Elevated growth temperatures reduce the carbon gain of black spruce [*Picea mariana* (Mill.) B.S.P.]

DANIELLE A. WAY and ROWAN F. SAGE
Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, ON, Canada M5S 3B2

Abstract

We explored the effect of high-growth temperatures on a dominant North American boreal tree, black spruce [*Picea mariana* (Mill.) B.S.P.]. In 2004 and 2005, we grew black spruce at either 22 °C/16 °C day/night temperatures [low temperature (LT)] or 30°/24 °C [high temperature (HT)] and determined how temperature affected growth, leaf morphology, photosynthesis, respiration and thermotolerance. HT spruce were 20% shorter, 58% lighter, and had a 58% lower root : shoot ratio than LT trees. Mortality was negligible in the LT treatment, but up to 14% of HT seedlings died by the end of the growing season. HT seedlings had a higher photosynthetic temperature optimum, but net photosynthesis at growth temperatures was 19–35% lower in HT than LT trees. HT seedlings had both a lower apparent maximum ribulose-1,5-bisphosphate carboxylation capacity (V_{cmax}) and a lower apparent maximum electron transport rate (J_{max}) than LT trees, indicating reduced allocation to photosynthetic components. Consistently, HT needles had 26% lower leaf nitrogen content than LT needles. At each measurement temperature, HT seedlings had 20–25% lower respiration rates than LT trees; however, this did not compensate for reduced photosynthetic rates at growth temperature, leading to a greater ratio of dark respiration to net carbon dioxide assimilation rate in HT trees. HT needles had 16% lower concentrations of soluble sugars than LT needles, but similar starch content. Growth at high temperatures increased the thermotolerance of black spruce. HT trees showed less PSII inhibition than LT seedlings and no increase in electrolyte leakage when briefly exposed to 40–57 °C. While trees that develop at high temperatures have enhanced tolerance for brief, extreme heat events, the reduction in root allocation indicates that seedlings will be more susceptible to episodic soil drying and less competitive for belowground resources in future climates of the boreal region.

Keywords: boreal forest, climate change, elevated temperature, leaf anatomy

Received 16 July 2007 and accepted 19 September 2007

Introduction

Black spruce (*Picea mariana*) is a dominant tree throughout much of the North American boreal forest, forming large monospecific stands that cover 55% of Alaska and most of northern Canada (ACIA, 2005). The distribution of black spruce extends through southern Canada and the northeastern US (Viereck & Johnston, 1990), where average summer temperatures are expected to increase between 3 and 8 °C by 2100 (Northeast Climate Impacts Assessment, 2006). Black spruce appears to be exceptionally temperature sensitive, with warmer growing seasons associated with declines in photosynthetic

Correspondence: Danielle A. Way, fax + 416 978 5878, e-mail: way@eeb.utoronto.ca

activity in the North American boreal forest (Angert *et al.*, 2005; Goetz *et al.*, 2005), and annual ring growth of black spruce in Alaska (ACIA, 2005). The Arctic Climate Impact Assessment (2005) used these tree ring data and climate models (Coupled Global Climate Model 2 and Climate System Model 1.4) to predict that black spruce would be lost from the central boreal forest by 2100. Given the dominance of black spruce in one of the world's most extensive biomes, such a loss could have enormous impacts on the ecological integrity and carbon storage potential of the terrestrial biosphere. It is, therefore, imperative to identify the physiological controls underlying the tree ring response.

Because high latitude tree species are often assumed to be temperature limited (Tucker *et al.*, 2001; ACIA, 2005), with warmer temperatures enhancing growth,

recent declines in photosynthetic activity in the boreal forest have been largely attributed to increasing drought stress caused by higher evapotranspiration (Angert et al., 2005; Goetz et al., 2005). However, if boreal species have a low thermal optimum for net photosynthesis, high growth temperatures may reduce carbon balance. Photosynthesis can decline sharply in red spruce (Picea rubens) as measurement temperatures rise above 32 °C (Day, 2000). In many species, acclimation shifts the photosynthetic temperature optimum towards the new growth temperature and thus improves leaf carbon balance (Mooney et al., 1978; Teskey & Will, 1999; Gunderson et al., 2000; Yamori et al., 2005; Sage & Kubien, 2007). Species such as black spruce that experience a wide range of temperatures over the year are generally thought to have greater acclimatory potential (Berry & Bjorkman, 1980). To our knowledge, acclimation of the photosynthetic temperature response curve has not been examined in black spruce, but it may not be great. When black spruce seedlings are grown at different temperatures, the net photosynthetic rate measured at growth temperature declines with increasing temperature from 18 to 30 °C (Tjoelker et al., 1998).

High temperatures can also increase carbon loss by promoting respiration. This response is greatest after a short-term increase in temperature, but acclimation often reduces respiration rates in plants grown at high temperatures compared with those grown at lower temperatures (Samuelson & Teskey, 1991; Tjoelker et al., 1999a; Gunderson et al., 2000; Atkin & Tjoelker, 2003). Respiratory acclimation can maintain a constant ratio between photosynthesis and respiration (Gifford, 1995; Gunderson et al., 2000; Loveys et al., 2003), but does not always, especially in plants adapted to stressful environments which often show reduced acclimation potential (Loveys et al., 2002; Atkin et al., 2006). Black spruce may be one of these species, given its preference for waterlogged soils and extreme climate (Viereck & Johnston, 1990). Without sufficient acclimation of respiration at elevated growth temperatures, plants cannot compensate for decreases in photosynthesis at the leaf level. If this occurs, growth at high temperatures will reduce net carbon gain, and may even cause carbon starvation. In red spruce (P. rubens), short-term measurement temperatures above 35 °C shift trees into negative carbon balance (Vann et al., 1994).

Climate warming is not only expected to increase average temperatures, but also to cause more frequent extreme heat events (IPCC, 2001). Thus, even if trees can tolerate higher growth temperatures, they may reach a lethal threshold temperature during one of these extreme events. Exposure to high, but sublethal, temperatures can increase the temperature at which damage occurs in plants (Colombo & Timmer, 1992; Havaux,

1993). Thus, acclimation to elevated temperatures could also increase thermotolerance, thereby reducing climate change-induced mortality.

In this study, we examined the effect of high growth temperatures on the morphology, photosynthesis and respiration of black spruce seedlings. We also determine whether high growth temperatures enhance seedling thermotolerance. The warm growth treatment used in this study (30 °C/24 °C day/night temperatures) is representative of the upper range of the growth season temperatures predicted for the boreal region in coming decades (IPCC, 2001; ACIA, 2005); it is realistic given that global warming to date is in line with the high end of the IPCC scenario range (Rahmstorf et al., 2007).

Materials and methods

In 2004 and 2005, 1000 black spruce seeds from seed zone 36 (southern Ontario) were germinated and grown in 3.8 L pots filled with peat moss and watered as needed to maintain a moist rooting medium. Seedlings were fertilized weekly with 100 ppm N conifer fertilizer (Plant Prod, 19-9-18 + 1.42% S). The 2004 cohort germinated in late May at day/night temperatures of 22 °C/16 °C [low temperature (LT)] in a greenhouse with compressor-based temperature control at 45% relative humidity. Twelve days after germination, half of the seedlings were moved to an adjacent 30 °C/24 °C greenhouse [high temperature (HT)] with 66% relative humidity. The difference in relative humidity between the treatments provided a similar daytime vapor pressure difference ($\sim$1.4 kPa). Peak daytime light intensity in the greenhouses on a clear day was $\sim$1800 μmol photons m^{-2} s^{-1}. Natural daylight was supplemented with high-pressure sodium lamps if light levels fell below 500 μmol photons m^{-2} s^{-1}. The 2005 seedlings were grown under similar conditions, but germinated in late April at their respective temperatures. Seedlings were thinned to one per pot a month after germination, leaving 400 trees per treatment per year. Needle temperatures were measured continuously on three trees per treatment with copper-constantan thermocouples attached to a datalogger (Spectrum 1700, Veriteq Instruments, Richmond, BC, Canada). Soil temperature was measured in three pots per treatment using thermistors (Priva Computers, Vineland Station, ON, Canada). Midday shoot water potentials were measured in October 2005 on six seedlings per treatment with a pressure chamber (PMS Instruments, Corvallis, OR, USA).

Morphology and leaf chemistry

Morphology and leaf chemistry were compared on seedlings of the same age. In both 2004 and 2005, shoot height was measured throughout the summer.

In September 2004 and October 2005, six random seedlings per treatment were separated into needles, stems and roots, dried at 60 °C and weighed. Leaf N from these samples was determined on a CN analyzer (Costech elemental combustion system CHNS-O, Valencia, CA, USA). In July 2005, total seedling leaf area was assessed on five random trees per treatment by photographing needles and calculating projected leaf area with IMAGEJ (v. 1.33u, NIH). Mortality was assessed by counting dead and surviving trees in the 2005 cohort.

Needles from 12 random trees per treatment per year were collected in early September, dried at 60 °C and ground. Samples were assayed for nonstructural carbohydrates according to Hendrix (1993). Glucose, fructose, sucrose and starch concentrations were determined spectrophometrically (Hewlett Packard 8452A, Palo Alto, CA, USA) using a glucose kit (GAHK-20, Sigma, St Louis, MO, USA). Phosphoglucose isomerase (P5381-1 KU, Sigma) was used to convert fructose to glucose, and invertase (I-4504, Sigma) was used to convert sucrose to glucose.

In 2005, the length of needles from the main leader from 10 random trees per treatment was measured with digital calipers. Samples of the middle third of these needles were prepared for anatomical study according to Muhaidat et al. (2007). Five-micrometre-thick sections were observed on a light microscope (Provis AX-70, Olympus, Tokyo, Japan), and images were analyzed with IMAGE PRO PLUS (v 6.0, Media Cybernetics, Bethesda, MD, USA). The height of the needle cross-section was assessed as the abaxial–adaxial needle diameter and needle width as the diameter perpendicular to needle height. The cross-sectional area of each tissue type (mesophyll, intercellular airspace, resin duct, vascular tissue) was estimated by tracing the tissue on the screen and calculating the delineated area. The total cross-sectional area of the needle was then used to calculate percentages of cross-sectional area for each tissue type. The length of the mesophyll cell wall facing the intercellular airspace was traced for each slide; this length was then divided by the cross-sectional mesophyll area to generate L_{mes}/area (Nelson et al., 2005). Projected leaf area and dried leaf mass from gas exchange needle samples were used to calculate leaf mass per area (LMA, $g cm^{-2}$). Leaf tissue density for each treatment was estimated as

$$Tissue density = (((h + w)/2) \times L \times LMA)/(L \times A \times (1 - IAS)), \quad (1)$$

where h and w are the mean needle height and width, L is the mean needle length, A is the mean cross-sectional needle area, and IAS is the fraction of cross-sectional area filled with intercellular airspace.

Gas exchange

In 2004, net carbon dioxide (CO_2) assimilation rates of 20 random trees per treatment were measured at 22 and 30 °C with an open photosynthesis system and a conifer chamber (Li-6400 and 6400-05, Li-Cor, Lincoln, NB, USA) in the greenhouse. The cuvette was kept at 1.25 kPa VPD, 400 $\mu mol mol^{-1}$ CO_2 and 400 μmol photons $m^{-2} s^{-1}$ (greenhouse growth light conditions). Respiration rates were measured on 18 random trees per growth temperature at 18 and 24 °C in a dark growth chamber (Bigfoot Model #GC-20, Enconair Ecological Chambers, Winnipeg, MB, Canada) with the cuvette maintained at 400 $\mu mol mol^{-1}$ CO_2 and 1.3 kPa VPD. Seedlings used for respiratory measurements were moved to the darkened growth chamber the evening before measurements began and were kept overnight at their nocturnal growth temperature.

In 2005, photosynthetic and respiration temperature curves were measured on two different sets of six random seedlings per treatment in growth chambers to provide temperature control of the gas exchange system. Net photosynthesis was measured at 400 $\mu mol mol^{-1}$ CO_2 and saturating light ($\sim$1000 μmol photons $m^{-2} s^{-1}$) provided by the chamber's fluorescent tubes and additional 150 W cool-beam floodlamps. Respiration measurements were made at 400 $\mu mol mol^{-1}$ CO_2 at the end of the dark period in a dark chamber with a cloth over the cuvette; there was no light detected in the cuvette (Li-1900 quantum sensor, Li-Cor). For both temperature response curves, the seedling and gas exchange system were put in a growth chamber and acclimated to 10 °C. Measurements were made every 5 °C from 10 to 40 °C once the CO_2 flux was stable. Leaf VPD ranged from 1.1 to 1.6 kPa, except at 40 °C where the VPD varied from 2.5 to 3 kPa. The Q_{10} of respiration was calculated every 5 °C as

$$Q_{10} = (R_2/R_1)^{[10/(T_2 - T_1)]}, \quad (2)$$

where R_1 and R_2 are the respiration rates at the lower (T_1) and higher temperature (T_2). The apparent activation energy of respiration (E_a) was calculated as per Berry & Raison (1981).

CO_2 response curves were measured in 2005 on six random seedlings per treatment; measurements were made at 25 °C, saturating light ($\sim$ 1000 μmol photons $m^{-2} s^{-1}$ supplied by 150 W cool-beam floodlamps), 1.5 kPa VPD and CO_2 concentrations from 50 to 1000 $\mu mol mol^{-1}$ CO_2. V_{cmax} and J_{max} were modeled at 25 °C using the method of Medlyn et al. (2002). V_{cmax} and J_{max} were not corrected for diffusion leaks (Rodeghiero et al., 2007), but the chamber was checked for leaks between measurements by ensuring that the chamber CO_2 did not change significantly when the CO_2 gradient

between the chamber and air was increased. Results are reported on a projected leaf area basis.

Thermotolerance

Randomly selected seedlings were dark acclimated overnight and needles were removed from the leader between 09:00 and 10:00 hours the following morning. Needles were aligned on clear tape (Major *et al.*, 2003) and dark-adapted F_v/F_m (variable fluorescence divided by maximal fluorescence, a measure of the maximum quantum yield of photosystem II) was measured with a pulse-amplitude-modulated fluorometer (PAM-101, Walz, Effeltrich, Germany). Seedlings were put in a dark temperature-controlled chamber (S-1.2 environmental test chamber, Thermotron, Holland, MI, USA) for 3 min in 2004 and 10 min in 2005 at temperatures ranging from 35 to 59 °C; the heat stress duration and temperatures were chosen to facilitate comparisons with previous black spruce thermotolerance work (Colombo & Timmer, 1992). Three to 10 seedlings were measured at each temperature. One hour later, F_v/F_m was measured on another set of needles. Trees from 2005 were returned to the greenhouses and reassessed 1 week later to determine if F_v/F_m declines were reversible.

Electrolyte leakage was measured on needles from 12 random trees per treatment in 2005. Needles were put in glass vials and heated in the darkened temperature chamber for 10 min at temperatures from 25 to 60 °C. Five millilitres of distilled water was added to each sample, and after 24 h of mixing, the electrical conductivity of the solution (c_{heated}) was measured (Ultrameter 4P, Myron L, Carlsbad, CA, USA). Samples were then boiled for 2 h, and the maximum solution conductivity ($c_{maximum}$) was measured 24 h later. Electrolyte leakage was measured as $c_{heated}/c_{maximum}$.

Data analysis

Data were analyzed using SIGMASTAT (v. 3.0.1, SPSS). Leaf anatomy traits, total leaf area and water potential differences between treatments were assessed with *t*-tests; mortality was tested with a χ^2 test. Differences in dry weights, LMA, leaf carbohydrates and shoot height were assessed with two-way ANOVAs with growth temperature and year as factors.

Dark respiration and net CO_2 assimilation rates at growth temperatures were each compared with a two-way ANOVA with growth temperature and year as factors. Because temperature response curves did not include points at 16 or 24 °C, rates for 2005 were generated by fitting curves to temperature response curves and solving for net CO_2 assimilation and respira-

tion rates at the growth temperatures. Respiration rates at night growth temperatures from 2005 were estimated by fitting exponential curves to temperature response curves. Net CO_2 assimilation rates at day growth temperatures for 2005 were derived from second-order polynomials fitted to temperature response curves. Thermal optima for photosynthesis were calculated as the photosynthetic temperature curve maxima; optima were tested with a *t*-test, as were differences in net CO_2 assimilation at each point on the temperature curve. The ratio of net CO_2 assimilation to dark respiration was calculated every 5 °C from 15 to 35 °C using the means and standard errors from the temperature response curves and analyzed with *t*-tests. Differences in F_v/F_m and electrolyte leakage were tested with three-way ANOVAs using exposure temperature, growth temperature and year as variables.

Results

Year by growth temperature interactions were not significant ($P > 0.05$), so only growth temperature results are reported. Leaf and air temperatures were generally close, except on sunny days when direct solar radiation could raise needle temperatures up to 6 °C above air temperatures (Fig. 1); afternoon temperatures did not spike as much because the greenhouses faced east. Daytime soil temperatures averaged 21.3 ± 0.3 and 27.3 ± 0.3 °C in the LT and HT treatments, respectively. There was no difference in midday water potentials between growth temperatures ($P > 0.1$; mean midday shoot water potential of -0.7 ± 0.1 MPa).
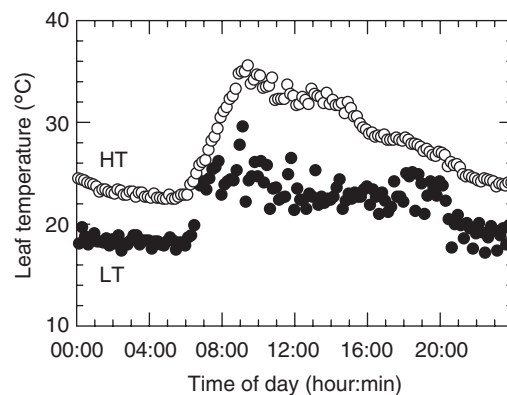

Fig. 1 Representative 24-h leaf temperature profiles from a black spruce seedling growing at either 22 °C/16 °C (LT; solid circles) or 30 °C/24 °C (HT; open symbols) day/night temperatures. LT, low temperature; HT, high temperature.

Morphology and leaf chemistry

By the end of the growing season, HT seedlings were smaller than LT seedlings, with 15–20% shorter shoots, 58% less dry biomass, and 54% less total needle area (Fig. 2a and Table 1). The root:shoot ratio was 58% lower in HT trees than LT trees, and HT trees had a 77% lower root mass than LT seedlings. Mortality was 14% in HT seedlings by the end of the growing season, but only 1% for LT seedlings (Fig. 2b).

Needles that developed in the elevated temperature treatment were short and thin, with a 24% lower leaf mass per area relative to LT leaves (Table 1). The reduction in leaf mass per area in HT needles was consistent throughout the growing season, indicating that the difference was not caused by ontogenetic effects (data not shown). The smaller cross-sectional area of the HT needles corresponded to reduced areas of intercellular airspace, resin ducts and vascular tissue relative to LT leaves (Fig. 3, Table 1). Despite having 32% less cross-sectional area, HT needles had a similar cross-sectional area of mesophyll tissue, and thus a greater proportion of leaf space was filled with mesophyll cells compared with LT needles (Table 1). The HT needles had relatively more mesophyll surface exposed to the intercellular airspace than LT mesophyll cells, resulting in a similar L_{mes}/area. HT seedlings had less dense needles than LT seedlings which likely reflects the reduction in the cell wall-rich vascular tissue and resin ducts.

Leaves from HT trees had similar leaf nitrogen concentrations, but 26% lower N content (per unit leaf area) and lower glucose, fructose and total soluble sugars concentrations than LT leaves (Table 2). There was no difference in sucrose, starch or total nonstructural carbohydrates levels between growth temperatures (Table 2).
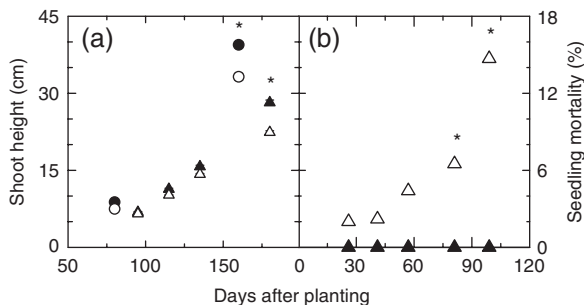

Fig. 2 Effects of growth temperature on (a) shoot height and (b) mortality of black spruce grown at either 22 °C/16 °C (LT; solid symbols) or 30 °C/24 °C (HT; open symbols) day/night temperatures. Circles are data from 2004, triangles are data from 2005. Means ± SE, $n = 172$–500 for shoot height. *Significant difference between growth temperatures on a given day ($P < 0.05$). LT, low temperature; HT, high temperature.

Photosynthesis, leaf conductance and respiration responses

Plants grown at elevated temperatures had lower net CO_2 assimilation rates at their growth temperature ($P < 0.001$; Fig. 4a). In 2004, when measured at growth temperatures and light levels, net CO_2 assimilation rates were 35% lower in HT seedlings than in LT seedlings. Light saturated net photosynthesis at growth temperature in 2005 was 19% lower in HT seedlings compared with LT seedlings. The photosynthetic thermal optimum shifted from 23–24 °C in LT seedlings to 26 °C in HT trees ($P < 0.001$). Below the thermal optimum, HT trees had 25–38% lower net CO_2 assimilation rates than LT trees ($P < 0.05$). There was no difference in net CO_2 assimilation rates between 25 and 40 °C, with photosynthesis in both treatments declining above 30 °C, and decreasing sharply near 40 °C. Stomatal conductance was constant in LT trees from 12 to 35 °C, with a slight decline at 40 °C, while conductance increased in HT trees from 12 to 25 °C and then remained constant up to 40 °C (Fig. 4b). The C_i/C_a ratio declined in the LT trees from 0.77 at 12 °C to 0.64 at 20 °C, and then increased slowly back up to 0.75 at 40 °C; the C_i/C_a ratio remained constant around 0.7 in HT trees between 12 and 35 °C, and then increased to 0.8 at 40 °C (Fig. 4c).

The CO_2 response curve of HT seedlings had a lower initial slope, leading to an estimated 26% lower apparent V_{cmax} than the LT seedlings (Fig. 5, Table 3). The HT trees also had 16% lower photosynthetic rates at saturating CO_2 concentrations (A_{sat}), leading to an estimated 12% lower apparent J_{max} than LT seedlings (Fig. 5, Table 3).

HT trees had lower respiration rates at all temperatures than LT trees ($P < 0.05$; Fig. 6a). There was no difference between respiration rates at night growth temperatures, but HT seedlings had 47% higher respiration rates at daytime growth temperatures ($P < 0.003$). The Q_{10} and E_a of dark respiration were similar in both treatments (Table 3). The ratio of net CO_2 assimilation to dark respiration decreased with temperature in both treatments and there was no evidence of any acclimation as the decline in the ratio of A/R is similar in both treatments (Fig. 6b).

Thermotolerance

HT seedlings were less damaged by both a 3- and 10 min heat stress than LT seedlings ($P < 0.001$; Fig. 7a–c). The maximum quantum yield of PSII was insensitive to temperatures up to 40 °C in both treatments, while above 40 °C, F_v/F_m declined, particularly after a 10-min heat stress (Fig. 7b). Between 45 and 57 °C, HT trees showed less inhibition of F_v/F_m than LT trees. Seedlings

Table 1 Biomass and needle characteristics of black spruce seedlings grown at 22 °C/16 °C (LT) or 30 °C/24 °C (HT) day/night temperatures

	LT	HT	% Difference
Seedling dry mass (g)	19.6 ± 4.4	8.3 ± 2.9**	−58
Needle dry mass (g)	7.8 ± 1.3	4.5 ± 1.9	ns
Stem dry mass (g)	5.4 ± 1.5	2.3 ± 0.7 **	−58
Root dry mass (g)	6.4 ± 4.1	1.5 ± 0.3***	−77
Root : shoot	0.53 ± 0.07	0.22 ± 0.07**	−58
Leaf mass per unit area (g m^{-2})	54 ± 2	41 ± 2***	−24
Projected leaf area (cm^2 seedling^{-1})	586 ± 33	268 ± 61**	−54
Needle length (mm)	22.1 ± 0.8	17.3 ± 1.0**	−22
Needle height (μm)	600 ± 20	485 ± 30**	−19
Needle width (μm)	720 ± 25	580 ± 20***	−19
Leaf cross-sectional area (mm^2)	0.28 ± 0.02	0.19 ± 0.02**	−32
Mesophyll area (mm^2)	0.11 ± 0.01	0.10 ± 0.01	ns
Intercellular air space (mm^2)	0.09 ± 0.01	0.05 ± 0.01**	−44
Resin duct area (μm^2)	6510 ± 680	3650 ± 390**	−44
Vascular tissue (μm^2)	4480 ± 510	3450 ± 130	ns
% Mesophyll tissue	38.3 ± 1.6	49.7 ± 5.1*	+ 23
% Intercellular air space	31.5 ± 1.7	24.7 ± 2.2*	−22
% Resin duct	2.3 ± 0.2	2.0 ± 0.3	ns
% Vascular tissue	1.6 ± 0.1	1.8 ± 0.2	ns
L_{mes}/area (μm^{-1})	0.05 ± 0.00	0.04 ± 0.00	ns
Needle density (g cm^{-3})	0.18	0.14	

Means ± SE, asterisks indicate significant differences between treatments.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
The percent difference is not shown for nonsignificant (ns) results. Leaf mass per area was determined on needles used for gas exchange. L_{mes}/area is the length of the mesophyll cell wall length exposed to the intercellular airspace, divided by the cross-sectional mesophyll area.
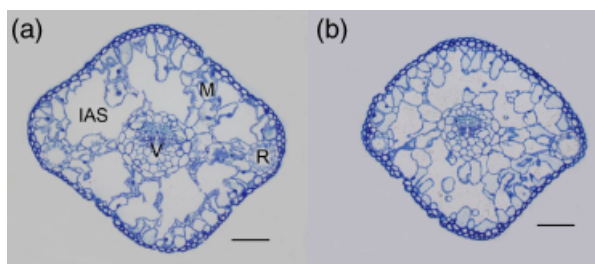HT, high temperature; LT, low temperature.

Fig. 3 Representative needle cross-sections from black spruce seedlings grown at (a) 22 °C/16 °C (LT) or (b) 30 °C/24 °C (HT) day/night temperatures. × 10 magnification, scale bar = 100 μm. Labels indicate intercellular airspace (IAS), mesophyll tissue (M), resin duct (R), and vascular tissue (V). LT, low temperature; HT, high temperature.

exposed to temperatures below 45 °C in the LT treatment, and below 47 °C in the HT treatment, showed little visible injury and had full recovery of F_v/F_m 1 week after heat exposure (Fig. 7c); the needles of seedlings exposed to temperatures above 49 °C turned brown within 24 h and were dead within a week. Between 45 and 49 °C, F_v/F_m in some seedlings showed

full recovery after 1 week, while other seedlings there was still significant inhibition of F_v/F_m, or death. Electrolyte leakage remained constant in HT leaves between 25 and 57 °C; LT leaves showed a linear increase in electrolyte leakage above 50 °C (Fig. 7d).

Discussion

The reduction in growth of black spruce grown at 30 °C/24 °C compared with 22 °C/16 °C was substantial, being close to 60%. The reduced growth was accompanied by lower root allocation in warm-grown trees, such that root : shoot ratios were also close to 60% less in warm-grown trees relative to cool-grown trees. The consequence of reduced root allocation in warm-grown trees would be lower overall carbon costs than cool-grown trees, partially offsetting the reduced carbon gain capacity associated with lower photosynthesis per unit leaf area, higher respiration rates, and a smaller leaf canopy. As a result, plants maintained a positive carbon balance as indicated by the negligible change in sucrose, starch and total nonstructural carbohydrate contents. The hypothesis that warm temperatures cause

Table 2 Carbohydrate concentrations and leaf nitrogen content of black spruce seedlings grown at 22 °C/16 °C (LT) or 30 °C/24 °C (HT) day/night temperatures

	LT	HT	% Difference
Carbohydrates (% dry leaf mass)			
Glucose	2.06 ± 0.1	1.73 ± 0.1*	−16
Fructose	1.60 ± 0.1	1.14 ± 0.1***	−29
Sucrose	1.04 ± 0.3	1.10 ± 0.2	ns
Soluble sugars	4.71 ± 0.3	3.97 ± 0.3*	−16
Starch	1.29 ± 0.3	1.46 ± 0.2	ns
Total nonstructural carbohydrates	5.82 ± 0.5	5.43 ± 0.4	ns
Leaf nitrogen concentration (%N)	2.19 ± 0.1	2.39 ± 0.1	ns
Leaf nitrogen content (mmol m^{-2})	116 ± 10	87 ± 10*	−26

Means ± SE, asterisks indicate significant differences between treatments.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

The percent difference is not shown for nonsignificant (ns) results. Values determined on needles used for gas exchange. HT, high temperature; LT, low temperature.

black spruce seedlings to experience carbon starvation is not supported; instead the reduction in growth appears to be related to a regulatory decline in sink demand, mediated by slower growth in general, and lower growth of roots in particular. This reduction in growth likely reflects a direct heat inhibition of cell division and expansion in meristematic regions in addition to reduced carbon acquisition by source tissues; both effects interact because carbohydrate supply to growing tissues antagonizes heat inhibition of cell division and cell expansion in a range of species (Koch, 1996; Cowling & Sage, 1998; Campbell & Sage, 2006). Regardless of the mechanism, heat inhibition will make black spruce seedlings more prone to failure in warm environments. The low root : shoot ratio in warm-grown trees will reduce the ability to withstand drought in this shallow-rooting species. Black spruce is severely injured by moderately low water potentials (−2.5 MPa), and has little capacity for osmotic adjustment (Johnsen & Major, 1999; Marshall *et al.*, 2000). With a lower root mass per shoot, less soil water can be mined, predisposing seedlings to drought injury; this effect would be more severe in a warmer climate where evapotranspiration and surface drying is more rapid. Black spruce also faces severe competition from broadleaved vegetation during stand establishment, such that lower canopy growth and reduced root mass will limit the ability of black spruce to gather light and soil resources before they are acquired by competitors. We thus conclude that
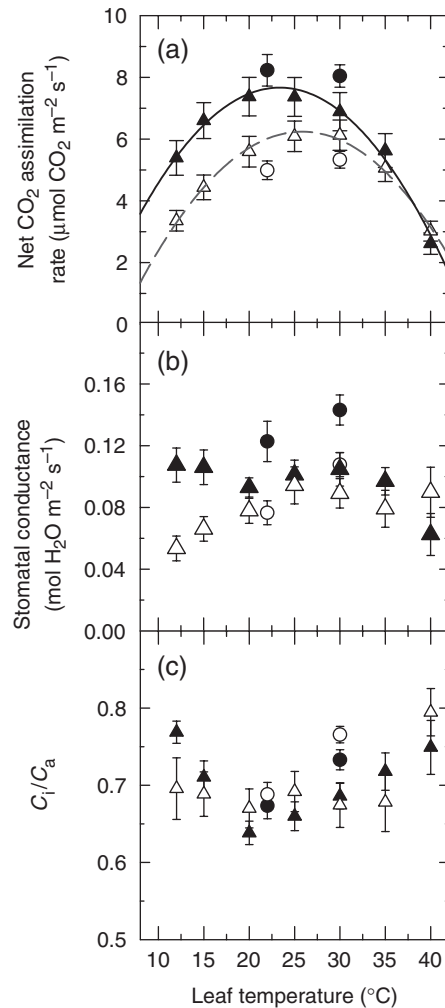

Fig. 4 The response of net carbon dioxide (CO_2) assimilation rate to temperature in black spruce grown at 22 °C/16 °C (LT; solid symbols) or 30 °C/24 °C (HT; open symbols) day/night temperatures: (a) net CO_2 assimilation rate; (b) stomatal conductance; (c) intercellular CO_2 concentration/ambient CO_2 concentration (C_i/C_a ratio). Circles are data from 2004, triangles are data from 2005. Measurements from 2004 were conducted at 400 μmol photons m^{-2} s^{-1} (growth light levels), while 2005 measurements were made at 1000 μmol photons m^{-2} s^{-1} (saturating light levels). Means ± SE, $n = 18$–20 trees per growth temperature (2004) and six trees per growth temperature (2005). LT, low temperature; HT, high temperature.

the primary mechanism of heat-induced growth reduction in black spruce seedlings is a direct suppression of photosynthesis and growth, and a rise in respiration. However, rather than causing negative carbon balance, plants modulate growth in such a manner that they become more susceptible to drought and competition, and these latter mechanisms will likely be significant contributors to a loss of black spruce-dominated stands as the climate warms.
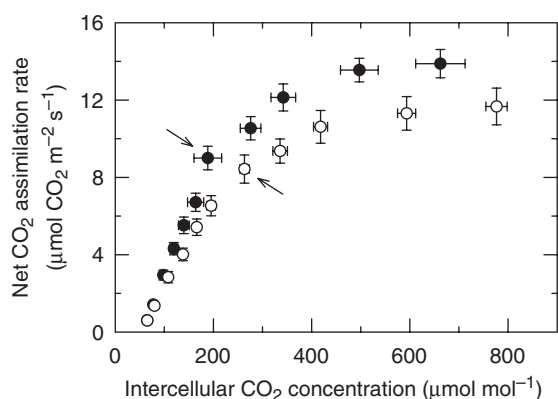
Fig. 5 The response of net carbon dioxide (CO_2) assimilation rate to CO_2 concentration for black spruce grown at $22\,°C/16\,°C$ (LT; solid symbols) or $30\,°C/24\,°C$ (HT; open symbols) day/night temperatures. Measurements were conducted at $25\,°C$ and light saturation. Arrows indicate intercellular CO_2 concentrations at an ambient CO_2 concentration of $400\,\mu mol\,mol^{-1}$. Means $\pm$ SE, $n = 6$ trees per growth temperature. LT, low temperature; HT, high temperature.

Mechanisms of growth inhibition at elevated temperature

Warmer temperatures increase cell division and elongation with slower synthesis of cell-wall components and proteins, leading to the thin, short needles and lower nitrogen content in warm-grown spruce seedlings and other conifers, such as *Pinus sylvestris* (Luomala *et al.*, 2005). These anatomical and biochemical changes reduce net CO_2 assimilation rates (Luomala *et al.*, 2003) which would lead to a reduction in nonstructural carbohydrate concentrations if there was no regulatory control. However, low carbohydrate status is sensed through sugar signaling pathways that reduce growth, suppress respiration and inhibit mitochondrial development in order to maintain homeostatic carbohydrate concentrations in leaves (Journet *et al.*, 1986; Koch, 1996; Giege *et al.*, 2005; Rolland *et al.*, 2006). The regulatory reductions in growth include lower allocation to roots relative to shoots, which reduces the carbon sink strength (Reich *et al.*, 1993; Teskey & Will, 1999; Atkin *et al.*, 2007). Under other carbon-limiting conditions,

Table 3 Net CO_2 assimilation (A) and respiration (R) rates and related gas exchange parameters of black spruce seedlings grown at $22\,°C/16\,°C$ (LT) or $30\,°C/24\,°C$ (HT) day/night temperatures

	LT	HT	% Difference
Net CO_2 assimilation rate ($\mu mol\,m^{-2}\,s^{-1}$)			
A at day growth temperature			
2004 ($400\,\mu mol\,photons\,m^{-2}\,s^{-1}$)	8.2 ± 0.5	$5.3 \pm 0.3^{***}$	-35
2005 ($1000\,\mu mol\,photons\,m^{-2}\,s^{-1}$)	7.5 ± 0.6	$6.1 \pm 0.5^{**}$	-19
A at $22\,°C$			
2004	8.2 ± 0.5	$5.0 \pm 0.3^{***}$	-39
2005	7.5 ± 0.6	$5.6 \pm 0.5^{**}$	-25
A at $30\,°C$			
2004	8.0 ± 0.4	$5.3 \pm 0.3^{***}$	-34
2005	6.9 ± 0.6	6.1 ± 0.5	ns
Initial slope of CO_2 response curve	0.06 ± 0.01	$0.04 \pm 0.01^{**}$	-33
A_{sat} (1000 ppm CO_2)	13.9 ± 0.7	$11.7 \pm 1.0^{*}$	-16
V_{cmax} ($\mu mol^{-1}\,m^{-2}\,s^{-1}$)	44.6 ± 4.4	$32.8 \pm 2.1^{**}$	-26
J_{max} ($\mu mol\,m^{-2}\,s^{-1}$)	43.7 ± 1.3	$38.4 \pm 2.0^{**}$	-12
Respiration rates ($\mu mol\,m^{-2}\,s^{-1}$)			
R at night growth temperature			
2004	0.3 ± 0.0	0.4 ± 0.0	ns
2005	0.4 ± 0.0	0.4 ± 0.0	ns
R at day growth temperature			
2005	0.6 ± 0.1	$0.9 \pm 0.2^{**}$	$+33$
Q_{10} of respiration ($20-25\,°C$)	2.0 ± 0.1	2.1 ± 0.3	ns
E_a of respiration ($KJ\,mol^{-1}$)	-49.6	-47.5	
A/R day growth temperature	13.1 ± 1.9	$7.0 \pm 1.0^{**}$	-47
A/R $22\,°C$	13.1 ± 1.9	12.9 ± 2.0	ns
A/R $30\,°C$	6.3 ± 1.0	7.0 ± 1.0	ns

Means $\pm$ SE, asterisks indicate significant differences between treatments.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
The percent difference is not shown for nonsignificant (ns) results.
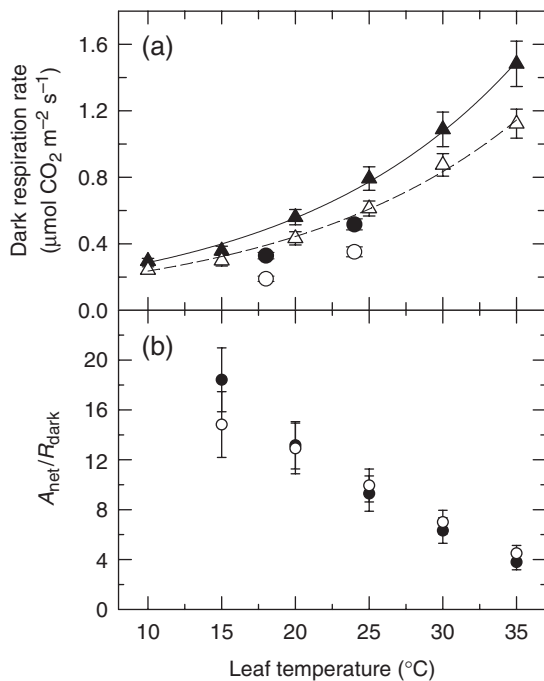HT, high temperature; LT, low temperature; CO_2, carbon dioxide.

Fig. 6 (a) The temperature response of dark respiration in black spruce grown at 22 °C/16 °C (LT; solid symbols) or 30 °C/24 °C (HT; open symbols) day/night temperatures. Circles are data from 2004, triangles are data from 2005. Means ± SE, $n = 18$–20 trees per growth temperature (2004) and six trees per growth temperature (2005). (b) The effect of temperature on the ratio of net CO_2 assimilation to dark respiration in black spruce grown at 22 °C/16 °C (LT; solid symbols) or 30 °C/24 °C (HT; open symbols) day/night temperatures. Means ± SE, six trees per growth temperature (2005).

such as growth at low CO_2, large reductions in photosynthesis and growth rate also occur, and carbohydrate levels also tend to remain stable, indicating regulatory control of growth (Cowling & Sage, 1998; Campbell et al., 2005). This control is also seen in studies of plants shifted into a low carbon status by extensive defoliation or transfer to deep shade, where growth rates immediately decline and carbohydrate levels show rapid stabilization (Veneklaas & den Ouden, 2005; Myers & Kitajima, 2007). The scaling back of growth when carbon becomes limiting correlates well with tree ring data of black spruce showing that warmer growing seasons are associated with less annual growth of trees (Brooks et al., 1998; ACIA, 2005).

Mechanisms contributing to the decline in net CO_2 assimilation rate

Growth of black spruce at 30 °C day temperatures caused substantial declines in net carbon gain. Warm-grown trees had 19–35% lower CO_2 assimilation rates
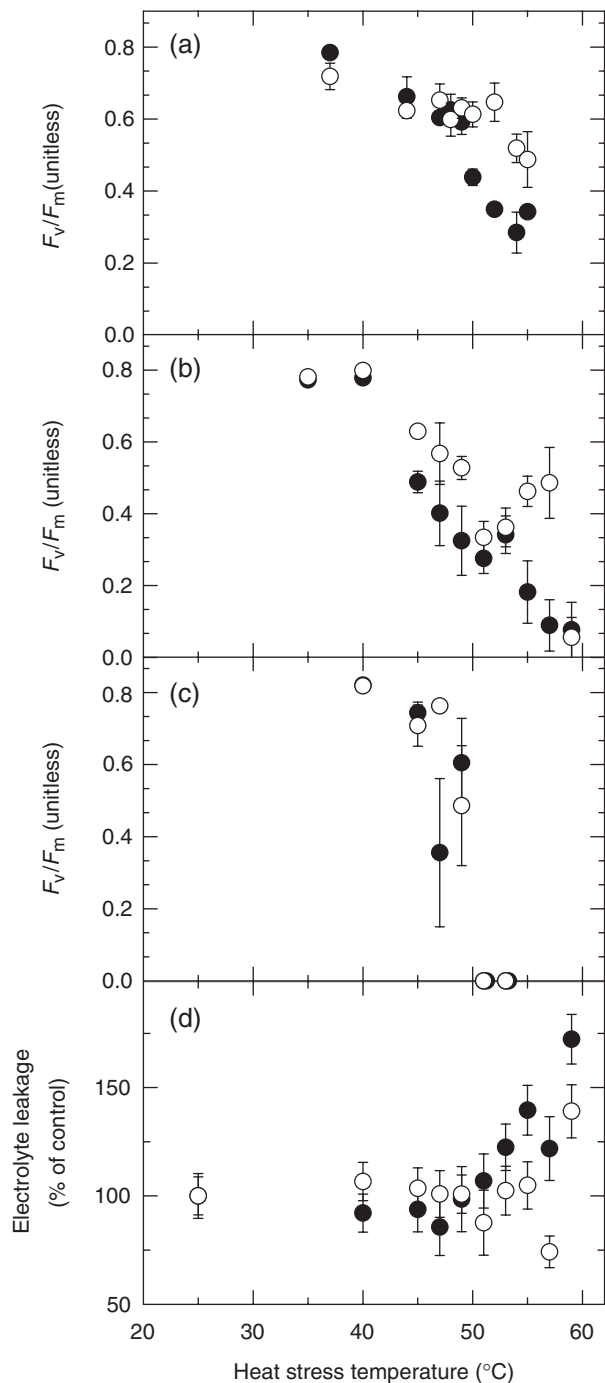

Fig. 7 The effect of growth temperature on the thermotolerance of black spruce to heat stress: (a) the response of maximal photochemical efficiency of PSII (F_v/F_m) following a 3-min heat exposure (2004); (b) the response of F_v/F_m following a 10-min heat exposure (2005); (c) the changes in F_v/F_m 1 week after a 10-min heat exposure (2005); (d) electrolyte leakage from leaves after a 10-min heat exposure (2005). Solid circles are for spruce grown at 22 °C/16 °C (LT), open circles are data from seedlings grown at 30 °C/24 °C (HT). Means ± SE, $n = 2$–8 trees per growth temperature per stress temperature. LT, low temperature; HT, high temperature.

per unit leaf area at their growth temperature and 47% higher respiration rates at daytime growth temperatures than trees grown at 22 °C days. Thus, the net CO_2 assimilation rate per unit leaf area was 12-fold greater than the dark respiration rate at 22 °C, but only fourfold greater at 30 °C for trees from both treatments. The lack of significant photosynthetic acclimation to high growth temperatures in black spruce meant that warm-grown trees acquired much less carbon than cool-grown trees. Plants that develop at warm temperatures often have lower level of leaf nitrogen and photosynthetic enzymes, such as Rubisco and cytochrome f (Tjoelker et $al.$, 1999b; Yamori et $al.$, 2005). Black spruce that developed at high temperatures had less nitrogen per unit leaf area, and reduced Rubisco and electron transport proteins as indicated by lower apparent V_{cmax} and J_{max} of HT seedlings. While lower photosynthetic protein content reduced net photosynthesis on a leaf area basis in warm-grown seedlings, their 54% smaller leaf canopy area compounded the lower photosynthetic capacity at the seedling level, thereby reducing whole tree carbon gain. This lower carbon gain in warm-grown trees was partially offset by greater allocation to photosynthetic tissue and less allocation to roots, as seen in the lower root : shoot ratio, which increases the source to sink ratio.

While we did not test the effect of elevated CO_2 on the temperature response of black spruce, it is unlikely that rising CO_2 concentrations will offset the results shown here. Black spruce strongly acclimates to elevated CO_2, with net CO_2 assimilation rates declining within weeks to a year of exposure to high CO_2 concentrations (Johnsen, 1993; Way & Sage, 2004). Tjoelker et $al.$ (1998) observed that black spruce seedlings grown and measured at elevated CO_2 and 30 °C days had lower net photosynthetic rates than those grown and measured at 30 °C and ambient CO_2 concentrations. Respiratory acclimation of black spruce also responds more strongly to high temperatures than to elevated CO_2 (Tjoelker et $al.$, 1999a). Studies on other conifers examining the effects of both elevated CO_2 and temperature have found that temperature effects dominate CO_2 effects on growth, anatomy, photosynthesis and respiratory acclimation (Kellomaki & Wang, 1998; Olszyk et $al.$, 1998, 2005; Tjoelker et $al.$, 1999a; Apple et $al.$, 2000; Lewis et $al.$, 2002, 2004; Luomala et $al.$, 2005).

Respiratory acclimation and the inability to maintain a constant A/R ratio

To prevent high respiration rates from depleting accumulated carbon, plants can acclimate respiration, often within a day of a temperature change (Teskey & Will, 1999; Atkin & Tjoelker, 2003; Yamori et $al.$, 2005; Arm-

strong et $al.$, 2006; Atkin et $al.$, 2006). Respiratory acclimation to warmer temperatures may be caused by a reduced availability of substrate or reduced demand for respiratory products (Atkin & Tjoelker, 2003). In $Arabidopsis$ $thaliana$, respiratory acclimation is associated with changes in mitochondrial ultrastructure and reduced mitochondrial abundance and volume (Armstrong et $al.$, 2006). We found that although dark respiration rates measured at daytime growth temperatures were 47% higher in warm-grown seedlings than cool-grown trees, respiration rates at night-time growth temperatures were identical. Despite respiratory acclimation, lower photosynthetic rates at high temperatures led to a decrease in the ratio of net CO_2 assimilation to dark respiration (A/R) with increasing temperature. This means that a greater proportion of daily fixed carbon is respired in warm-grown trees. In other species, a low A/R correlates with slow growth rates (Poorter et $al.$, 1990; Atkin et $al.$, 1996). A similar inability to balance A/R across different growth temperatures was found in an alpine $Plantago$ species, although lowland $Plantago$ species maintained a constant A/R at growth temperatures from 13 to 27 °C (Atkin et $al.$, 2006). These results suggest that species, such as black spruce, from stressful environments may have less acclimatory capability in maintaining carbon homeostasis across growth temperatures than species from moderate climates.

Black spruce and boreal forest ecology in a warmer world

The slow growth of black spruce at high temperatures will reduce their competitive ability in a warmer world. Small plants are more vulnerable to stochastic events, including drought, herbivory and extreme heat events, because they lack reserves to sustain themselves through the stress (Campbell et $al.$, 2005; Myers & Kitajima, 2007). As well, the reduced root mass of warm-grown trees will be less able to obtain water and nutrients before their acquisition by competitors. In our study, seedlings that grew at elevated temperatures also had 13% mortality, despite being grown under otherwise optimal conditions. Thus, warmer temperatures will not only reduce growth of mature black spruce, but will also accelerate species decline via increased seedling mortality. As black spruce growth and competitive ability decline, southern spruce-dominated forests may be replaced by pine-dominated forests in western Canada, with concomitant declines in forest productivity (tree basal area) of 30–40% (Burton & Cumming, 1995). Mixed deciduous–conifer forests are likely to move into the current boreal zone in eastern North America, based on climate envelope models and experimental seed transplants. In particular, sugar

maple (*Acer saccharum* Marsh.) and oak-hickory dominated vegetation should expand their ranges northwards where soils are compatible (Hansen *et al.*, 2001; Kellman, 2004; Goldblum & Rigg, 2005). A shift in boreal forest species composition could strongly impact the global atmosphere, given black spruce's low albedo compared with the vegetation which may replace it (Bala *et al.*, 2007).

While severe heat events will be more common in coming decades (IPCC, 2001), we see no evidence that these will directly harm black spruce. Temperatures as low as 44 °C reduced the maximum quantum yield of PSII, which is at least 5 °C cooler than previously shown to induce damage in black spruce (Colombo & Timmer, 1992). Despite reductions in F_v/F_m at these temperatures, damage from heat stresses <45 °C was generally reversible within a week. High growth temperatures increased thermotolerance, as the rise in F_v/F_m and electrolyte leakage occurred between 45 and 50 °C in LT seedlings but at 50–55 °C in HT seedlings. Because the temperatures where injury occurs are above those likely to be encountered by black spruce, we conclude that direct heat injury will not be a major cause of decline. Instead, low net carbon gain per needle area, associated with growth responses that limit canopy development and root growth will be the more important physiological mechanisms contributing to black spruce decline. Thus, in a warmer climate, black spruce will grow more slowly, be less competitive, and be more prone to drought-induced damage than in its present climate.

Acknowledgements

References

ACIA (2005) *Forests, Land Management and Agriculture. Arctic Climate Impact Assessment*. Cambridge University Press, Cambridge, UK, pp. 781–862.

Angert A, Biraud S, Bonfils C *et al.* (2005) Drier summers cancel out the CO$_2$ uptake enhancement induced by warmer springs. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 10823–10827.

Apple ME, Olszyk DM, Ormrod DP, Lewis A, Southworth D, Tingey DT (2000) Morphology and stomatal function of Douglas fir needles exposed to climate chance: elevated CO$_2$ and temperature. *International Journal of Plant Sciences*, **161**, 127–132.

Armstrong AF, Logan DC, Tobin AK, O'Toole P, Atkin OK (2006) Heterogeneity of plant mitochondrial responses underpinning respiratory acclimation to the cold in Arabidopsis thaliana leaves. *Plant, Cell and Environment*, **29**, 940–949.

Atkin OK, Botman B, Lambers H (1996) The causes of inherently slow growth in alpine plants: an analysis based on the underlying carbon economies of alpine and lowland Poa species. *Functional Ecology*, **10**, 698–707.

Atkin OK, Scheurwater I, Pons TL (2006) High thermal acclimation potential of both photosynthesis and respiration in two lowland *Plantago* species in contrast to an alpine congener. *Global Change Biology*, **12**, 500–515.

Atkin OK, Scheurwater I, Pons TL (2007) Respiration as a percentage of daily photosynthesis in whole plants is homeostatic at moderate, but not high, growth temperatures. *New Phytologist*, **174**, 367–380.

Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science*, **8**, 343–351.

Bala G, Caldeira K, Wickett M, Phillips TJ, Lobell DB, Delire C, Mirin A (2007) Combined climate and carbon-cycle effects of large-scale deforestation. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 6550–6555.

Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **31**, 491–543.

Berry JA, Raison JK (1981) Responses of macrophytes to temperature. In: *Physiological Plant Ecology. I: Responses to the Physical Environment* (eds Lange OL, Nobel PS, Osmond CB, Ziegler H), pp. 277–338. Springer-Verlag, Berlin.

Brooks JR, Flanagan LB, Ehleringer JR (1998) Responses of boreal conifers to climate fluctuations: indications from tree-ring widths and carbon isotope analyses. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **28**, 524–533.

Burton PJ, Cumming SG (1995) Potential effects of climatic-change on some Western Canadian forests, based on phenological enhancements to a patch model of forest succession. *Water Air and Soil Pollution*, **82**, 401–414.

Campbell CD, Sage RF (2006) Interactions between the effects of atmospheric CO$_2$ content and P nutrition on photosynthesis in white lupin (*Lupinus albus* L.). *Plant, Cell and Environment*, **29**, 844–853.

Campbell CD, Sage RF, Kocacinar F, Way DA (2005) Estimation of the whole-plant CO$_2$ compensation point of tobacco (*Nicotiana tabacum* L.). *Global Change Biology*, **11**, 1956–1967.

Colombo SJ, Timmer VR (1992) Limits of tolerance to high temperatures causing direct and indirect damage to black spruce. *Tree Physiology*, **11**, 95–104.

Cowling SA, Sage RF (1998) Interactive effects of low atmospheric CO$_2$ and elevated temperature on growth, photosynthesis and respiration in *Phaseolus vulgaris*. *Plant, Cell and Environment*, **21**, 427–435.

Day ME (2000) Influence of temperature and leaf-to-air vapor pressure deficit on net photosynthesis and stomatal conductance in red spruce (*Picea rubens*). *Tree Physiology*, **20**, 57–63.

Giege P, Sweetlove LJ, Cognat V, Leaver CJ (2005) Coordination of nuclear and mitochondrial genome expression during

mitochondrial biogenesis in Arabidopsis. *Plant Cell*, **17**, 1497–1512.

Gifford RM (1995) Whole plant respiration and photosynthesis of wheat under increased CO_2 concentration and temperature: long-term vs short-term distinctions for modelling. *Global Change Biology*, **1**, 385–396.

Goetz SJ, Bunn AG, Fiske GJ, Houghton RA (2005) Satellite-observed photosynthetic trends across boreal North America associated with climate and fire disturbance. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 13521–13525.

Goldblum D, Rigg LS (2005) Tree growth response to climate change at the deciduous-boreal forest ecotone, Ontario, Canada. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **35**, 2709–2718.

Gunderson CA, Norby RJ, Wullschleger SD (2000) Acclimation of photosynthesis and respiration to simulated climatic warming in northern and southern populations of *Acer saccharum*: laboratory and field evidence. *Tree Physiology*, **20**, 87–96.

Hansen AJ, Neilson RR, Dale VH *et al.* (2001) Global change in forests: responses of species, communities, and biomes. *Bioscience*, **51**, 765–779.

Havaux M (1993) Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. *Plant, Cell and Environment*, **16**, 461–467.

Hendrix DL (1993) Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. *Crop Science*, **33**, 1306–1311.

IPCC (2001) *Climate Change 2001: Working Group II: Impacts, Adaptation and Vulnerability*. Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge.

Johnsen KH (1993) Growth and ecophysiological responses of black spruce seedlings to elevated CO_2 under varied water and nutrient additions. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **23**, 1033–1042.

Johnsen KH, Major JE (1999) Shoot water relations of mature black spruce families displaying a genotype × environment interaction in growth rate. I. Family and site effects over three growing seasons. *Tree Physiology*, **19**, 367–374.

Journet EP, Bligny R, Douce R (1986) Biochemical-changes during sucrose deprivation in higher-plant cells. *Journal of Biological Chemistry*, **261**, 3193–3199.

Kellman M (2004) Sugar maple (*Acer saccharum* Marsh.) establishment in boreal forest: results of a transplantation experiment. *Journal of Biogeography*, **31**, 1515–1522.

Kellomaki S, Wang KY (1998) Sap flow in Scots pines growing under conditions of year-round carbon dioxide enrichment and temperature elevation. *Plant, Cell and Environment*, **21**, 969–981.

Koch KE (1996) Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **47**, 509–540.

Lewis JD, Lucash M, Olszyk DM, Tingey DT (2002) Stomatal responses of Douglas-fir seedlings to elevated carbon dioxide and temperature during the third and fourth years of exposure. *Plant, Cell and Environment*, **25**, 1411–1421.

Lewis JD, Lucash M, Olszyk DM, Tingey DT (2004) Relationships between needle nitrogen concentration and photosynthetic responses of Douglas-fir seedlings to elevated CO_2 and temperature. *New Phytologist*, **162**, 355–364.

Loveys BR, Atkinson LJ, Sherlock DJ, Roberts RL, Fitter AH, Atkin OK (2003) Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast- and slow-growing plant species. *Global Change Biology*, **9**, 895–910.

Loveys BR, Scheurwater I, Pons TL, Fitter AH, Atkin OK (2002) Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast- and slow-growing plant species. *Plant, Cell and Environment*, **25**, 975–987.

Luomala EM, Laitinen K, Kellomaki S, Vapaavuori E (2003) Variable photosynthetic acclimation in consecutive cohorts of Scots pine needles during 3 years of growth at elevated CO_2 and elevated temperature. *Plant, Cell and Environment*, **26**, 645–660.

Luomala EM, Laitinen K, Sutinen S, Kellomaki S, Vapaavuori E (2005) Stomatal density, anatomy and nutrient concentrations of Scots pine needles are affected by elevated CO_2 and temperature. *Plant, Cell and Environment*, **28**, 733–749.

Major JE, Barsi DC, Mosseler A, Campbell M, Rajora OP (2003) Light-energy processing and freezing-tolerance traits in red spruce and black spruce: species and seed-source variation. *Tree Physiology*, **23**, 685–694.

Marshall JG, Rutledge RG, Blumwald E, Dumbroff EB (2000) Reduction in turgid water volume in jack pine, white spruce and black spruce in response to drought and paclobutrazol. *Tree Physiology*, **20**, 701–707.

Medlyn BE, Dreyer E, Ellsworth D *et al.* (2002) Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. *Plant, Cell and Environment*, **25**, 1167–1179.

Mooney HA, Bjorkman O, Collatz GJ (1978) Photosynthetic acclimation to temperature in desert shrub, *Larrea divaricata*.1. Carbon-dioxide exchange characteristics of intact leaves. *Plant Physiology*, **61**, 406–410.

Muhaidat R, Sage RF, Dengler ND (2007) Key innovations in the evolution of Kranz anatomy and C4 vein pattern in *Flaveria* (Asteraceae). *American Journal of Botany*, **94**, 382–399.

Myers JA, Kitajima K (2007) Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *Journal of Ecology*, **95**, 383–395.

Nelson EA, Sage TL, Sage RF (2005) Functional leaf anatomy of plants with crassulacean acid metabolism. *Functional Plant Biology*, **32**, 409–419.

Northeast Climate Impacts Assessment (2006) *Climate Change in the US Northeast*. UCS Publications, Cambridge, MA.

Olszyk D, Apple M, Gartner B *et al.* (2005) Xeromorphy increases in shoots of *Pseudotsuga menziesii* (Mirb.) Franco seedlings with exposure to elevated temperature but not elevated CO_2. *Trees-Structure and Function*, **19**, 552–563.

Olszyk D, Wise C, VanEss E, Tingey D (1998) Elevated temperature but not elevated CO_2 affects long-term patterns of stem diameter and height of Douglas-fir seedlings. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **28**, 1046–1054.

Poorter H, Remkes C, Lambers H (1990) Carbon and nitrogen economy of 24 wild-species differing in relative growth-rate. *Plant Physiology*, **94**, 621–627.

Rahmstorf S, Cazenave A, Church JA, Hansen JE, Keeling RF, Parker DE, Somerville RCJ (2007) Recent climate observations compared to projections. *Science*, **316**, 709.

Reich PB, Walters MB, Krause SC, Vanderklein DW, Raffa KF, Tabone T (1993) Growth, nutrition and gas-exchange of pinus-resinosa following artificial defoliation. *Trees-Structure and Function*, **7**, 67–77.

Rodeghiero M, Niinemets U, Cescatti A (2007) Major diffusion leaks of clamp-on leaf cuvettes still unaccounted: how erroneous are the estimates of Farquhar et al. model parameters? *Plant, Cell and Environment*, **30**, 1006–1022.

Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annual Review of Plant Biology*, **57**, 675–709.

Sage RF, Kubien DS (2007) The temperature response of C-3 and C-4 photosynthesis. *Plant, Cell and Environment*, **30**, 1086–1106.

Samuelson LJ, Teskey RO (1991) Net photosynthesis and leaf conductance of Loblolly pine seedlings in 2 percent and 21 percent oxygen as influenced by irradiance, temperature and provenance. *Tree Physiology*, **8**, 205–211.

Teskey RO, Will RE (1999) Acclimation of loblolly pine (*Pinus taeda*) seedlings to high temperatures. *Tree Physiology*, **19**, 519–525.

Tjoelker MG, Oleksyn J, Reich PB (1998) Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO_2 and temperature. *Tree Physiology*, **18**, 715–726.

Tjoelker MG, Oleksyn J, Reich PB (1999a) Acclimation of respiration to temperature and CO_2 in seedlings of boreal tree species in relation to plant size and relative growth rate. *Global Change Biology*, **5**, 679–691.

Tjoelker MG, Reich PB, Oleksyn J (1999b) Changes in leaf nitrogen and carbohydrates underlie temperature and CO_2 acclimation of dark respiration in five boreal tree species. *Plant, Cell and Environment*, **22**, 767–778.

Tucker CJ, Slayback DA, Pinzon JE, Los SO, Myneni RB, Taylor MG (2001) Higher northern latitude normalized difference vegetation index and growing season trends from 1982 to 1999. *International Journal of Biometeorology*, **45**, 184–190.

Vann DR, Johnson AH, Casper BB (1994) Effect of elevated temperatures on carbon dioxide exchange in *Picea rubens*. *Tree Physiology*, **14**, 1339–1349.

Veneklaas EJ, den Ouden F (2005) Dynamics of non-structural carbohydrates in two Ficus species after transfer to deep shade. *Environmental and Experimental Botany*, **54**, 148–154.

Viereck LA, Johnston WF (1990) *Picea mariana* (Mill.) B.S.P. In: *Silvics of North America: 1. Conifers* (eds Burns RM, Honkala BH), pp. 877. US Department of Agriculture, Forest Service, Washington, DC.

Way DA, Sage RF (2004) Short-term exposure to elevated CO_2 causes downwards regulation of photosynthesis in black spruce seedlings. In: *Photosynthesis: Fundamental Aspects to Global Perspectives. 13th International Congress of Photosynthesis* (eds van der Est A, Bruce D), pp. 624–626. Allen Press, Montreal.

Yamori W, Noguchi K, Terashima I (2005) Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. *Plant, Cell and Environment*, **28**, 536–547.