



Research paper

Higher growth temperatures decreased net carbon assimilation and biomass accumulation of northern red oak seedlings near the southern limit of the species range

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If an increase in temperature will limit the growth of a species, it will be in the warmest portion of the species distribution. Therefore, in this study we examined the effects of elevated temperature on net carbon assimilation and biomass production of northern red oak (*Quercus rubra* L.) seedlings grown near the southern limit of the species distribution. Seedlings were grown in chambers in elevated CO₂ (700 μmol mol⁻¹) at three temperature conditions, ambient (tracking diurnal and seasonal variation in outdoor temperature), ambient +3 °C and ambient +6 °C, which produced mean growing season temperatures of 23, 26 and 29 °C, respectively. A group of seedlings was also grown in ambient [CO₂] and ambient temperature as a check of the growth response to elevated [CO₂]. Net photosynthesis and leaf respiration, photosynthetic capacity (V_{cmax} , J_{max} and triose phosphate utilization (TPU)) and chlorophyll fluorescence, as well as seedling height, diameter and biomass, were measured during one growing season. Higher growth temperatures reduced net photosynthesis, increased respiration and reduced height, diameter and biomass production. Maximum net photosynthesis at saturating [CO₂] and maximum rate of electron transport (J_{max}) were lowest throughout the growing season in seedlings grown in the highest temperature regime. These parameters were also lower in June, but not in July or September, in seedlings grown at +3 °C above ambient, compared with those grown in ambient temperature, indicating no impairment of photosynthetic capacity with a moderate increase in air temperature. An unusual and potentially important observation was that foliar respiration did not acclimate to growth temperature, resulting in substantially higher leaf respiration at the higher growth temperatures. Lower net carbon assimilation was correlated with lower growth at higher temperatures. Total biomass at the end of the growing season decreased in direct proportion to the increase in growth temperature, declining by 6% per 1 °C increase in mean growing season temperature. Our observations suggest that increases in air temperature above current ambient conditions will be detrimental to *Q. rubra* seedlings growing near the southern limit of the species range.

Keywords: photosynthesis, *Quercus rubra*, respiration, temperature acclimation.

Introduction

Almost all studies of the effects of elevated temperature on the growth of deciduous tree species have reported that warming enhances growth (Way and Oren 2010). For example, in both ambient and elevated [CO₂], saplings of *Fagus sylvatica* had increased stem diameter, biomass and leaf area when grown at +2 and +4 °C above the current ambient temperature

(Overdieck et al. 2007). A similar response was observed in *Quercus myrsinaefolia* saplings when grown at temperatures of +3 and +5 °C above ambient (Usami et al. 2001). Positive growth responses can be substantial: in elevated [CO₂], elevated air temperatures increased the annual biomass increment by 11–12% per 1 °C (Usami et al. 2001, Overdieck et al. 2007). In cold temperate regions, in elevated [CO₂], the

height, diameter and biomass growth of *Betula pendula* seedlings increased with a +3 °C increase in temperature (Kuokkanen et al. 2004), and the height, diameter, leaf area and biomass growth of *Populus cathayana* increased with +2 and +4 °C increases in temperature (Xu et al. 2007). The average growth response among all studies reviewed by Way and Oren (2010) was appreciable: a 10 °C increase in growth temperature produced a 1.7-fold increase in biomass.

Many models simulating future tree distributions in North America have predicted that most tree species will expand their range in the north and contract their range in the south (e.g., Iverson and Prasad 2001, 2002, McKenney et al. 2007, Morin et al. 2008, Tang and Beckage 2010). This outcome is largely based on the concept that temperature is a principal factor controlling north-south distributions of tree species (He et al. 2005, Xu et al. 2007). Evidence of rapid species migrations in response to global warming after the last ice age supports this idea (Clark et al. 1998, Svenning and Skov 2007) as do provenance trials examining the correlation between temperature and growth across tree species' ranges (Schmidtling 1994, Xu et al. 2007).

However, empirical studies on temperate deciduous tree species do not support the concept, since warming has consistently been shown to increase growth (Way and Oren 2010), suggesting that deciduous trees are well equipped to cope with elevated temperature, or that they are growing in suboptimal temperature environments. Most tree species exhibit substantial acclimation of respiration to temperature, thereby reducing carbohydrate losses from maintenance respiration at higher temperatures (Atkin and Tjoelker 2003). Additionally, most tree species generally have a broad optimum temperature range for photosynthesis (Berry and Bjorkman 1980, Teskey 1995), which can extend to even higher temperatures when atmospheric [CO₂] is elevated because the temperature optimum of photosynthesis shifts upward as [CO₂] increases (Sage et al. 2002). Acclimation of net photosynthesis to growth temperature has also been observed in some tree species (Gunderson et al. 2010), but it has generally not been found in most tree species studied (Ow et al. 2010).

If high temperature limits the growth of a species in any part of its range, it will be in the southern (or warmest) portion. Therefore, we specifically chose to study the response of a deciduous tree species to warming near the southern limit of its distribution to determine whether, under certain conditions, a deciduous tree species will have a negative response to warming. The objective of this study was to examine the effects of elevated growth temperatures of +3 and +6 °C on net photosynthesis, leaf respiration and biomass production of seedlings of northern red oak (*Quercus rubra* L.), a temperate deciduous species, grown in elevated [CO₂] in the southernmost part of its range. The study was

conducted in elevated atmospheric [CO₂] (700 μmol mol⁻¹) because increases in air temperature and atmospheric [CO₂] are predicted to occur concurrently. By the year 2100, [CO₂] is expected to reach 700 μmol mol⁻¹ (Model A1B, IPCC 2007a) and air temperatures in the southeastern USA are expected to increase between 3 and 6 °C (Regional Climate Projections, IPCC 2007b). We tested the hypothesis that near the southern limit of the range of *Q. rubra*, elevated air temperatures of +3 and +6 °C would decrease the net carbon assimilation and biomass production of seedlings grown in elevated [CO₂].

Materials and methods

Experimental setup

Two-year-old northern red oak (*Quercus rubra* L.) seedlings were grown in four treatment chambers located at the University of Georgia Whitehall Experimental Forest in Athens, GA (33 °57'N, 83 °19'W, elevation 230 m) for one growing season. The current distribution of northern red oak stretches from southern Canada to central Georgia, USA; the experimental site is located near the southern edge of the species range (Figure 1). Half-cylinder domed treatment chambers measuring 3.6 m long × 3.6 m wide × 2.4 m high were constructed of wood and PVC pipe and covered with poly-film (6 ml clear GT Performance Film, Green-Tek Inc., Edgerton, WI, USA) (Boyette and Bilderback 1996). The chambers were constructed in a large open field, oriented facing south and placed side-by-side 2.5 m apart.

Temperature was independently measured and controlled in each chamber. A differential thermostat (Model DSD-2, Kera Technologies Inc., Mississauga, Ontario, Canada) in each chamber controlled an air conditioner and an electric resistance heater to maintain the temperatures at ambient, ambient +3 °C and ambient +6 °C. The mean ambient site temperature was 23.7 °C and the mean ambient chamber temperature was 23.1 °C over the course of the experiment. The mean elevated temperatures achieved with the control system were 26.3 and 28.9 °C, i.e., ambient +3.2 °C and ambient +5.8 °C. Carbon dioxide concentration was also measured and controlled independently in each chamber using a non-dispersive infrared CO₂ sensor (Model GMT222, Vaisala Inc., Woburn, MA, USA) and a solenoid valve connected to a cylinder of compressed CO₂. Three chambers were maintained at elevated [CO₂] (targeted 700 μmol mol⁻¹ CO₂). The actual mean [CO₂] achieved over the course of the experiment was 680 μmol mol⁻¹ CO₂. The fourth chamber was maintained at ambient temperature and [CO₂] (targeted 380, achieved 400 μmol mol⁻¹ CO₂) to serve as a check of seedling response to elevated CO₂. The experiment was analyzed using linear regression, taking into account the lack of replication of temperature and CO₂ conditions (see the subsection Data analysis).

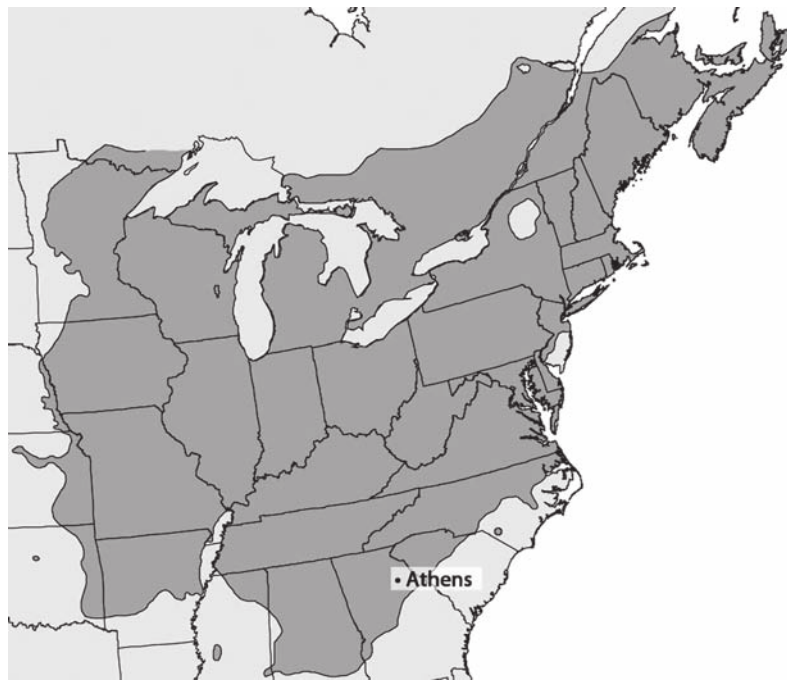


Figure 1. Distribution of northern red oak and location of the experimental site at Athens, Georgia, USA (Little 1971).

Temperature and $[\text{CO}_2]$ treatments were initiated on 1 April, just as the seedlings began to leaf out. Northern red oak is a winter-deciduous species, so all foliage through the course of the experiment developed in the treatment conditions. Air was circulated in each chamber with an oscillating fan to promote uniform $[\text{CO}_2]$ dispersion and a stable temperature throughout the treatment chamber. Seedlings were rotated within chambers several times throughout the study.

Two-year-old bare-root northern red oak seedlings (South Carolina Forestry Commission, Taylor Nursery, Trenton, SC, USA) were planted on 3 March 2009 in 12 l pots in a commercial potting substrate (Fafard Nursery Mix, Conrad Fafard Inc., Agawam, MA, USA). The seed source was natural stands in the South Carolina Piedmont, which is geographically close and climatically similar to the study location. The average stem height and diameter of the seedlings at planting were 0.47 m and 54 mm, respectively ($n = 13$ per treatment). All seedlings were fertilized with ~ 30 g of 15-9-12 extended-release fertilizer (Osmocote Plus #903286, Scotts-Sierra Horticultural Products, Marysville, OH, USA) on 26 April and 28 July 2009. On 28 May 2009, 0.04 ml Imidacloprid (Bayer Advanced 12 Month Tree and Shrub Insect Control; Bayer; Monheim am Rhein, Germany) was applied topically to the soil in each pot to prevent insect infestation. Seedlings were watered to saturation four times a day with an automated irrigation system and drip emitters (Supertif—PLASTRO, Kibbutz Gvat D. N. Ha'Amakim, Israel). Sensor data (temperature and $[\text{CO}_2]$) were

measured every 30 s, and 15 min averages were recorded with a datalogger (23X, Campbell Scientific, Logan, UT, USA).

Gas exchange

Temperature response curves of light-saturated photosynthesis (A_{net}) and leaf respiration (R_d) were measured three times during the growing season: on 1 June, 20 July and 8 September. To avoid the effects of both seasonal and diurnal temperature variation on foliar gas exchange and to ensure that the whole plant was at the desired temperature, these measurements were conducted in environmentally controlled conditions. The procedure was similar to that outlined in Ghannoum et al. (2010). On each measurement date, four seedlings were randomly selected from each treatment chamber and rapidly transported to two growth chambers (EGC 36; Environmental Growth Chambers, Chargin Falls, OH, USA) which were located <8 km from the treatment chambers. All measurements were completed in one day. Initial growth chamber conditions were set at 20 °C, 55% relative humidity, either 500 (A_{net}) or 0 (R_d) $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR), and either 380 or 700 $\mu\text{mol mol}^{-1}$ $[\text{CO}_2]$ (depending on growth conditions). Seedlings were allowed to equilibrate to chamber conditions for at least 45 min and then foliar gas exchange was measured on a representative leaf in the most recent fully developed flush. When measurements at one temperature were complete, chamber temperature conditions were adjusted, seedlings were allowed to equilibrate again and measurements

were repeated. Measurements of A_{net} and R_d were made at 20, 27, 34 and 41 °C with a portable photosynthesis system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA) using a broadleaf cuvette with a standard red/blue light-emitting diode light source set at 1500 (A_{net}) or 0 (R_d) $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Cuvette [CO_2] was maintained at growth conditions with a CO_2 mixer.

One day after the temperature response curves were completed, the response of A_{net} to intercellular [CO_2] (A_{net}/C_i) was measured using the same portable photosynthesis system. Five seedlings were randomly selected from each treatment, excluding seedlings used for the most recent temperature response curves. The same growth chambers were used, with conditions maintained at 25 °C, 55% relative humidity and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Seedlings were allowed to equilibrate to chamber conditions for 1 h. Measurements were conducted on a leaf in the most recent fully developed flush. The environmental conditions in the leaf cuvette were 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR and 25 °C with the cuvette [CO_2] controlled at seven concentrations (380, 200, 100, 35, 550, 700 and 1000 $\mu\text{mol mol}^{-1}$ [CO_2]).

Chlorophyll fluorescence

Leaf fluorescence was measured to determine if growth temperature affected photosynthetic capacity. Following the completion of the A_{net}/C_i curves on 8 June, 27 July and 3 September, dark-acclimated leaf fluorescence (F_v/F_m) was measured in situ at 05:00 h on a leaf from the most recent fully developed flush of five randomly selected seedlings in each treatment. Light-acclimated steady-state fluorescence (F_s ; made at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR), basic fluorescence after induction (F'_o) and maximal fluorescence during a saturating light flash (F'_m) were measured between 12:00 and 15:00 h. All fluorescence measurements were made with a portable photosynthesis system fitted with a fluorescence chamber (LI-6400-40, LI-COR Biosciences, Lincoln, NE, USA). From these measurements, the effective quantum yield of PSII (Φ_{PSII}) ($(F'_m - F_s)/F'_m$) and photochemical quenching (qP) ($(F'_m - F_s)/(F'_m - F'_o)$) were calculated.

Growth measurements

Seedlings were harvested just prior to the end of the growing season (30 September 2009) to prevent the loss of foliage. Stem height (H) and diameter at the base (D_{base}) of each seedling were measured using a meter stick and a digital caliper (ABSOLUTE Digimatic 500-196-20, Mitutoyo USA, Aurora, IL, USA). Foliage was removed from the seedlings and total leaf area was measured with a leaf area meter (LI-3000, LI-COR Biosciences, Lincoln, NE, USA). Roots were hand washed to remove all potting material. Foliage, stem and root biomass was dried at 60 °C for 1 week and weighed.

Data analysis

The three elevated CO_2 chambers were considered independent populations and the seedlings within them were treated

as subsamples (Neter and Wasserman 1985). Temperature response curves of A_{net} were fitted with a second-order polynomial function, and the value for the optimum temperature (T_{opt}) of A_{net} was calculated as the temperature corresponding to maximum net photosynthesis. The temperature response curves of R_d were fitted with an Arrhenius function. Linear regression analysis with a t -test for slopes was conducted on the relationship between mean daytime temperature of the 30 days prior to measurement (independent variable), mean A_{net} from the temperature response curves, T_{opt} , V_{cmax} , J_{max} , TPU, F_v/F_m , qP and PSII, as well as the relationship between 30-day mean nighttime temperature and mean R_d from the temperature response curves. These relationships were also examined using the mean temperature of 7 and 14 days prior to measurement, but the correlations were not as good, hence these data were not included. Additionally, significant differences between T_{opt} and R_d at 41 °C were tested using repeated measures analysis of variance with temperature treatment (three levels) and date (three levels) as the fixed effects.

Total biomass accumulation, seedling height and seedling diameter were analyzed by linear correlation (t -test for slope). Changes in biomass partitioning in response to growth temperature were also tested by analysis of covariance for separate intercepts and slope using natural log transformations of component biomass following Bongarten and Teskey (1987) (SAS v. 9.2, SAS Institute, Cary, NC, USA).

Results

Gas exchange

Averaged across all measurement temperatures of the temperature response curves, net photosynthesis (A_{net}) in June was lower than in July or August for the ambient and +3 °C growth temperatures (Figure 2). Seedlings grown in the highest temperature treatment did not exhibit an increase in A_{net} as the growing season progressed. Seedlings grown in, and measured in, elevated [CO_2] had a higher A_{net} compared with seedlings grown and measured in ambient [CO_2], although the effect of elevated [CO_2] on photosynthesis diminished as the growing season progressed. A_{net} was generally highest from 20 to 30 °C, but was always positive at 41 °C. There was no apparent difference in the temperature response of A_{net} among the three temperature treatments in elevated CO_2 in June. In July, A_{net} was higher in seedlings in the ambient temperature than those at +3 or +6 °C. In September the temperature response curves were again similar among the treatments, although A_{net} at 20 °C was noticeably lower in the +6 °C seedlings than in the other treatments.

We did not observe any signs of acclimation of A_{net} to growth temperature. When the optimum temperature of A_{net}

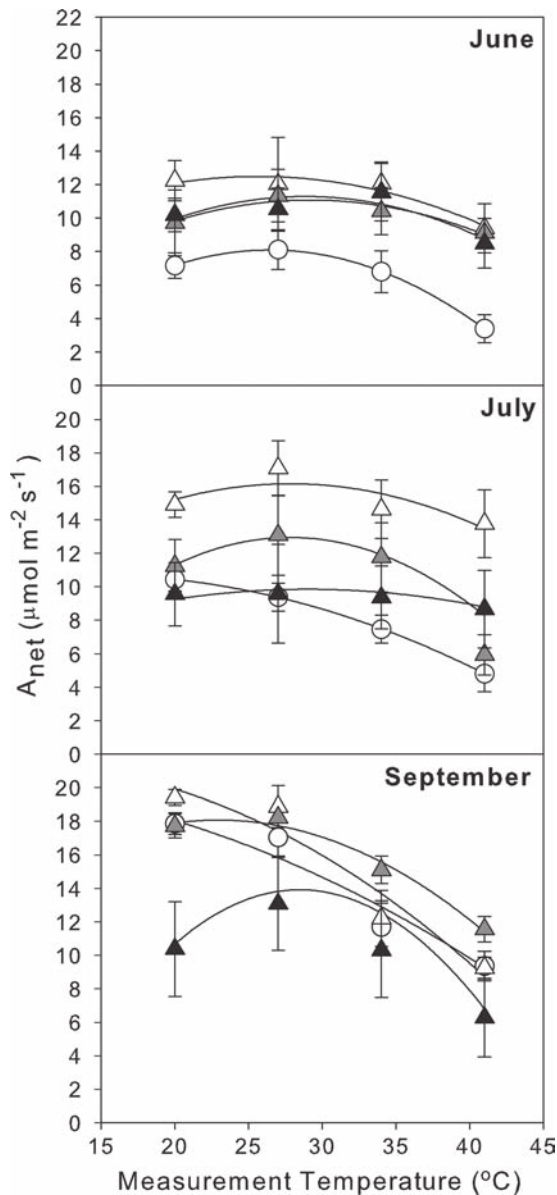


Figure 2. Temperature response of light-saturated net photosynthesis (A_{net}) of northern red oak seedlings. Plants grown at ambient $[\text{CO}_2]$ indicated by circles, elevated $[\text{CO}_2]$ by triangles; ambient temperature indicated by white symbols, ambient temperature +3 °C by gray symbols and ambient temperature +6 °C by black symbols. Error bars represent ± 1 SE; $n = 4$.

(extrapolated from the temperature response curves) was plotted against the mean daytime temperature for the 30 days prior to measurements, no significant relationship was determined for any growth temperature regime (Figure 3). Across all measurement dates and growth temperatures, there was no significant relationship between daytime growth temperature for the 30 days prior to measurements and the optimum temperature of A_{net} (t -test for slope: $P = 0.21$). Additionally, there was no significant difference in the optimum temperature of A_{net} between the three temperature treatments at any date (repeated measures analysis: June: $P = 0.41$; July: $P = 0.14$),

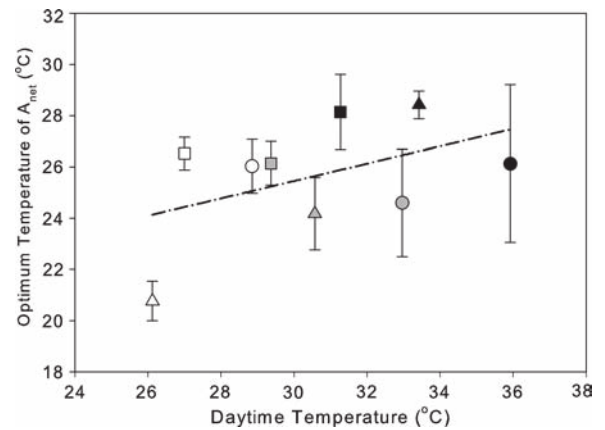


Figure 3. Optimum temperature of net photosynthesis (A_{net}) of northern red oak seedlings, calculated from temperature response curves, plotted against mean daytime temperature for the 30 days prior to measurements. Measurements were made on a leaf of the most recent fully developed flush in June (squares, $P = 0.48$), July (circles, $P = 0.97$) and September (triangles, $P = 0.12$); ambient temperature indicated by white symbols, ambient temperature +3 °C by gray symbols and ambient temperature +6 °C by black symbols. The dotted line represents the trend of optimum temperature of A_{net} across all measurements (t -test for slope: $P = 0.21$). Error bars represent ± 1 SE.

with one exception: in September the optimum temperature for seedlings grown in the +6 °C treatment was significantly higher than that for seedlings grown in the ambient ($P = 0.006$) or +3 °C ($P = 0.035$) treatment, which did not vary ($P = 0.4$).

Foliar dark respiration (R_d) increased as the growing season progressed regardless of growth temperature, with one exception: R_d did not increase from July to September in seedlings grown in the ambient +3 °C temperature (Figure 4). Exposure to elevated $[\text{CO}_2]$ did not appear to have any effect on R_d . There was no evidence of acclimation of R_d to growth temperature, even though a consistent difference of approximately 3 and 6 °C above ambient temperature was maintained day and night. There was no significant treatment effect on R_d measured at 41 °C in June or July (repeated measures analysis: $P = 0.76$ and $P = 0.15$, respectively). However, there was a significant difference in R_d measured at 41 °C in September, with R_d being significantly higher in seedlings grown in the ambient temperature treatment compared with the +3 or +6 °C treatment (repeated measures analysis: $P = 0.02$ and $P = 0.04$, respectively), which did not significantly vary ($P = 0.84$).

To assess the potential effect of growth temperature on daily mean A_{net} and R_d , we used the temperature response curves and the mean daytime or nighttime temperature for the 30 days prior to the three measurement periods to estimate average A_{net} or R_d for the three temperature treatments during the three periods (Figure 5). Across the growing season, estimated A_{net} was negatively correlated with growth temperature (Figure 5a). The estimated A_{net} of seedlings grown at current ambient temperature was generally higher than that of those grown at elevated temperatures, suggesting they had a lower potential for carbon

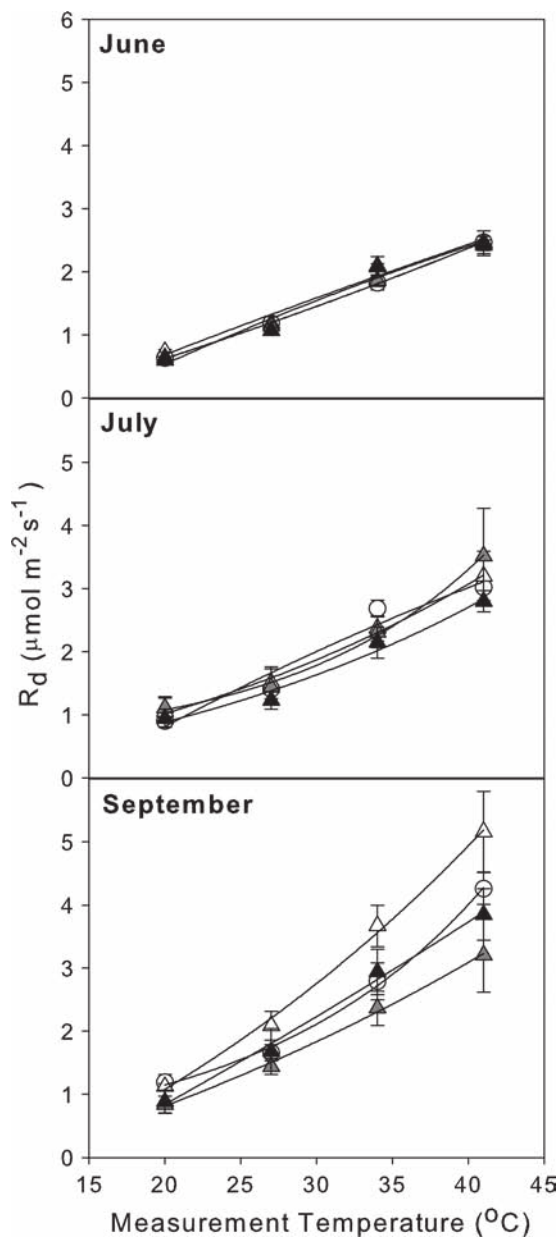


Figure 4. Temperature response curves of foliar dark respiration (R_d) of northern red oak seedlings. Plants grown at ambient $[\text{CO}_2]$ indicated by circles, elevated $[\text{CO}_2]$ by triangles; ambient temperature indicated by white symbols, ambient temperature $+3^\circ\text{C}$ by gray symbols and ambient temperature $+6^\circ\text{C}$ by black symbols. Error bars represent ± 1 SE; $n = 4$.

gain at higher temperatures. The opposite pattern was apparent in R_d ; seedlings grown at higher temperatures had higher estimated R_d in their growth conditions, indicating greater loss of carbohydrates due to maintenance respiration at higher temperatures (Figure 5b). The calculated decreases in A_{net} and increases in R_d both indicate that net carbon assimilation was lower in seedlings grown at elevated temperatures.

Maximum A_{net} , determined as the CO_2 -saturated rate of fixation from the A/C_i curves, was consistently lower in seedlings

grown in the warmer conditions, compared with those grown at current ambient temperature (Figure 6). Initially, seedlings grown at $+3^\circ\text{C}$ also had lower maximum A_{net} than those grown at ambient temperature, but in July and September the A/C_i curves of seedlings from those two treatments were nearly identical. There was no effect of growth temperature of the 30 days prior to measurements on V_{cmax} , J_{max} and TPU (t -test for slope: V_{cmax} : $P = 0.83$; J_{max} : $P = 0.81$; TPU: $P = 0.70$) (Table 1).

Across the growing season, dark-acclimated fluorescence (F_v/F_m) was not affected by nighttime temperature for the 30 days prior to measurement (t -test of slope: $P = 0.14$, data not shown). All measurements of F_v/F_m were consistently higher than 0.75, which we interpreted to mean that the foliage remained healthy (Genty et al. 1989). Measurements of both qP and Φ_{PSII} , when averaged across the growing season, were not affected by daytime temperature (t -test of slope: $P = 0.21$ and $P = 0.20$, respectively). The lack of an effect of higher growth temperatures on leaf fluorescence suggests that the relationship between A_{net} and daytime temperature was not driven by heat-induced foliar damage, but instead was due to temporary stresses related to the growing conditions.

Biomass

There was a significant negative correlation between mean growth temperature and total seedling biomass, height and diameter at harvest (Figure 7) (t -test for slope: $P < 0.001$). Each increase of 1°C in mean growth temperature corresponded to a 6% decrease in biomass accumulation. Under current ambient temperature, as expected, seedlings grown in elevated $[\text{CO}_2]$ accrued more biomass, were taller and had a larger stem diameter than seedlings grown in ambient $[\text{CO}_2]$. Leaf biomass partitioning was significantly affected by growth temperature, with seedlings allocating more biomass to leaves when grown at $+6^\circ\text{C}$ (correlation analysis: $P = 0.01$); the effect was not dependent upon seedling size ($P = 0.61$). Leaf area ratio was not affected by growth temperature (t -test for slope: $P = 0.43$). Stem and root biomass partitioning were not significantly affected by growth temperature (correlation analysis: $P = 0.35$ and $P = 0.63$, respectively; Figure 8), indicating that increased growth temperature did not alter carbon allocation.

Discussion

Many models of species distributions use the current temperatures in a species range to predict future distributions (e.g., Iverson and Prasad 2001, 2002, McKenney et al. 2007). While there appears little doubt that low air temperature has a controlling influence on a tree species' northernmost extent (in the northern hemisphere) (Sakai and Weiser 1973, Morin et al. 2007, Walther et al. 2007), there is uncertainty about the role of high temperature in controlling a tree species' southernmost extent (Woodward 1987, Bonan and Sirois

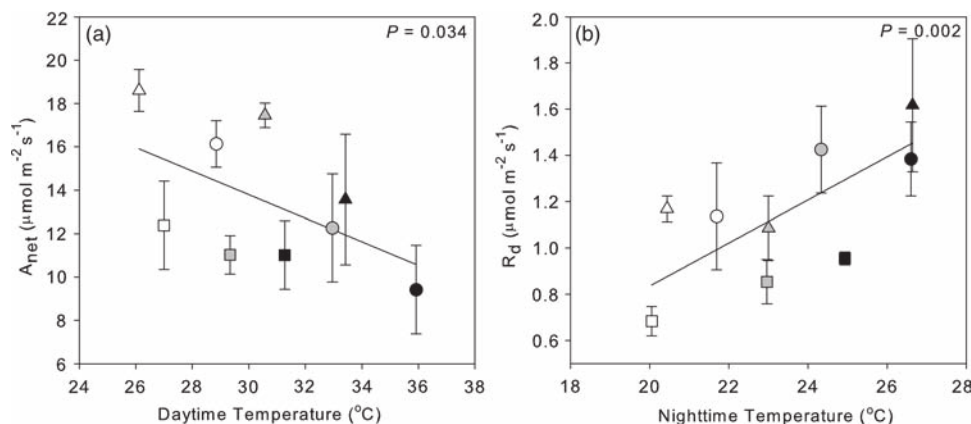


Figure 5. (a) Estimated net photosynthesis (A_{net}) and (b) foliar dark respiration (R_d) of northern red oak seedlings calculated from temperature response curves and the mean daytime or nighttime temperatures for the 30 days prior to measurements made on 1 June, 20 July and 8 September 2009. Measurements were made on a leaf of the most recent fully developed flush in June (squares), July (circles) and September (triangles). Values from seedlings grown in ambient temperature indicated by white symbols, ambient temperature +3 °C by gray symbols and ambient temperature +6 °C by black symbols. Error bars represent ± 1 SE.

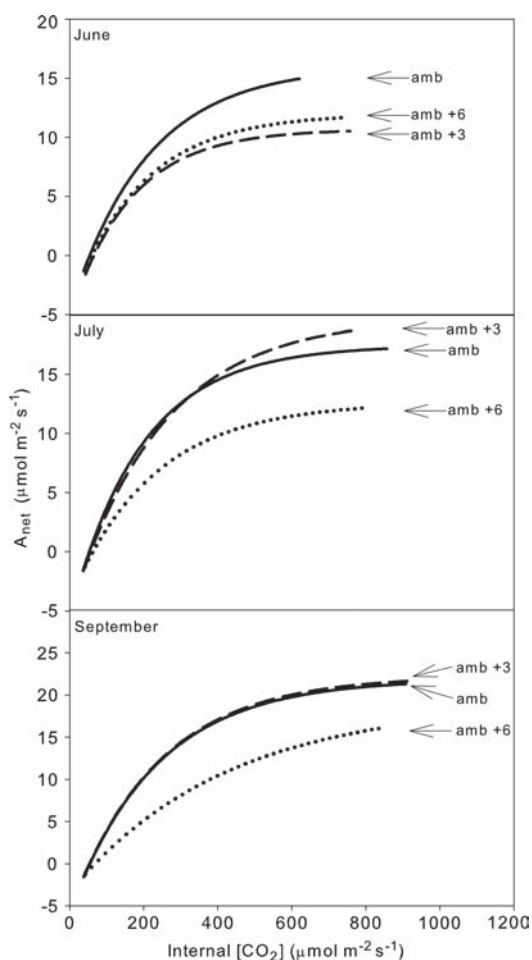


Figure 6. Response of net photosynthesis (A_{net}) to intercellular $[\text{CO}_2]$ (C_i) of northern red oak seedlings grown at $700 \mu\text{mol mol}^{-1}$ $[\text{CO}_2]$ in three temperature regimes. Measurements made on a leaf of the most recently developed flush in June, July and September 2009. Plants grown at ambient temperature indicated by solid line, ambient temperature +3 °C by long dashed line and ambient temperature +6 °C by short dashed line.

Table 1. V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$), J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and TPU ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (calculated from A_{net}/C_i curves) (+SE) measured on a leaf of the most recent fully developed flush of northern red oak seedlings on 3 June, 22 July and 10 September 2009. Growth treatments were: ambient temperature and ambient $[\text{CO}_2]$ ($T_A C_A$), ambient temperature and elevated $[\text{CO}_2]$ ($T_A C_E$), ambient temperature +3 °C and elevated $[\text{CO}_2]$ ($T_{+3} C_E$), and ambient temperature +6 °C and elevated $[\text{CO}_2]$ ($T_{+6} C_E$).

	V_{cmax}	J_{max}	TPU
June			
$T_A C_A$	85 (9.7)	90 (9.2)	6.2 (0.45)
$T_A C_E$	63 (6.5)	80 (5.5)	5.6 (0.40)
$T_{+3} C_E$	39 (8.4)	57 (6.2)	4.1 (0.49)
$T_{+6} C_E$	46 (5.4)	62 (5.9)	4.4 (0.49)
July			
$T_A C_A$	69 (13)	90 (12)	6.3 (0.90)
$T_A C_E$	65 (5.3)	86 (10)	6.1 (0.58)
$T_{+3} C_E$	86 (13)	100 (5.7)	6.9 (0.49)
$T_{+6} C_E$	55 (6)	67 (4.6)	4.7 (0.12)
September			
$T_A C_A$	87 (6.7)	113 (5.9)	7.5 (0.56)
$T_A C_E$	92 (15)	109 (5.9)	7.7 (0.36)
$T_{+3} C_E$	94 (8.7)	110 (5.7)	7.7 (0.44)
$T_{+6} C_E$	97 (4.1)	98 (3.8)	5.7 (0.19)

1992). Our observations support the hypothesis that in the southern portion of the range of *Q. rubra*, elevated growth temperature will be detrimental to net carbon assimilation and biomass accumulation, even in elevated $[\text{CO}_2]$. These results also provide support for the hypothesis that temperature has an influence on the extent of temperate deciduous tree species distributions in the warmer part of their range.

Although elevated $[\text{CO}_2]$ increased photosynthesis and growth as expected, elevated air temperature reduced the beneficial effect of higher $[\text{CO}_2]$ on growth. At current ambient temperature, final biomass increased by 38% in elevated $[\text{CO}_2]$

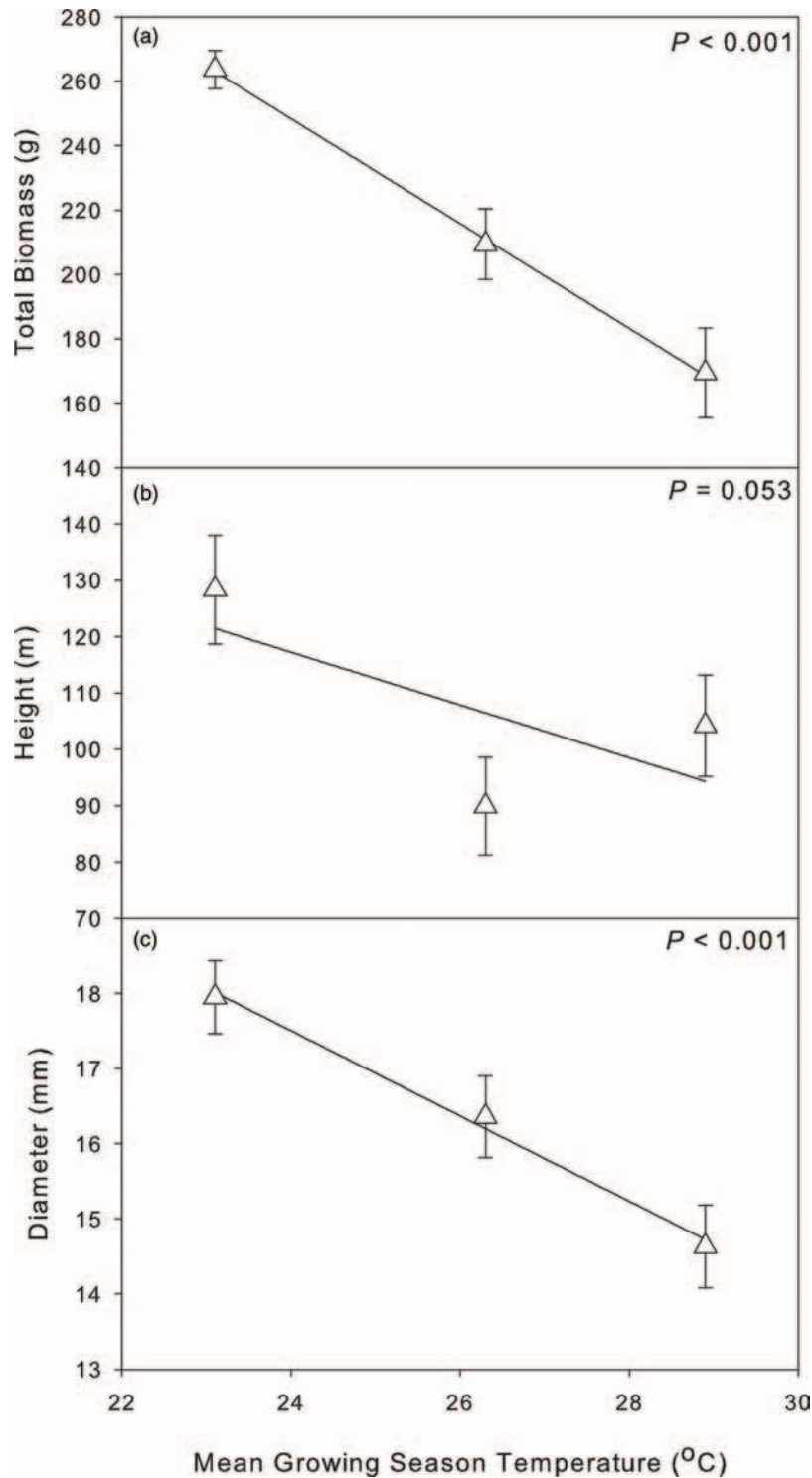


Figure 7. (a) Total biomass, (b) height and (c) diameter of northern red oak seedlings at the end of the growing season plotted against average growing season temperature. P values represent t -test for slope.

compared with ambient $[\text{CO}_2]$. When grown at $+3^\circ\text{C}$, this increase was almost completely negated, and at $+6^\circ\text{C}$ final biomass was reduced by 12% compared with the baseline value obtained at current ambient $[\text{CO}_2]$ and temperature conditions. The negative effects of elevated growth temperature were

linear, suggesting that the impact on *Q. rubra* seedlings in the warmest parts of the species range will depend directly on the magnitude of future warming. In agreement with our findings, some other studies have observed a negative effect of increasing temperature on A_{net} (Callaway et al. 1994, Wang et al. 1995).

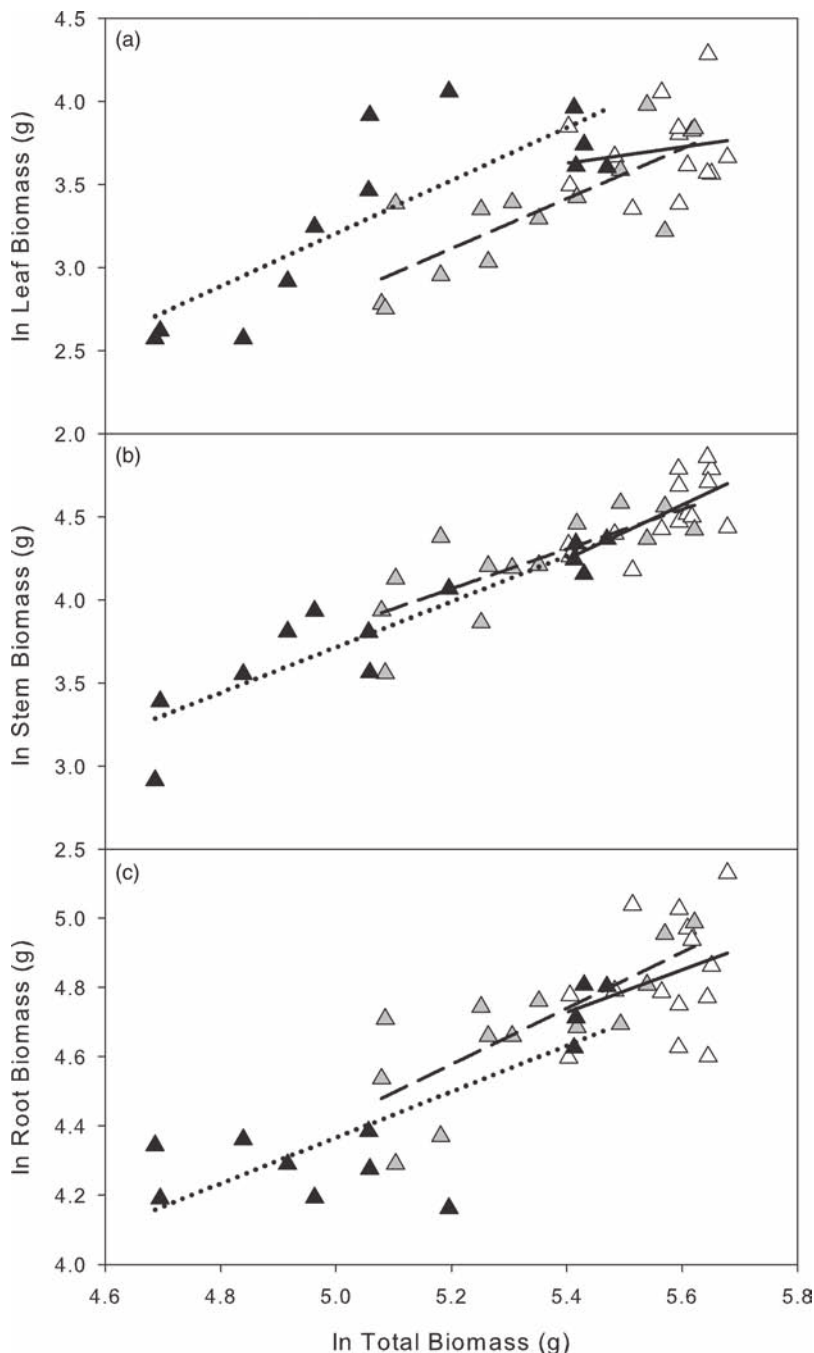


Figure 8. (a) Leaf, (b) stem and (c) root biomass partitioning of northern red oak seedlings grown in elevated $[\text{CO}_2]$. Plants grown at ambient temperature indicated by white symbol and solid regression line, ambient temperature +3 °C by gray symbol and long dashed regression line, and ambient temperature +6 °C by black symbol and dotted regression line.

However, these findings are not universal, and other studies on different species have reported either no effect, or even a positive effect, of increased growth temperature on A_{net} (Way and Oren 2010). For instance, Ghannoum et al. (2010) did not report any negative effects of elevated temperature on light-saturated photosynthesis (measured at growth $[\text{CO}_2]$), maximum photosynthesis (at saturating $[\text{CO}_2]$) or J_{max} in eucalypt seedlings grown in either ambient or elevated $[\text{CO}_2]$.

The reduction in A_{net} coupled with the increase in R_d with increased growth temperatures may explain the linear decrease in biomass accumulation, stem height and stem diameter. Many studies have reported an increase in biomass accumulation with increased growth temperature in deciduous trees (e.g., Kellomaki and Wang 2001, Usami et al. 2001, Overdieck et al. 2007, Allen and Vu 2009, Way and Oren 2010) and evergreen trees (e.g., Callaway et al. 1994, Cantin et al. 1997, Danby and

Hik 2007, Yin et al. 2008, Zhao and Liu 2009, Way and Oren 2010). However, a few studies have reported a reduction in growth with increased growth temperature (Wang et al. 1995, Norby et al. 2000, Way and Sage 2008, Nedlo et al. 2009).

The only other report we are aware of that examined the effect of elevated temperature on a temperate deciduous tree species near its southern limit was by Norby et al. (2000). They observed that in elevated $[\text{CO}_2]$ with an increase in air temperature of 3.5 °C, *Acer saccharum* seedlings had a 21% reduction in biomass growth. Our results, and those of Norby et al. (2000), suggest that temperatures in the southern (warmer) portion of a species range can be supra-optimal for growth of temperate deciduous species. However, it is also likely that different species will respond differently to increased temperature, perhaps related to their ecological niche, inherent growth rate or ability to acclimate physiologically to changes in temperature (Saxe et al. 2001, Lo et al. 2010). Species-specific responses to changes in temperature, coupled with differences in species ranges and the location of plants within their ranges, will likely change competitive relationships and the relative importance and dominance among species in the forest community (van der Putten et al. 2004).

An atypical response observed in this study was the lack of acclimation of foliar respiration (R_d) to growth temperature. Both short- and long-term acclimation have been clearly demonstrated in numerous tree species (Atkin and Tjoelker 2003), including *Q. rubra*, which has been reported to rapidly and reversibly acclimate to changes in temperature (Bolstad et al. 2003). Additionally, Lee et al. (2005) showed acclimation of respiration with exposure to varying diurnal and seasonal temperatures in *Q. rubra*, *Q. alba* and *Acer rubrum*. We cannot be certain of the reasons for the lack of acclimation observed in this study, nor are the findings completely novel. Ayub et al. (2011) reported no significant effect of temperature or $[\text{CO}_2]$ on R_d of *Eucalyptus saligna* seedlings. It is important to point out that in our study, September R_d was lower in seedlings grown at the ambient temperature than in seedlings grown at the elevated temperatures, suggesting that there may be seasonal plasticity in acclimation of foliar respiration. Indeed, seasonal declines in R_d have been reported in *Eucalyptus globulus* (O'Grady et al. 2010) and *Populus canadensis* (Searle and Turnbull 2011). Using the same measurement approach, we found distinct temperature acclimation of respiration in *Pinus taeda* seedlings (Wertin et al. in preparation), suggesting methodology was not the cause. It may be possible that different genotypes of northern red oak have a greater or lesser capability to acclimate to temperature. Although we used an open pollinated seed source rather than a single family or clone, the seed source was limited to a narrow geographical area near the southern edge of the species distribution. Although genetic variation in acclimation has not been observed previously (Teskey and Will 1999, Bolstad et al. 2003), the number of

reports exploring this phenomenon is very limited and further verification is necessary.

Another interesting finding in this study was the lack of apparent reduction to photosynthetic capacity at higher growth temperatures, as determined by both fluorescence measurements and A_{net}/C_i curves. Darbah et al. (2010) demonstrated that while exposure to high temperature can significantly reduce photosynthetic capacity, the reduction is dependent on both the degree of heat stress exposure and whether the tree is capable of emitting isoprene to cope with thermal stress. Our A_{net}/C_i curves indicated that elevated temperatures had only a small effect on V_{cmax} and J_{max} , although we did observe a substantial suppression in A_{max} with increasing growth temperature. It should be noted that in this study the ratio of $J_{\text{max}}:V_{\text{cmax}}$ was lower than that reported by Medlyn et al. (2002), due primarily to below average rates of J_{max} . We are uncertain why our rates of J_{max} were lower than those usually reported, especially given that the seedlings were well watered and well fertilized. Fluorescence measurements did not provide any evidence of temperature-induced reduction to either photochemical quenching or the effective quantum yield of PSII. Some species of *Quercus* can emit substantial amounts of isoprene and it is possible that elevated $[\text{CO}_2]$ or isoprene emissions may have protected the photosynthetic apparatus (Haldimann and Feller 2004, Darbah et al. 2010), although Way et al. (2011) reported that elevated CO_2 may reduce isoprene production.

This study was focused on the effect of elevated growth temperature in elevated $[\text{CO}_2]$ because air temperature and $[\text{CO}_2]$ are coupled, and both are expected to continue to increase throughout this century. The ambient $[\text{CO}_2]$ treatment was included to verify that there was a response to elevated $[\text{CO}_2]$ alone. The effects of $[\text{CO}_2]$ on photosynthesis and growth of trees are now well established and were not the focus of this study. The effects of $[\text{CO}_2]$ alone in this study were consistent with previous reports (e.g., Ainsworth and Long 2005, Bader et al. 2010, Ghannoum et al. 2010).

In this study, only a single seed source was used and only the seedling stage was evaluated. The seed source was obtained from the southern edge of the species range and was presumably locally adapted, but it is possible that different genotypes or plants at other stages of maturity will respond differently to increased growth temperature. However, several studies of temperature acclimation failed to detect significant differences in response among genotypes (Teskey and Will 1999, Bolstad et al. 2003). With respect to stage of maturity, in a review of 63 studies, Way and Oren (2010) found the same general response to temperature irrespective of whether a tree was grown in a pot or in the field; the reviewed studies included trees at many life stages. The seedling growth stage is one of the most critical times for survival of an individual tree. This study suggests that northern red oak seedlings will

undergo additional stress due to elevated temperature near the southern limits of the species' natural range.

Conclusion

In this study we demonstrated that elevated growth temperature imposed stress that reduced the growth of seedlings of *Q. rubra*. This finding had previously been observed in only two other temperate deciduous tree species, *A. rubrum* and *A. saccharum* (Norby et al. 2000). The negative effect of elevated temperature on growth was linear and substantial, suggesting that any increase in air temperature will be detrimental to *Q. rubra* near the southern limit of its range. Although growth is a complicated phenomenon, the lack of temperature acclimation of respiration appears to have been a contributing factor to our results. Along with the degree of future warming, whether a species, or a genotype within a species, exhibits temperature acclimation of respiration, and the degree of acclimation, appear to be a key physiological indicators of how the species or genotype will respond to future temperature increases.

Results from this study indicate that plants may respond both positively and negatively to higher growth temperatures, which we suggest may be partly related to the location of the growing site within a species' geographic range. These findings support the assumption commonly used in models of current and future plant distributions that temperature is one of the key factors controlling the northern and southern extent of a species' natural distribution.

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References

Ainsworth, E.A. and S.P. Long. 2005. What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytol.* 165: 351–371.

Allen, L.H. and J.C.V. Vu. 2009. Carbon dioxide and high temperature effects on growth of young orange trees in a humid, subtropical environment. *Agric. Forest Meteorol.* 149:820–830.

Atkin, O.K. and M.G. Tjoelker. 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends Plant Sci.* 8:343–351.

Ayub, G., R.A. Smith, D.T. Tissue and O.K. Atkin. 2011. Impacts of drought on leaf respiration in darkness and light in *Eucalyptus saligna* exposed to industrial-age atmospheric CO₂ and growth temperature. *New Phytol.* 190:1003–1018.

Bader, M.K.F., R. Siegwolf and C. Korner. 2010. Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO₂ enrichment. *Planta* 232:1115–1125.

Berry, J. and O. Bjorkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 31:491–543.

Bolstad, P.V., P. Reich and T. Lee. 2003. Rapid temperature acclimation of leaf respiration rates in *Quercus alba* and *Quercus rubra*. *Tree Physiol.* 23:969–976.

Bonan, G.B. and L. Sirois. 1992. Air temperature, tree growth, and the northern and southern range limits to *Picea mariana*. *J. Veg. Sci.* 3:495–506.

Bongarten, B.C. and R.O. Teskey. 1987. Dry weight partitioning and its relationship to productivity in loblolly pine seedlings from seven sources. *For. Sci.* 33:255–267.

Boyette, M.D. and T.E. Bilderback. 1996. A small backyard greenhouse for the home gardener. North Carolina Cooperative Extension Service. AG-426. p 1–4.

Callaway, R.M., E.H. Delucia, E.M. Thomas and W.H. Schlesinger. 1994. Compensatory responses of CO₂ exchange and biomass allocation and their effects on the relative growth-rate of Ponderosa pine in different CO₂ and temperature regimes. *Oecologia.* 98:159–166.

Cantin, D., M.F. Tremblay, M.J. Lechowicz and C. Potvin. 1997. Effects of CO₂ enrichment, elevated temperature, and nitrogen availability on the growth and gas exchange of different families of jack pine seedlings. *Can. J. For. Res.* 27:510–520.

Clark, J.S., C. Fastie, G. Hurtt, S.T. et al. 1998. Reid's paradox of rapid plant migration—dispersal theory and interpretation of paleoecological records. *Bioscience* 48:13–24.

Danby, R.K. and D.S. Hik. 2007. Responses of white spruce (*Picea glauca*) to experimental warming at a subarctic alpine treeline. *Global Change Biol.* 13:437–451.

Darbuh, J.N.T., T.D. Sharkey, C. Calfapietra and D.F. Karnosky. 2010. Differential response of aspen and birch trees to heat stress under elevated carbon dioxide. *Environ. Pollut.* 158:1008–1014.

Genty, B., J.M. Briantais and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 990:87–92.

Ghannoum, O., N.G. Phillips, J.P. Conroy, R.A. Smith, R.D. Attard, R. Woodfield, B.A. Logan, J.D. Lewis and D.T. Tissue. 2010. Exposure to preindustrial, current and future atmospheric CO₂ and temperature differentially affects growth and photosynthesis in *Eucalyptus*. *Global Change Biol.* 16:303–319.

Gunderson, C.A., K.H. O'Hara, C.M. Campion, A.V. Walker and N.T. Edwards. 2010. Thermal plasticity of photosynthesis: the role of acclimation in forest responses to a warming climate. *Global Change Biol.* 16:2272–2286.

Haldimann, P. and U. Feller. 2004. Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant Cell Environ.* 27:1169–1183.

He, H.S., Z.Q. Hao, D.J. Mladenoff, G.F. Shao, Y.M. Hu and Y. Chang. 2005. Simulating forest ecosystem response to climate warming incorporating spatial effects in north-eastern China. *J. Biogeogr.* 32:2043–2056.

IPCC. 2007a. Climate change 2007: working group I: the physical science basis: global climate projections. Cambridge University Press, Cambridge, UK.

IPCC. 2007b. Climate change 2007: working group I: the physical science basis: regional climate projections. Cambridge University Press, Cambridge, UK.

Iverson, L.R. and A.M. Prasad. 2001. Potential changes in tree species richness and forest community types following climate change. *Ecosystems* 4:186–199.

- Iverson, L.R. and A.M. Prasad. 2002. Potential redistribution of tree species habitat under five climate change scenarios in the eastern US. *For. Ecol. Manag.* 155:205–222.
- Kellomaki, S. and K.Y. Wang. 2001. Growth and resource use of birch seedlings under elevated carbon dioxide and temperature. *Ann. Bot.* 87:669–682.
- Kuokkanen, K., P. Niemela, J. Matala, R. Julkunen-Tiitto, J. Heinonen, M. Rousi, H. Henttonen, J. Tahvanainen and S. Kellomaki. 2004. The effects of elevated CO₂ and temperature on the resistance of winter-dormant birch seedlings (*Betula pendula*) to hares and voles. *Global Change Biol.* 10:1504–1512.
- Lee, T.D., P.B. Reich and P.V. Bolstad. 2005. Acclimation of leaf respiration to temperature is rapid and related to specific leaf area, soluble sugars and leaf nitrogen across three temperate deciduous tree species. *Funct. Ecol.* 19:640–647.
- Little, E.L. 1971. Atlas of United States trees: Volume 1. Conifers and important hardwoods. United States Government Printing Office, Washington, DC.
- Lo, Y.H., J.A. Blanco and J.P. Kimmins. 2010. A word of caution when planning forest management using projections of tree species range shifts. *Forestry Chronicle* 86:312–316.
- McKenney, D.W., J.H. Pedlar, K. Lawrence, K. Campbell and M.F. Hutchinson. 2007. Potential impacts of climate change on the distribution of North American trees. *Bioscience* 57:939–948.
- Medlyn, B.W., E. Dreyer, D. Ellsworth, M. Forstreuter, P.C. Harley, U.F. Kirshbaum, X. Le Roux, P. Montpied, J. Strassmeyer, A. Walcroft, K. Wang and D. Loustau. 2002. Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. *Plant, Cell Environ.* 25:1167–1179.
- Morin, X., T. Ameglio, R. Ahas, C. Kurz-Besson, V. Lanta, F. Lebourgeois, F. Miglietta and I. Chuine. 2007. Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. *Tree Physiol.* 27:817–825.
- Morin, X., D. Viner and I. Chuine. 2008. Tree species range shifts at a continental scale: new predictive insights from a process-based model. *J. Ecol.* 96:784–794.
- Nedlo, J.E., T.A. Martin, J.M. Vose and R.O. Teskey. 2009. Growing season temperatures limit growth of loblolly pine (*Pinus taeda* L.) seedlings across a wide geographic transect. *Trees-Struct. Funct.* 23:751–759.
- Neter, J. and W. Wasserman. 1985. Applied linear statistical models. Irwin, Nomewood, IL, USA.
- Norby, R.J., T.M. Long, J.S. Hartz-Rubin and E.G. O'Neill. 2000. Nitrogen resorption in senescing tree leaves in a warmer, CO₂-enriched atmosphere. *Plant Soil.* 224:15–29.
- O'Grady, A.P., A. Eyles, D. Worledge and M. Battaglia. 2010. Seasonal patterns of foliage respiration in dominant and suppressed *Eucalyptus globulus* canopies. *Tree Physiol.* 30:957–968.
- Overdieck, D., D. Ziche and K. Bottcher-Jungclaus. 2007. Temperature responses of growth and wood anatomy in European beech saplings grown in different carbon dioxide concentrations. *Tree Physiol.* 27:261–268.
- Ow, L.F., D. Whitehead, A.S. Walcroft and M.H. Turnbull. 2010. Seasonal variation in foliar carbon exchange in *Pinus radiata* and *Populus deltoides*: respiration acclimates fully to changes in temperature but photosynthesis does not. *Global Change Biol.* 16:288–302.
- Sage, R.F., D.A. Way and D.S. Kubien. 2002. Rubisco, rubisco activase, and global climate change. *J. Exp. Bot.* 59:1581–1595.
- Sakai, A. and C.J. Weiser. 1973. Freezing resistance of trees in North America with reference to tree regions. *Ecology* 54:118–126.
- Saxe, H., M.G.R. Cannell, B. Johnsen, M.G. Ryan and G. Vourlitis. 2001. Tree and forest functioning in response to global warming. *New Phytol.* 149:369–399.
- Schmidtling, R.C. 1994. Use of provenance tests to predict response to climate change—loblolly pine and Norway spruce. *Tree Physiol.* 14:805–817.
- Searle, S.Y. and M.H. Turnbull. 2011. Seasonal variation of leaf respiration and the alternative pathway in field-grown *Populus × canadensis*. *Physiol. Plantarum* 141:332–342.
- Svenning, J.C. and F. Skov. 2007. Could the tree diversity pattern in Europe be generated by postglacial dispersal limitation? *Ecol. Lett.* 10:453–460.
- Tang, G.P. and B. Beckage. 2010. Projecting the distribution of forests in New England in response to climate change. *Divers. Distrib.* 16:144–158.
- Teskey, R.O. 1995. A field study of the effects of elevated CO₂ on carbon assimilation, stomatal conductance and leaf and branch growth of *Pinus taeda* trees. *Plant Cell Environ.* 18:565–573.
- Teskey, R.O. and R.E. Will. 1999. Acclimation of loblolly pine (*Pinus taeda*) seedlings to high temperatures. *Tree Physiol.* 19:519–525.
- Usami, T., J. Lee and T. Oikawa. 2001. Interactive effects of increased temperature and CO₂ on the growth of *Quercus myrsinaefolia* saplings. *Plant Cell Environ.* 24:1007–1019.
- van der Putten, W.H., P.C. de Ruiter, T.M. Bezemer, J.A. Harvey, M. Wassen and V. Wolters. 2004. Trophic interactions in a changing world. *Basic Appl. Ecol.* 5:487–494.
- Walther, G.R., E.S. Gritti, S. Berger, T. Hickler, Z.Y. Tang and M.T. Sykes. 2007. Palms tracking climate change. *Global Ecol. Biogeogr.* 16:801–809.
- Wang, K.Y., S. Kellomaki and K. Laitinen. 1995. Effects of needle age, long-term temperature and CO₂ treatments on the photosynthesis of Scots pine. *Tree Physiol.* 15:211–218.
- Way, D.A. and R. Oren. 2010. Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree Physiol.* 30:669–688.
- Way, D.A. and R.F. Sage. 2008. Thermal acclimation of photosynthesis in black spruce [*Picea mariana* (Mill.) BSP]. *Plant Cell Environ.* 31:1250–1262.
- Way, D.A., J.P. Schnitzler, R.K. Monson and R.B. Jackson. 2011. Enhanced isoprene-related tolerance of heat- and light-stressed photosynthesis at low, but not high, CO₂ concentrations. *Oecologia* 166:233–282.
- Woodward, F.I. 1987. Climate and plant distribution (Cambridge studies in Ecology). Cambridge University Press, Cambridge, UK.
- Xu, C.G., G.Z. Gertner and R.M. Scheller. 2007. Potential effects of interaction between CO₂ and temperature on forest landscape response to global warming. *Global Change Biol.* 13:1469–1483.
- Yin, H.J., Q. Liu and T. Lai. 2008. Warming effects on growth and physiology in the seedlings of the two conifers *Picea asperata* and *Abies faxoniana* under two contrasting light conditions. *Ecol. Res.* 23:459–469.
- Zhao, C.Z. and Q. Liu. 2009. Growth and photosynthetic responses of two coniferous species to experimental warming and nitrogen fertilization. *Can. J. For. Res.* 39:1–11.