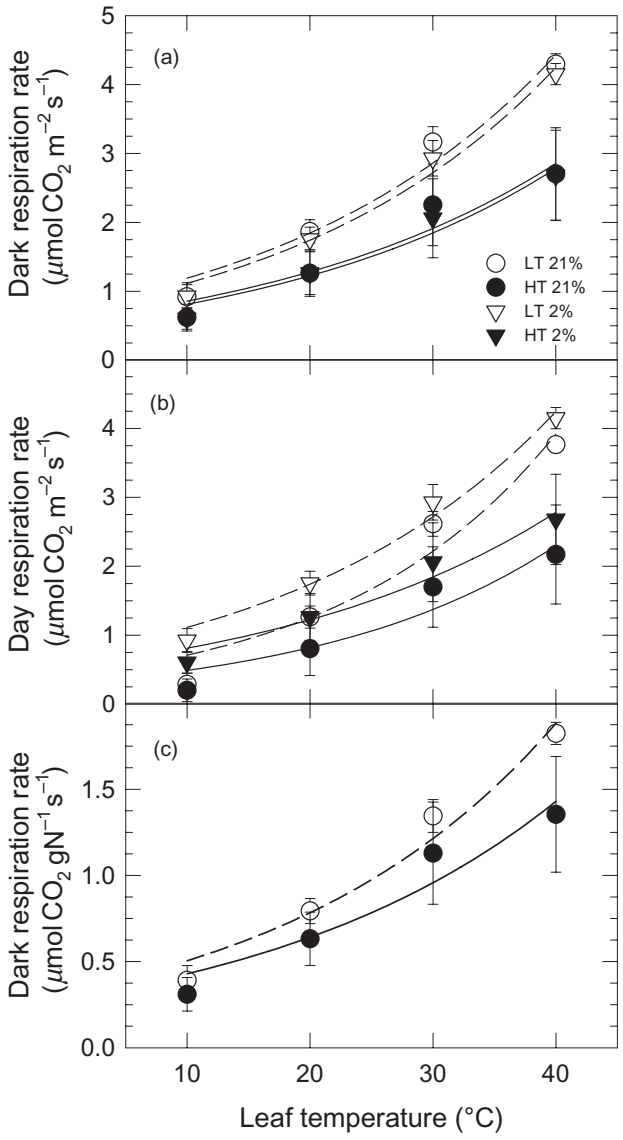

Figure 2. The temperature response of respiration for low-temperature (LT) (22/14 °C day/night growth temperatures) and high-temperature (HT) (30/22 °C) black spruce seedlings measured at 21 and 2% O_2. (a) dark respiration and (b) day respiration rates on a projected leaf area basis; (c) dark respiration rates on a leaf nitrogen basis. Means ± SE; $n = 2$ chambers, 3 seedlings per chamber. Regressions: (a) LT 21%: $y = 0.768e^{0.044x}$ ($r^2 = 0.97$), HT 21%: $y = 0.574e^{0.040x}$ ($r^2 = 0.93$), LT 2%: $y = 0.714e^{0.045x}$ ($r^2 = 0.98$), HT 2%: $y = 0.538e^{0.041x}$ ($r^2 = 0.96$); (b) LT 21%: $y = 0.403e^{0.057x}$ ($r^2 = 0.95$), HT 21%: $y = 0.292e^{0.052x}$ ($r^2 = 0.91$), LT 2%: $y = 0.714e^{0.045x}$ ($r^2 = 0.98$), HT 2%: $y = 0.538e^{0.041x}$ ($r^2 = 0.96$); (c) LT 21%: $y = 0.326e^{0.044x}$ ($r^2 = 0.99$), HT 21%: $y = 0.288e^{0.040x}$ ($r^2 = 0.99$).

DISCUSSION

We demonstrate that net photosynthesis in black spruce has a modest ability to acclimate to a warm growth regime, while respiration shows substantial acclimation. The photosynthetic acclimation response predominantly reflects changes in leaf nitrogen and, therefore, the quantity of photosynthetic enzymes, rather than changes in the ratio of enzymes in the photosynthetic apparatus. In this regard, black spruce is more of a quantitative acclimator that maintains the same relative allocation to the various photosynthetic processes, and is less of a qualitative acclimator that shifts allocation between photosynthetic enzymes. Qualitative changes are also apparent, however, particularly at elevated temperatures, as indicated by differences between the treatments in A_{gross}/N above 30 °C.

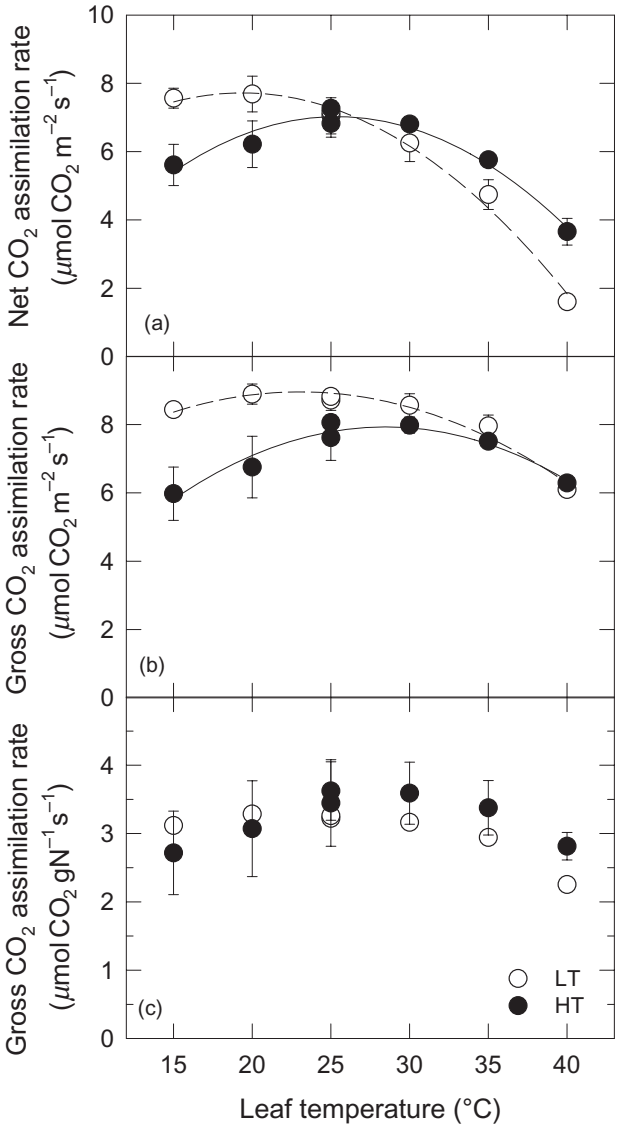

Figure 3. The temperature response of CO_2 assimilation in low-temperature (LT) (22/14 °C day/night growth temperatures) and high-temperature (HT) (30/22 °C) black spruce seedlings. (a) net CO_2 assimilation and (b) gross CO_2 assimilation on a projected leaf area basis; (c) gross CO_2 assimilation on a leaf nitrogen basis. Means ± SE; $n = 2$ chambers, 3 seedlings per chamber. Regressions: (a) LT: $y = 2.55 + 0.53x - 0.14x^2$ ($r^2 = 0.99$), HT: $y = -2.68 + 0.77x - 0.015x^2$ ($r^2 = 0.96$); (b) LT: $y = 4.07 + 0.43x - 0.009x^2$ ($r^2 = 0.96$), HT: $y = -1.55 + 0.67x - 0.012x^2$ ($r^2 = 0.94$); (c) no regression given as there was no difference between curves ($P = 0.11$).

Table 2. Analysis of variance (ANOVA) results of thermal responses of photosynthesis and respiration in black spruce seedlings grown at 22/14 °C [low temperature (LT)] or 30/22 °C [high temperature (HT)] day/night temperatures

	R_{dark} (μmol m^{-2} s^{-1})	R_{dark} (μmol gN^{-1} s^{-1})	R_{day} (μmol m^{-2} s^{-1})	A_{gross} (μmol m^{-2} s^{-1})	A_{gross} (μmol gN^{-1} s^{-1})
T_{growth}	**<0.001**	**<0.005**	**<0.001**	0.99	0.51
Rep	0.11	**<0.05**	**<0.05**	0.067	**<0.001**
T_{leaf}	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.63
$T_{growth} \times Rep$	**<0.001**	**<0.001**	**<0.001**	0.51	**<0.05**
$T_{growth} \times T_{leaf}$	**<0.01**	0.053	**<0.05**	**<0.001**	0.11
$T_{leaf} \times Rep$	0.20	0.15	0.17	0.12	0.83
$T_{growth} \times T_{leaf} \times Rep$	0.26	0.19	0.31	0.33	**<0.05**

Measurements are either on a leaf area basis or a leaf nitrogen basis.
Values are *P*-values; significant values are in bold.
T_{growth}, growth temperature; *Rep*, chamber replicate; T_{leaf}, measurement leaf temperature; R_{dark}, dark respiration; R_{day}, day respiration.

Net CO_2 assimilation rates showed a classic temperature acclimation pattern where LT seedlings had higher rates at low temperatures, while HT seedlings had higher rates at elevated temperatures. However, LT seedlings had higher respiration rates, and when respiration was factored out to yield A_{gross}, the relative differences in the temperature responses of photosynthesis changed. The LT seedlings had higher gross photosynthetic rates below 30 °C, while above 30 °C, both sets of seedlings had similar responses of A_{gross} to temperature. When expressed on a nitrogen basis, A_{gross} in LT and HT seedlings had identical responses below 30 °C, while LT seedlings had a lower A_{gross}/N above 35 °C. The convergence of A_{gross}/N below 30 °C supports the hypothesis that much of the acclimation of A_{net} to temperature in black spruce is a result of changes in needle nitrogen content and acclimation of respiration. Because leaf nitrogen is proportional to the nitrogen investment in photosynthetic enzymes (Evans 1989), the reduced needle nitrogen content indicates lower photosynthetic enzyme content in HT than in LT needles, which is consistent with their reduced estimates of apparent V_{cmax} and J_{max}. Lower needle nitrogen also reduces respiration rates (Tjoelker, Reich & Oleksyn 1999b), which explains in part the lower respiration rates of HT relative to LT seedlings.

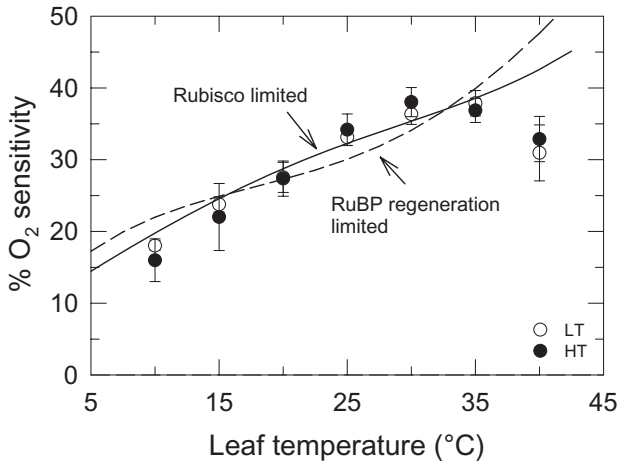

Figure 4. The temperature response of O_2 sensitivity of gross photosynthesis in low-temperature (LT) (22/14 °C day/night growth temperatures) and high-temperature (HT) (30/22 °C) black spruce seedlings measured at a constant C_i of 300 μbar CO_2. Circles are measured values; the solid line is the modelled O_2 sensitivity of ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco)-limited photosynthesis, and the dashed line is the modelled O_2 sensitivity of RuBP regeneration-limited photosynthesis (Sage & Sharkey 1987). Means ± SE; $n = 2$ chambers, 4 seedlings per chamber. C_i, intercellular CO_2.

While lower respiration rates reduced carbon loss in HT seedlings, the reduction in photosynthetic capacity reduced carbon intake, so that the overall rate of carbon gain in HT seedlings at 30 °C was less than that of LT seedlings at 22 °C. This loss in photosynthetic capacity, coupled with reduced canopy size, indicates that the capacity for carbon uptake in black spruce seedlings will be impaired where climate change pushes the growth temperature above the thermal optimum. This reduction in carbon balance likely contributes to the growth reductions observed during warmer growing seasons (Brooks *et al.* 1998; Arctic Climate Impact Assessment 2005; Way & Sage 2008).

Respiratory acclimation to temperature

Acclimation of respiration to temperature is common and often leads to homeostasis of plant carbon balance and biomass (Teskey & Will 1999; Atkin & Tjoelker 2003), but this does not occur in black spruce. Thermal acclimation of respiration in conifer seedlings, including black spruce, has been correlated with reduced leaf nitrogen (Tjoelker *et al.* 1999b). Lower nitrogen content can reduce costs of protein turnover, and may reflect reduced cell number and density (Amthor 2000). In black spruce, mesophyll cell volume was 23% higher in HT than in LT seedlings (Way & Sage 2008), indicating that cell density in needles explains little of the leaf nitrogen and respiratory changes. The Q_{10} of respiration was similar between LT and HT seedlings up to 30 °C, further supporting the argument that changes in respiration largely reflect quantitative changes in leaf protein. Atkin &

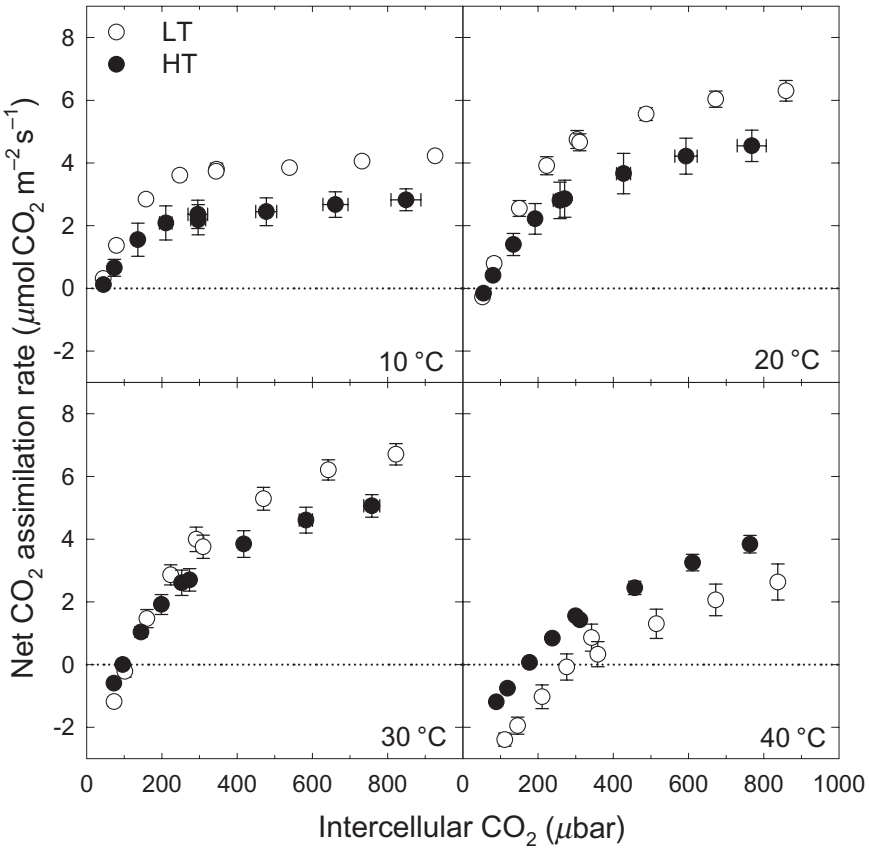

Figure 5. The response of net CO_2 assimilation rate to variation in intercellular CO_2 partial pressure in low-temperature (LT) (22/14 °C day/night growth temperatures) and high-temperature (HT) (30/22 °C) black spruce seedlings measured at 10, 20, 30 and 40 °C. Means ± SE; $n = 2$ chambers, 3 seedlings per chamber.

Tjoelker (2003) proposed that species that shift the Q_{10} of respiration in response to a change in temperature are type I acclimators; type II acclimation involves a proportional shift in respiration at both low and high temperature but does not necessitate a change in the Q_{10}. By this definition, black spruce is predominantly a type II acclimator, with much of the acclimation accounted for by a decline in leaf nitrogen. However, some qualitative acclimation of respiration is apparent, as indicated by differences in R_{dark}/N and in the E_a of respiration above 30 °C between the treatments.

Biochemical acclimation of photosynthesis to temperature

Despite an 8 °C difference in growth temperature, we saw little qualitative thermal acclimation of the three biochemical limitations to photosynthesis. The 20% lower A_{net} of HT seedlings at cooler temperatures (10–20 °C) was caused by lower leaf nitrogen, as reflected in the similarity of the thermal response of photosynthesis on a leaf nitrogen basis. While assimilation is often P_i regeneration limited at low temperatures (Sage & Sharkey 1987; Savitch, Gray & Huner 1997; Hendrickson, Chow & Furbank 2004), the low photosynthetic rates of HT seedlings at low temperatures were not caused by P_i regeneration limitations. O_2 sensitivity of photosynthesis never fell below zero, and below 30 °C, it was similar to the modelled thermal response of O_2 sensitivity assuming Rubisco and RuBP regeneration capacities were limiting A_{gross}. Instead, we observed that RuBP regeneration capacity and Rubisco capacity were approximately co-limiting in both LT and HT seedlings between 10 and 30 °C, as shown by the similarity of the modelled and measured responses at a C_a of 400 μbar CO_2. This analysis indicates no pronounced shift in photosynthetic limitations between 10 and 30 °C. As demonstrated by species with antisense constructs to Rubisco, a disproportional change in the Rubisco to RuBP regeneration capacity shifts the C_i where these processes co-limit photosynthesis. This crossover C_i rises if Rubisco capacity declines relative to RuBP regeneration capacity and falls if RuBP regeneration capacity declines relative to Rubisco (Ruuska *et al.* 1998). While many plants, such as spinach (Yamori, Noguchi & Terashima 2005) and *Plantago asiatica* (Hikosaka 2005; Ishikawa, Onoda & Hikosaka 2007), shift the thermal response of the J_{max}/V_{cmax} ratio when their growth temperature is changed, the ratio of J_{max}/V_{cmax} at a common measurement temperature in black spruce did not differ greatly between growth temperatures. This, along with the proportionally similar change in needle nitrogen content, is indicative of quantitative acclimation.

Photosynthetic limitations at high temperature

Above 30 °C, photosynthesis declines in both treatments. Net photosynthesis is reduced in LT relative to HT seedlings, reflecting the higher respiration rates of LT seedlings.

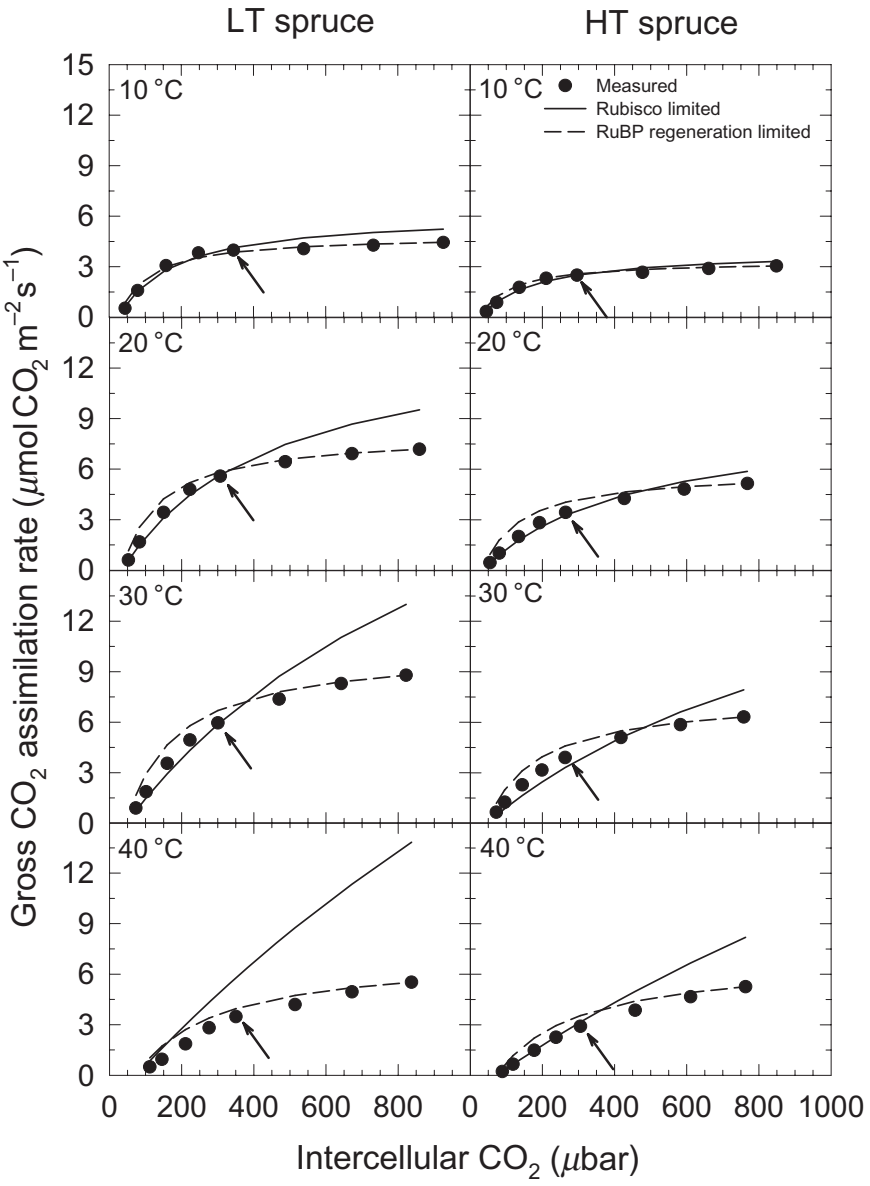

Figure 6. The modelled CO_2 response of gross photosynthesis between 10 and 40 °C assuming either ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) capacity or RuBP regeneration capacity is limiting. Filled circles are mean measured values; solid lines are modelled Rubisco-limited photosynthesis, and dashed lines are modelled RuBP regeneration-limited photosynthesis. Arrows indicate points corresponding to an ambient CO_2 partial pressure of 400 μbar CO_2.

Gross photosynthesis has an identical response to temperature above 30 °C in each treatment, which could lead to the erroneous conclusion that a common limitation controls the thermal response of photosynthesis at elevated temperature. However, because LT seedlings have more needle nitrogen, they should have higher gross CO_2 assimilation rates if common limitations controlled photosynthesis at elevated temperatures. As shown by the lower A_{gross}/N in LT seedlings above 30 °C, one of the photosynthetic controls is impaired to a greater degree in LT than HT seedlings. The gas exchange evidence does not clearly show what the limitation is in LT seedlings above 30 °C. However, the data clearly demonstrate that acclimation to warmer growth conditions involves a partial release from this limitation and indicates some possibilities regarding the nature of the limitation at elevated temperatures.

Rubisco is stable to over 50 °C, and thus the predicted V_{cmax} should rise with temperature if the fully activated capacity of Rubisco becomes limiting (Salvucci *et al.* 2001; Sage 2002). The observed decline in apparent V_{cmax} in LT seedlings at 40 °C could reflect a limitation in RuBP regeneration if electron transport capacity was so low that it limited photosynthesis in the initial slope region of the A/C_i response (Sage, Sharkey & Pearcy 1990). This is unlikely because the modelled RuBP regeneration value of A_{gross} is greater than the observed A_{gross} at low C_i. Modelled Rubisco-limited photosynthesis is also greater than the observed A_{gross} at low CO_2 in LT seedlings, indicating that fully activated Rubisco capacity does not limit photosynthesis. One explanation for the low observed initial slope and low apparent V_{cmax} in LT seedlings at 40 °C is a reduction in the activation state of Rubisco, because of

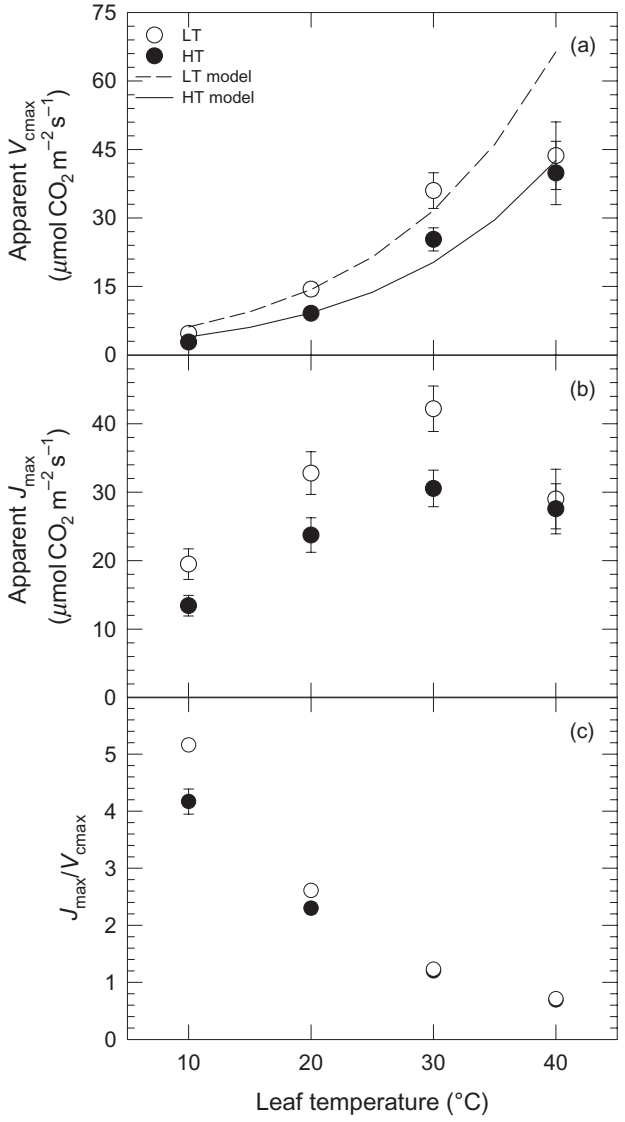

Figure 7. The temperature response of (a) apparent V_{cmax}, (b) apparent J_{max} and (c) the ratio of apparent J_{max}/V_{cmax} of low-temperature (LT) (22/14 °C day/night growth temperatures) and high-temperature (HT) (30/22 °C) black spruce seedlings. In (a), circles are measured apparent V_{cmax} values from the A/C_i (CO_2 assimilation versus intercellular CO_2) response, while lines are the apparent V_{cmax} estimated using an Arrhenius function to adjust the V_{cmax} measured at 20 °C (see Materials and Methods). Means ± SE; $n = 2$ chambers, 3 seedlings per chamber. V_{cmax}, maximum carboxylation rate of Rubisco; J_{max}, maximum electron transport rate.

heat-induced impairment of Rubisco activase. Deactivation of Rubisco is widely observed in plants over 40 °C (Feller, Crafts-Brandner & Salvucci 1998; Salvucci & Crafts-Brandner 2004b), and if this occurs, the initial slope should decline in proportion to the degree of Rubisco deactivation. The observed decline in the initial slope relative to modelled predictions is therefore consistent with heat lability of Rubisco activase causing a limitation on A_{gross} in LT seedlings exposed to 40 °C.

By contrast, in HT seedlings, the modelled and measured V_{cmax} were similar. Growth at HTs can induce the production of a more thermally tolerant isoform of Rubisco activase in some species, such as spinach (Crafts-Brandner, van de Loo & Salvucci 1997), but not others, such as tobacco (Salvucci *et al.* 2001). While we cannot determine whether HT seedlings produced a new isoform of Rubisco activase, the higher A_{gross}/N in HT seedlings at high temperatures is consistent with the hypothesis that HT seedlings had a more stable activase. Growth at high temperatures can also increase the thermal stability of the thylakoid membrane and the thermal optimum of RuBP regeneration (Badger, Bjorkman & Armond 1982; Haldimann & Feller 2005); if this occurred, HT seedlings may have had sufficient ATP from electron transport to support activase function.

Implications for boreal ecosystems

The inability to balance photosynthetic carbon gain and respiratory carbon loss appears to constrain the growth of black spruce in warm conditions at current atmospheric CO_2 levels (Way & Sage 2008). Current CO_2 levels are already elevated relative to historic norms, and are now double the values predominant at the end of the Pleistocene; CO_2 concentrations were as low as 180 μbar 18 000 years ago and rose to 280 μbar in the late Holocene (Petit *et al.* 1999). Because acclimation to low CO_2 is generally weak (Sage & Coleman 2001), the temperature response of photosynthesis measured at low CO_2 is relevant to photosynthetic functioning of black spruce during recent glacial time. To evaluate how temperature may have affected carbon gain in black spruce in low CO_2 atmospheres, we estimated A_{net} at 200 μbar CO_2 from A/C_i curves (Fig. 5). The temperature response of A_{net} in LT seedlings measured at a C_a of 200 μbar shows a thermal optimum near 10 °C, with a steady decline in carbon gain at warmer temperatures, until 35 °C when A_{net} is zero for LT seedlings. These results indicate that carbon limitation at warmer temperatures would have been much more severe than today, and growth above 30 °C would likely have been impossible. In the Pleistocene, the dense boreal forest dominated by black spruce did not exist, despite the greater distribution of cold temperatures; instead, the boreal zone consisted of a spruce savanna, where isolated stands of spruce grew in a matrix of tussock grasses (Shuman *et al.* 2002; Edwards *et al.* 2005). The reasons why the modern boreal biome was absent are unclear. The poor carbon balance of black spruce at 200 μbar CO_2 could limit the ability of spruce to establish and grow, and may explain why the modern boreal forest did not develop until CO_2 levels rose at the end of the Pleistocene (Shuman *et al.* 2002). This implies that future CO_2 increases will further favour black spruce; however, elevated CO_2 may not offset the decline in growth caused by warm growing temperatures. Responses of vegetation to a range of CO_2 values are not linear, showing a greater response from low to current CO_2 concentrations than from current to higher CO_2 concentrations (Gill *et al.* 2002). As well, studies of conifers that combine high CO_2 and

temperature treatments often find that the temperature response overwhelms the response to CO_2 (Kellomaki & Wang 1998; Tjoelker *et al.* 1999b; Apple *et al.* 2000; Lewis *et al.* 2002, 2004). Confirming the response of spruce species to a combination of elevated temperature and CO_2 should therefore be a major research goal, given the heat sensitivity and widespread dominance of spruce in the boreal forest.

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