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Growth temperature modulates the spatial variability of leaf morphology and chemical elements within crowns of climatically divergent *Acer rubrum* genotypes

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Summary Our understanding of leaf acclimation in relation to temperature of fully grown or juvenile tree crowns is mainly based on research involving spatially uncontrolled growth temperature. In this study, we test the hypothesis that leaf morphology and chemical elements are modulated by within-crown growth temperature differences. We ask whether within-species variation can influence acclimation to elevated temperatures. Within-crown temperature dependence of leaf morphology, carbon and nitrogen was examined in two genotypes of *Acer rubrum* L. (red maple) from different latitudes, where the mean annual temperature varies between 7.2 and 19.4 °C. Crown sections were grown in temperature-controlled chambers at three daytime growth temperatures (25, 33 and 38 °C). Leaf growth and resource acquisition were measured at regular intervals over long-term (50 days) controlled daytime growth temperatures. We found significant intraspecific variation in temperature dependence of leaf carbon and nitrogen accumulation between genotypes. Additionally, there was evidence that leaf morphology depended on inherited adaptation. Leaf dry matter and nitrogen content decreased as growth temperature was elevated above 25 °C in the genotype native to the cooler climate, whereas they remained fairly constant in response to temperature in the genotype native to the warmer climate. Specific leaf area (SLA) was correlated positively to leaf nitrogen content in both genotypes. The SLA and the relative leaf dry matter content (L_M), on the other hand, were correlated negatively to leaf thickness. However, intraspecific variation in SLA and L_M versus leaf thickness was highly significant. Intraspecific differences in leaf temperature response between climatically divergent genotypes yielded important implications for convergent evolution of leaf adaptation. Comparison of our results with those of previous studies showed that leaf carbon allocation along a vertical temperature gradient was modulated by growth temperature in the genotype native to the cooler climate. This indicates that within-crown temperature-induced variations in leaf morphology and chemical content should be accounted for in forest ecosystem models.

Keywords: intraspecific variability, leaf anatomy, leaf nitrogen, leaf thickness, specific leaf area.

Introduction

Concern about the gradual rise in atmospheric temperature, predicted to range from 1 to 7 °C by 2100, has brought about several temperature-related studies that investigate the crown responses of trees (e.g., Roden and Ball 1996, Teskey and Will 1999, Medlyn et al. 2002, Haldimann and Feller 2004, Bauerle et al. 2007). Tree crowns are spatially and temporally heterogeneous environments. Therefore, analyzing variation in leaf morphology and physiology in response to temperature gradients within a crown is often confounded by concomitant changes in irradiance, vapour pressure deficit and wind speed (Kira and Yoda 1989, Bazzaz and Wayne 1994, Jifon and Syvertsen 2003, Niinemets 2007). Although researchers hypothesized that foliar characteristics are primarily modified by gradients in crown light availability (Brooks et al. 1996, Bond et al. 1999, Niinemets et al. 1999, 2004), the sheer size of the tree crowns make the manipulation of temperature difficult, thus preventing the researchers from controlling the within-crown atmospheric temperature. As a result, conclusions are primarily based on the correlative evidence.

Vertical temperature changes ranging from 0.25 to 1.8 °C every 1 m have been observed in both coniferous (Zweifel et al. 2002) and mixed hardwood-conifer (Harley et al. 1996) forests. The foliage carbon balance within such a variable canopy microclimate is alleged to depend on temperature acclimation (Dewar et al. 1999). Similarly, leaf nitrogen content is thought to vary in response to local irradiance exposure and acclimatize to the predominant

conditions (Evans and Poorter 2001, Grassi and Bagnaresi 2001, Warren et al. 2003). In a continuous canopy, however, our current knowledge of leaf acclimation is mainly based on studies that were conducted in uncontrolled outdoor conditions. In this situation, within-crown shading can cause a decrease in irradiance and a consequential temperature reduction that complicates understanding the effects of individual environmental factors (Niinemets and Valladares 2004). Researchers have also relied on models to estimate temperature acclimation (Dewar et al. 1999). Singling out the effects of temperature along a crown height gradient is difficult due to the multiple interacting environmental variables. The authors are unaware of studies that were able to control for growth temperature to investigate leaf morphology and chemical content temperature acclimation within a continuous crown. Thus, there is still a need to separate growth irradiance from temperature and examine the growth temperature spatial effects on leaf carbon and nitrogen content.

Nitrogen productivity (N_P) at the whole-plant level depends on leaf nitrogen use efficiency (NUE; N_L, the amount of leaf dry mass per unit nitrogen taken up) and N_P at the leaf level (the rate of leaf dry mass production per unit leaf nitrogen), both of which are closely related to NUE. Due to the large portion of nitrogen investment in leaves, whole-plant NUE can mostly be explained by leaf-level attributes (Escudero et al. 1992, Garnier et al. 1995). In this context, the relationship between leaf nitrogen content and specific leaf area (SLA, the ratio of leaf area to leaf dry mass) provides a means to investigate the relative leaf dry matter content (L_M, the ratio of leaf dry mass to fresh mass) response to growth temperature. Equally important to this study, the relationship allows differentiation between the production of biomass (high SLA and low L_M) and NUE (low SLA and high L_M). Therefore, we set out to characterize the spatial distribution of leaf carbon and nitrogen within a tree crown, while taking care to truly separate out the environmental temperature effect.

Higher leaf carbon assimilation rates are usually associated with higher leaf nitrogen, SLA and thickness. With this background in mind, understanding leaf thickness responses to elevated temperature can give an indication of carbon assimilation modulation. However, leaf thickness is not easy to measure. Several estimation methods have been proposed, and recently Vile et al. (2005) validated a simple and robust multiplicative method that uses two easy-to-measure variables $(SLA \cdot L_M)^{-1}$. This analysis estimates leaf thickness using Vile et al.'s (2005) methodology to consider the interplay between temperature and leaf structural features, and examines how these affect leaf nitrogen and carbon content.

Intraspecific variation in leaf thermotolerance explains, in part, the differences in the elevated temperature growth response of climatically divergent perennial populations (e.g., Weston and Bauerle 2007, Weston et al. 2007). In addition, exploring genotypes of a forest tree with different leaf thermotolerance characteristics may contribute to our understanding of how the evolutionary consequences of climatically divergent populations might be linked to differences in plant anatomy and constituent content. The purpose of this study, therefore, was to mimic the relative natural crown daytime temperature gradient of tall trees ($\sim$ 13 °C) to (i) separate temperature from crown light interception and wind; (ii) quantify the leaf morphologic and chemical content spatial acclimation to temperature gradients and (iii) test the hypotheses that a within-crown growth temperature gradient affects leaf carbon, nitrogen and water relations. Specifically, we controlled crown section growth temperature in two climatically contrasting genotypes of a common eastern biome continuously flushing species (*Acer rubrum* L.). We then measured leaf resource acquisition, including carbon, nitrogen and water status at regular intervals on leaves acclimated to a gradient in daytime growth temperature.

Materials and methods

Plant materials and study site layout

We compared two genotypes of *A. rubrum* (red maple), the most wide-spread native tree in the Eastern US, from different latitudes. Measurements were carried out during the 2003 growing season in a 0.58 ha outdoor gravel pad of open terrain at the Clemson University Calhoun Field Laboratory in Clemson, SC (latitude 34°40′8″and longitude 82°50′40″). A full description of the site is given in Bauerle et al. (2002). Two genotypes from thermally contrasting parentage were used for intensive sampling in this study: red maple cv. 'October glory' (OG), native to MA (latitude 40°27′1″ and longitude 74°29′3″), USA and red maple cv. 'summer red' (SR), native to southern GA (latitude 31°27′27″ and longitude 83°33′41″). The mean annual temperature differs from 7.2 to 19.4 °C between the MA and GA sites, however, the annual precipitation is similar (117 and 120 cm, respectively). Before transplant and experimentation, both genotypes were grown from cuttings in Georgetown, SC under well-watered common garden nursery conditions. In April 2003, 60 (30 per genotype) 3.5-m tall equal age saplings were shipped to Clemson, SC and transplanted into 114 l plastic pots containing a mixture of 20:1 pine bark:sand (v/v) with 8.3 kg m^{-3} of 10 N-3P-8.3 K Nutricote (type 360; Chisso-Asahi Fertilizer Co., Tokyo). Trees were spaced at 1.5 × 1.5 m. To ensure that the trees never experienced substrate water-limiting conditions, substrate volumetric water content was measured daily at 10 and 20 cm below the substrate surface in four predrilled locations on the opposite sides of the container (Theta Probe type ML2, Delta-T Devices, Cambridge, UK). Each tree was watered three times daily to near container capacity with 360° pressure-compensating micro emitters (ML Irrigation Inc., Laurens,

SC), maintaining the root zone volumetric water content in each container within a previously determined well-watered range (0.4–0.5 m^3 m^{-3}).

Chamber construction, temperature control and within-crown leaf temperature measurement

In this study, the daytime growth temperature of whole crown sections was controlled from 08:00 to 20:00 h, where three temperature treatments were applied to each of the four replicate trees per genotype. Temperature profiles were randomly assigned within each crown where each crown had three different temperatures controlled at 25, 33 or 38 °C. Whole crown chambers were placed on replicate trees ($n = 4$) of each genotype. The chambers were subdivided into three volumetrically equal area layers per crown (Figure 1). Each tree sub-crown chamber, dimensions $1 \times 1 \times 2$ m^3, was constructed from polyvinyl chloride tubing of 5-cm diameter and covered with clear 0.025 mm Mylar® (DuPont, Wilmington, DE). To create the self-contained crown sections, horizontal sheets of Mylar® divided each crown layer and were secured to the trunk with foam rubber gaskets. The Mylar® photon flux density (PFD) characteristics were checked with a spectroradiometer (model 1800, Li-Cor Inc., Lincoln, NE). Results similar to Corelli and Magnanini (1993) were found where PFD was > 90% of outside incident PFD, with midday levels exceeding 1800 μmol m^{-2} s^{-1}, and the spectral composition was unchanged over the 400–900 nm range. Each layer sub-chamber was plumbed independently to maintain growth temperature on an individual crown layer basis (Figure 1). Three Twintemp 16300/10700 BTU/h cooling and heating air conditioners (model ES16, Friedrich Inc., San Antonio, TX) were plumbed to the crown sections (three sections per crown) and together they continuously controlled temperature in each crown section. Each chamber's temperature was sampled at 10 s intervals with fine wire thermocouples (CR21X, Campbell Sci. Inc., Logan, UT) and a control switch triggered the air conditioners to either heat, cool or run at ambient temperature (fan only) to maintain the temperature within 1 °C of the set point. The airflow produced > 3 volume exchanges min^{-1} per layer and created a slight positive pressure on the Mylar®, which kept it in a wrinkle-free state for maximum light penetration. A preliminary experiment found the amount of air exchange more than adequate to ensure that CO_2 levels did not deviate from outside ambient conditions. At night, temperature returned to ambient for two reasons (1) to prevent variation in temperature acclimation of dark respiration (Turnbull et al. 2002), and (2) to mimic natural diurnal conditions where temperature gradients primarily arise during the daytime due to irradiance. Leaf temperature was measured in all crown sections with Type T (Thermo Electric Wire and Cable, L.L.C, Newark, NJ) thermocouples of 0.255 mm diameter affixed to the abaxial leaf surface with breathable athletic tape (Johnson and Johnson Inc., New Brunswick, NJ). Four leaves per crown section, one in each cardinal direction, were continuously monitored every minute and 15-min averages were computed and stored (CR7X, Campbell Scientific Inc., Logan, UT).

Calculation of leaf-intercepted radiation and optical characteristics

Crown spacing and leaf photosynthetically active radiation absorption were set up to minimize lower crown shading, where PAR variation between the upper and lower crown position was < 10%. Red maple leaf reflectance,

Figure 1. A side-view diagram of the Mylar® crown section temperature treatment chambers. Simultaneously, each of the three prescribed temperatures was controlled on four separate replicate tree crowns per genotype. The controlled temperature of each crown section is denoted to the immediate left of the section in °C (25, 33 and 38). Arrows with reference numbers denote the following: (1) separately plumbed air ducts per crown section, (2) micro irrigation emitters, (3) ventilation and crown access ports and (4) location of air conditioners. Note that the transparency of chambers is darkened compared to actual experimental conditions for visual clarity of crown sections. Unrestricted reproduction from Bauerle et al. (2007).

transmittance and absorption values were determined using the non-linear correlation equations of Bauerle et al. (2004*a*) by averaging five SPAD readings per leaf (Minolta SPAD 502 chlorophyll meter, Minolta Camera Co., Ramsey, NJ). Photosynthetically active radiation was estimated for each crown layer with a three-dimensional light absorption model validated on red maple (Bauerle et al. 2004*b*) and absorbed PAR was calculated by summing the crown subvolumes. Under the outdoor experimental conditions, leaf PAR was light saturating for red maple at all canopy positions when incident radiation was > 550 μmol m^{-2} s^{-1} (Bauerle et al. 2003).

Leaf sampling protocol and chlorophyll, mass, nitrogen and water potential measurements

One year before the experiment, a Western-blot analysis was conducted to determine the relative Rubisco activase abundance among red maple within season leaf age classes. No differences were found that would indicate the need to sample along a leaf age gradient (Weston et al. unpublished data). In addition, leaf net photosynthesis values were monitored daily under saturated light conditions for 60 continuous days, resulting in no significant difference in net photosynthesis values (Bauerle unpublished data). Therefore, to assess the extent to which long-term (50 continuous days of temperature control) temperature exposure influences leaf characteristics, leaf measurements took place on leaves that developed under treatment conditions. Red maple, a continuous flushing species, produced at least one new fully expanded leaf per branch every 7 days during the experiment, thus permitting the sampling strategy. Twice weekly from 27 May to 8 September 2003 (Julian days 147–251), one fully expanded leaf was excised from each tree and crown section at solar noon and immediately measured for fresh mass, water potential (Ψ_l) using a Scholader-Hammel pressure chamber (Soil moisture, Santa Barbara, CA), relative chlorophyll content with a SPAD 502 chlorophyll meter and leaf area (LA) (LI-3000 Li-Cor Inc., Lincoln, NE). The SLA and L_M were then determined following the proposed standard protocol of Garnier et al. (2001). The leaf was then dried (70 °C for 72 h) and weighed. Nitrogen concentration on a dry mass basis (N_M) was determined with a LECO model FP528 nitrogen combustion analyzer (LECO Corp., St Joseph, MI). The SLA (cm^2 g^{-1}) was expressed as the ratio of LA to leaf dry mass (Garnier et al. 2001, Vile et al. 2005). Relative L_M was determined as the ratio of leaf dry mass to fresh mass (Garnier et al. 2001). Leaf thickness was determined as in Vile et al. (2005) as $(SLA \cdot L_M)^{-1}$. Leaf NUE was calculated according to Berendse and Aerts (1987) as leaf dry mass (g cm^{-2})/nitrogen content (g^{-1}).

Statistical analysis

Analysis of variance (ANOVA) was used to test the effect of temperature on genotype Ψ_l (MPa), leaf fresh mass (g cm^{-2}), leaf dry mass (g cm^{-2}), L_M (g cm^{-2}), LA (cm^2), SLA (cm^2 g^{-1}), SPAD, nitrogen content (%) and N_L (g g^{-1} N) (SAS Institute, 2005). Mean separation was performed at $P = 0.05$ by Fisher's LSD test. Linear regression was used to calculate % nitrogen from SLA for both genotypes (Meziane and Shipley 2001). Data for SLA, L_M and estimated leaf thickness were normalized by log-transformation and subjected to regression analysis. Leaf thickness data were analyzed by an analysis of variance on the slopes of estimated thickness versus SLA, and L_M and genotypes were compared with regression covariance analysis (SAS Institute, 2005).

Results

Leaf temperatures in the controlled daytime temperature treatments of 25, 33 or 38 °C were within ± 1.6 °C of the chamber set point (Table 1). Leaf night temperatures (20:00–08:00 h) tracked ambient climatic conditions and average monthly nighttime temperatures were similar over the course of the study months (Table 1).

Independent effects of the canopy layer were not significant and regardless of the crown position (top, middle or bottom), long-term exposure to different daytime growth temperatures resulted in leaf acclimation to a specific temperature (25, 33 or 38 °C). In addition, an analysis of time-dependent leaf characteristics under controlled daytime temperature did not show a significant effect and, therefore, the data were pooled. Elevated temperature (33 or 38 °C), however, had a significant effect on L_M and SLA as compared to 25 °C and was significantly different between the two genotypes (Table 2). Separate from any potential temperature effects, genotypes were different in that OG maintained a slightly more negative Ψ_l than SR under all treatment temperatures (Figure 2A). However, Ψ_l did not vary significantly among treatment temperature for SR, whereas there was a significant variation among treatments for OG (Figure 2A). Under well-watered conditions, the lowest Ψ_l value occurred in the OG genotype at 38 °C (−1.3 MPa). The lowest Ψ_l value in SR (−1.1 MPa) occurred at the coolest controlled temperature (25 °C).

Leaf fresh mass differences among treatments were not evident (Figure 2B); however, leaf dry mass and L_M were significantly different among treatments and between

Table 1. Average monthly leaf temperature for leaves of red maple genotypes SR and OG under controlled temperature (25, 33 and 38 °C) and ambient nighttime (A_N) conditions ± *SE*.

Temperature (°C)	June	July	August	September
25	25 ± 1.1	25 ± 1.4	25 ± 1.2	25 ± 0.9
33	33 ± 1.4	33 ± 1.5	33 ± 1.1	33 ± 0.8
38	38 ± 1.2	38 ± 1.6	38 ± 1.3	38 ± 0.5
A_N	19.4 ± 0.5	21.8 ± 0.2	22.2 ± 0.2	21.5 ± 0.7

Table 2. Two-way ANOVA with mean squares and treatment significance of two thermally divergent *A. rubrum* genotypes (G) (cv. SR and OG) subjected to three different temperature treatments (T) (25, 33 and 38 °C) and their interactions (T × G).

Parameter	Source	df	Mean squares	*P* value
Leaf water potential (MPa)	Treatment	2	18.4	0.019
	Genotypes	1	293.83	0.0001
	Replications	47	28.76	< 0.0001
	T × G	2	7.6	0.6
Leaf fresh mass (g cm^{-2})	Treatment	2	0.000018	0.026
	Genotypes	1	0.0006	< 0.0001
	Replications	47	0.000006	0.31
	T × G	2	0.000002	0.72
Leaf dry mass (g cm^{-2})	Treatment	2	0.00001	0.014
	Genotypes	1	0.00012	< 0.0001
	Replications	47	0.000008	< 0.0001
	T × G	2	0.0000012	0.58
Relative leaf dry matter content (g g^{-1})	Treatment	2	0.071	0.012
	Genotypes	1	5.92	< 0.0001
	Replications	47	0.056	0.001
	T × G	2	0.0062	0.83
Leaf area (cm^2)	Treatment	2	868.02	0.0023
	Genotypes	1	3321.99	< 0.0001
	Replications	47	126.44	0.64
	T × G	2	230.32	0.19
Specific leaf area (cm^2 g^{-1})	Treatment	2	223794	0.011
	Genotypes	1	2430672	0.03
	Replications	47	131959	0.003
	T × G	2	47055	0.23
Relative leaf chlorophyll content (unitless)	Treatment	2	164.19	0.014
	Genotypes	1	444.27	0.0007
	Replications	47	94.52	< 0.0001
	T × G	2	79.59	0.12
Leaf nitrogen content (%)	Treatment	2	2.98	< 0.0001
	Genotypes	1	1.7	0.0011
	Replications	47	0.81	< 0.0001
	T × G	2	1.26	0.02
Leaf-level nitrogen use efficiency (g g^{-1} N)	Treatment	2	174.2	0.136
	Genotypes	1	457.1	< 0.0001
	Replications	47	208	0.2
	T × G	2	42.8	0.15

genotypes (Figure 2C and D). October glory had a 7.3% higher L_M at 25 °C as compared to SR (Figure 2D). However, at 33 and 38 °C, L_M of OG averaged 15.6% and 42.8% < 25 °C. In fact, relative to the 25 °C value, OG had a 14% and 33% greater reduction in L_M at 33 and 38 °C than that of SR. Other than 25 °C, OG leaf dry mass and L_M were lower than those of SR, where OG leaf dry mass ranged from 0.0029 to 0.0055 g cm^{-2} and L_M ranged from 0.27 to 0.5 g g^{-1}. In comparison, SR dry mass ranged from 0.0057 to 0.0064 g cm^{-2} and L_M ranged from 0.42 to 0.46 g g^{-1}.

Temperature had a significant effect on LA and SLA (Figure 2E and F). In addition, as compared to 25 °C, LA and SLA at 33 and 38 °C were different between genotypes, where SR SLA increased at elevated temperature and OG declined (Figure 2E and F). Moreover, at 33 and 38 °C, SR had a 32.4% and 41.9% higher SLA than OG. At 25 °C, OG SLA was also < SR but by a lesser amount (15.5%).

Relative chlorophyll content measured indirectly via SPAD and nitrogen content (%) declined in OG as temperature increased from 25 to 38 °C (Figure 2G). Summer red, on the other hand, consistently had a slightly higher relative chlorophyll content and increased both relative chlorophyll content and leaf nitrogen content (%) at 38 as opposed to 25 and 33 °C (Figure 2G and H).

Temperature effects on leaf thickness did not always follow a temperature gradient (Figure 3). A significantly higher leaf thickness was found at the two extreme temperatures (25 and 38 °C) in both genotypes as compared to 33 °C.

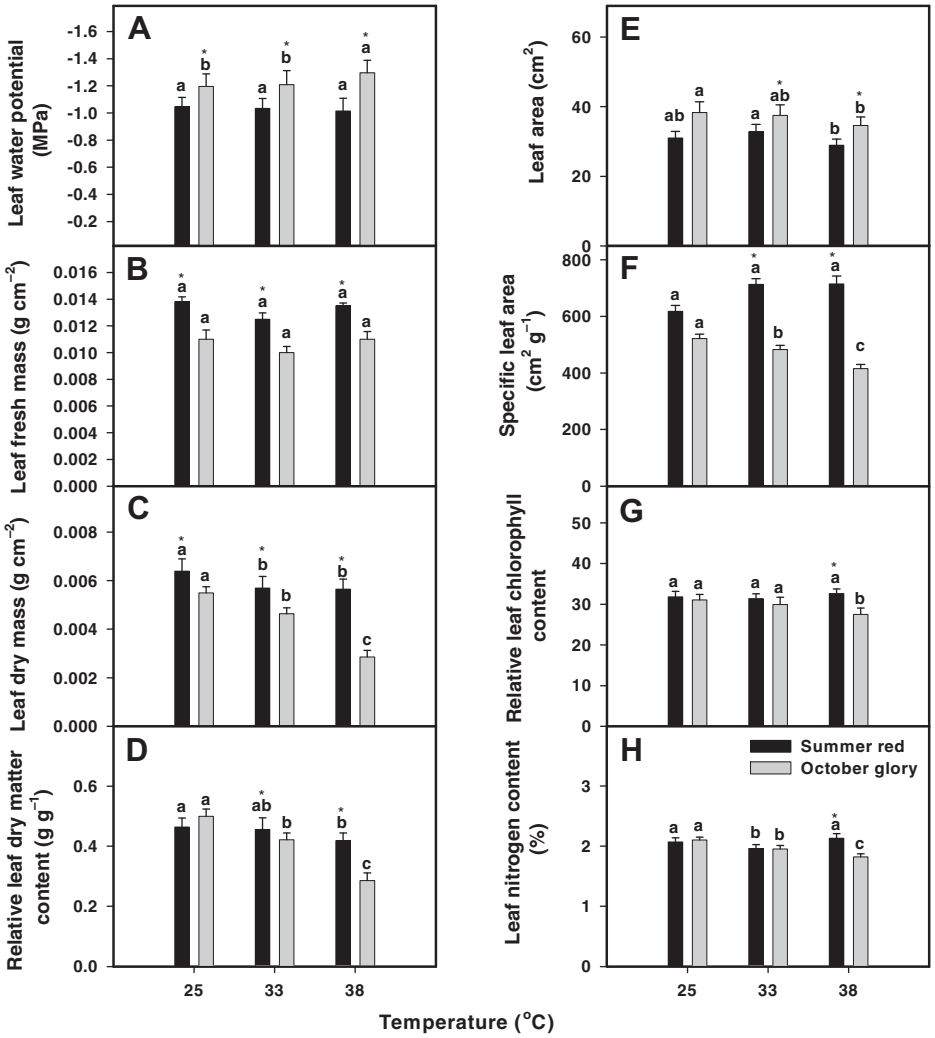

Figure 2. Effect of temperature treatments (25, 33 and 38 °C) on (A) leaf water potential, (B) leaf fresh mass, (C) leaf dry mass, (D) relative leaf dry matter content, (E) leaf area, (F) specific leaf area, (G) relative leaf chlorophyll content – unitless and (H) leaf nitrogen content on two thermally divergent *A. rubrum* genotypes (cv. SR and OG). Columns labelled with the same letter are not significantly different at $P = 0.05$ within the same genotype. Columns labelled with an * are significantly higher ($P = 0.05$) for genotype comparison within each temperature treatment. Vertical bars at the top of the columns represent ± *SE*.

Summer red had higher leaf thickness under all temperature treatments, where SR leaf thickness was 18.8, 37.8 and 33.3% higher than OG at 25, 33 and 38 °C, respectively. Temperature treatments had no effect on N_L in both genotypes, however, a significant difference was evident between genotypes within each temperature treatment (Table 2). In OG, N_L was 38.4, 37.2 and 35.8 g g^{-1} N, while in SR it was 31.0, 27.6 and 28.0 g g^{-1} N at 25, 33 and 38 °C, respectively.

Figure 4 illustrates the significant positive linear relationship between SLA and leaf nitrogen content in both genotypes. It should be noted that linear as opposed to non-linear regression analysis was performed using the data given in Figure 4 due to an analysis that indicated linearity. The coefficient of determination between SLA and nitrogen content was higher in OG ($R^2 = 0.96$) than in SR ($R^2 = 0.84$). Also, the relationships between leaf thickness and both SLA and L_M were significant. Regression equations for each genotype were obtained to predict leaf thickness (LT) knowing either SLA [SR: Log (LT) = 7.38−1.71 Log (SLA) and OG: Log (LT) = 5.55−1.27 Log (SLA)] or L_M [SR: Log (LT) = 0.40−1.597 Log (L_M) and OG: Log (LT) = 0.399−2.72 Log (SLA)]. The slopes were significantly different ($P < 0.0001$) between the two genotypes, indicating that as SLA increases, SR maintains a higher LT as compared to OG. The opposite occurred in comparison to L_M, where SR maintains a lower LT as L_M increases as compared to OG.

Discussion

Elevated global temperatures raise numerous concerns regarding ecosystem function and the influence on carbon

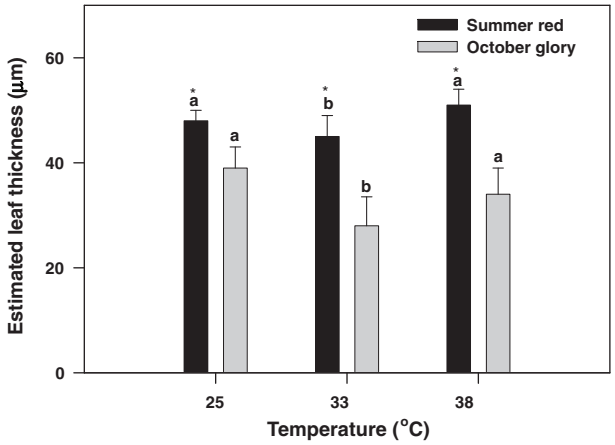

Figure 3. Effect of temperature treatments (25, 33 and 38 °C) on estimated leaf thickness of two thermally divergent *A. rubrum* genotypes (cv. SR and OG). Columns labelled with the same letter are not significantly different at $P = 0.05$ within the same genotype. Columns labelled with an * are significantly higher ($P = 0.05$) for genotype comparison within each temperature treatment. Vertical bars at the top of the columns represent ± *SE*.

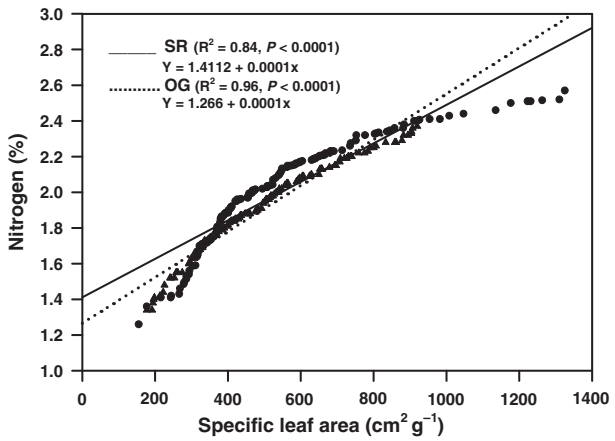

Figure 4. The relationship between SLA and leaf nitrogen content in the leaves of two thermally divergent *A. rubrum* genotypes cv. SR (•) and OG (▲) subjected to different growth temperature treatments (25, 33 and 38 °C).

exchange (IPCC 2007). Trees comprise the majority of the carbon-sequestering biomass in terrestrial ecosystems; thus, their responses to environment and climate change are a key determinant of global net primary production and carbon sequestration (Valentini et al. 2000, Barford et al. 2001, Breshears et al. 2005). However, the difficulty of separating environmental driver interactions and acclimation to interacting stresses confounds our understanding of the spatial distribution of carbon and nitrogen within a tree crown. Our temperature-controlled system provided a means of separating the effects of temperature and investigating the long-term leaf growth temperature responses within crowns of genotypes native to different thermal environments.

In nature, the light availability for plants varies spatially and temporally by over two orders of magnitude. However, the heterogeneous nature of light within a canopy interacts with temperature from the understory to the canopy surface, presenting steep environmental gradients (Niinemets and Valladares 2004). Within our study crowns, growth temperature had different effects on leaf carbon content. The responses were in line with in-depth physiological reports on genotypes with differences in leaf thermotolerance (Weston and Bauerle 2007, Weston et al. 2007), but broaden the instantaneous leaf physiological response observations in those studies to include the potential for long-lasting temperature-induced morphological and chemical element gradients within a crown. Regardless of the effect of any genotype differences, the temperature response reflects the importance of acclimation to daytime growth temperature. Our results illustrate that growth temperature within a crown can cause changes in SLA and L_M, and possibly Ψ_l, N (%), relative chlorophyll content and NUE. In the genotype native to the cooler climate, we observed a leaf dry matter response to a gradient in daytime growth temperature that was comparable to that observed in response to a gradient in canopy light as described by Niinemets et al. (1999). Moreover, the commonly observed magnitude of change in SLA that occurs in response to canopy light gradients (e.g., Walters et al. 1993, Beaudet and Messier 1998, Niinemets 2007) is similar to our OG observed SLA response to an imposed canopy temperature gradient. Related to SLA and the finding that changes in SLA alter the amount of photons available to intercept light per unit of leaf mass (Evans and Poorter 2001), a lower SLA in response to elevated temperature would reduce intercepted light in OG and potentially lower leaf temperature due to less overall photon absorption. The SLA increase in SR, on the other hand, could be attributed to elevated thermal tolerance and inherited adaptation to warmer climates (Bauerle et al. 2007).

It has been known for some time that leaf dimension can influence convective heat transfer (Vogel 1968, 1970, Taylor and Sexton 1972); therefore, variation in leaf morphological characteristics may serve as an indicator of environmental adaptations. Furthermore, leaves on the same plant that develop in full sunlight are not only smaller than those that mature in shaded conditions, but tend to be thicker and have more mesophyll cell surface area in relation to external leaf surface area (Smith and Nobel 1977). Although the range of leaf nitrogen concentrations in our experiment is comparable to that reported in the study of Meziane and Shipley (2001), nitrogen content was less responsive to temperature than morphological attributes indicating that leaf nitrogen content might be predominately determined by growth irradiance (Han et al. 2004). Alternatively, ontogenetic changes in crown exposure may be related to ontogenetic plasticity rather than light-related plasticity (Markesteijn et al. 2007).

In this study, we investigated the potential effects of variable growth temperature along an experimentally created temperature gradient and suggested that the magnitude in temperature variability that could be experienced in a natural forest is large enough to significantly modify leaf morphology and chemical elements. However, temperature-induced growth variations in leaf morphology and chemical content were much more substantial in the genotype native to the cooler climate, whereas temperature effects were less evident and sometimes nonexistent in the genotype native to the warmer climate. To date, there has been an absence of acclimation studies that have been conducted across a controlled crown temperature gradient. Accordingly, the current analyses indicated that crown sections (or canopy layers) acclimate to daytime growth temperature. The results imply that temperature can alter the leaf morphology and chemical composition throughout forest canopies and that these temperature-dependent effects can be included in forest ecosystem models. Unlike others who did not control temperature within the crown and speculated that acclimation was a result of irradiance gradients (e.g., Han et al. 2004), this study accounted for growth temperature acclimation and identified within-crown temperature induced variations in leaf morphology and chemical content. Controlled environment chambers are useful for developing hypotheses; however, these findings should still be tested in natural ecosystems due to the persistent challenge of integrating various scales of heterogeneous processes to yield system-level knowledge. The results of this study may also apply to shifts in seasonal climate, as temperature in forest canopies varies not only vertically but also daily, seasonally and annually (Harley et al. 1996, Singsaas et al. 1999, Zweifel et al. 2002).

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