Tree Physiology 27, 1083–1092

Inhibition and acclimation of C_3 photosynthesis to moderate heat: a perspective from thermally contrasting genotypes of *Acer rubrum* (red maple)

DAVID J. WESTON[1,2] and WILLIAM L. BAUERLE[3,4]

[1] *Oak Ridge National Laboratory, Environmental Sciences Division, Oak Ridge, TN 37831-6422, USA*

[2] *Corresponding author (westondj@ornl.gov)*

[3] *Clemson University, Department of Horticulture, Clemson, SC 29634, USA*

[4] *Clemson University, Department of Forestry and Natural Resources, Clemson, SC 29634, USA*

Received May 19, 2006; accepted September 11, 2006; published online May 1, 2007

Summary Effects of moderate heat on growth and photosynthesis were investigated in two clonal genotypes of *Acer rubrum* L., originally collected from the thermally contrasting habitats of Florida and Minnesota, USA, and known in the horticultural trade for sensitivity and insensitivity to heat, respectively. Under both common garden and warm greenhouse conditions (day/night temperature of 33/25 °C), the Florida genotype exhibited more growth than the Minnesota genotype. To determine the physiological parameters associated with this response, plants were acclimated to ambient (27/25 °C) or moderately elevated (33/25 °C) temperatures for 21 days before measurement of net photosynthesis at temperatures ranging from 25 to 48 °C. In vivo measurements of gas exchange and chlorophyll a fluorescence of ambient-acclimated plants revealed that, compared with the Minnesota genotype, the Florida genotype maintained a higher photosynthetic rate, higher stomatal conductance, more open PSII reaction centers, a greater PSII quantum yield and a lower quantum requirement for photosystem II (ϕ_{PSII}) per mole of CO_2 fixed (ϕ_{CO_2}) throughout the measurement temperature range. When both genotypes were acclimated at 33/25 °C and measured at 33 °C, analysis of the response of net photosynthesis to calculated intercellular CO_2 concentration indicated that the maximal rate of Rubisco carboxylation (V_{cmax}) decreased more in the Minnesota genotype than in the Florida genotype in response to elevated temperature. Additionally, ϕ_{PSII}/ϕ_{CO_2} at 33 °C was markedly higher for Minnesota plants under photorespiratory conditions, but similar to Florida plants under non-photorespiratory conditions. The results indicate that the higher net photosynthetic rate at 33/25 °C of the Florida genotype compared with the Minnesota genotype could be a result of several mechanisms, including the maintenance of a higher V_{cmax} and a more efficient quantum requirement of PSII per mole of CO_2 fixed, which is likely the result of lower photorespiration.

Keywords: drummondii, genotypic variation, temperature inhibition.

Introduction

The increase in *Acer rubrum* L. (red maple) dominance among softwood and hardwood stands marks one of the most dramatic changes in post-colonized eastern North American forests (Abrams 1998). Currently, the range of red maple is bound to the north by Newfoundland, the south by Florida and the west by Minnesota and eastern Texas (Burns and Honkala 1990). Included in this range are habitats differing dramatically in light, nutrition, water status and temperature. As a result, red maple has segregated into specific ecotypes or races adapted to a wide range of local endemic environments (Abrams and Kubiske 1990, Bauerle et al. 2003). However, ecotypic differences in leaf physiological characteristics (e.g., gas exchange, osmotic adjustment and N concentration) of red maple are relatively modest and fail to explain its newfound prominence among such diverse habitats. Therefore, the species' range expansion is unexplained by variation in leaf physiology and has been termed "the red maple paradox" (Abrams 1998).

Understanding the physiological constraints on a species' ability to adapt to environmental change, particularly change due to the elevation of temperature, is of interest in the context of climate warming. Temperature trends in the United States show an overall increase (Houghton et al. 2001). Forest ecosystems are thus being subjected to distributional and phenological changes, and species' range shifts (Easterling et al. 2000). Thomas et al. (2004) investigated the responses of diverse terrestrial ecosystems to predicted climate change and concluded that estimates of species "committed to extinction" will range from 18 to 32% by 2100. Such studies, while insightful, have failed to consider the physiological traits that allow plants to acclimate to warmer habitats.

One possibility whereby plants could moderate otherwise negative effects of local environment is through the response of photosynthesis to temperature. It is known that even moderately elevated temperatures (< 35 °C) inhibit photosynthesis, and this relationship is considered a major ecological driving

force in plant distributions (Berry and Björkman 1980). Despite decades of investigation into heat-limiting photosynthetic processes, the traits selected for high-temperature habitats remain elusive. Traditionally, photosystem II (PSII) has been considered the most heat-labile component of photosynthesis (Berry and Björkman 1980), but PSII damage is usually restricted to temperatures above 40 °C (Berry and Björkman 1980). More recently, it has been suggested that moderate heat affects thylakoid membrane permeability, and leaky membranes have an indirect effect on electron transport, reducing ATP and NADPH availability (Bukov et al. 1999). Support for this hypothesis was obtained by Wise et al. (2004), who obtained evidence that the functional photosynthetic limitation to moderate leaf temperatures could be explained by ribulose-1,5-bisphosphate (RuBP) regeneration via electron transport limitation. In a follow-up study, Schrader et al. (2004) reported that moderate heat stress affected thylakoid membrane permeability, resulting in stimulated cyclic photosystem I activity at the expense of stromal reductants. Subsequently, Rubisco was deactivated, thereby reducing photorespiratory metabolite accumulation.

One enzyme responsible for Rubisco's catalytic activity, Rubisco activase, is heat labile (Robinson and Portis 1989, Eckardt and Portis 1997, Salvucci et al. 2001) and may be a primary cause of reduced photosynthetic performance at moderately elevated temperatures (Salvucci and Crafts-Brandner 2004*a*). Support for this hypothesis was recently provided by a study comparing transgenic *Arabidopsis* with improved membrane integrity (lower membrane lipid saturation) and various isoforms of Rubisco activase (Kim and Portis 2005). In that study, plants subjected to heat (including a moderate heat of 38 °C) were measured for photosynthetic characteristics that included metabolites, gas exchange and fluorescence parameters. The results led the authors to conclude that inhibition of photosynthesis by moderate heat was a result of Rubisco deactivation, which concomitantly lowers electron transport. These results, considered in conjuction with those of studies of thermally contrasting species (Salvucci and Crafts-Brander 2004*c*), suggest that the physiochemical properties of Rubisco activase may underlie the geographic distribution of higher plants.

To investigate the photosynthetic traits that may infer adaptation to elevated temperatures in a long-lived forest species, in vivo gas exchange and chlorophyll a fluorescence were measured to determine mechanisms of heat-induced reductions of photosynthesis in two red maple genotypes known in the horticultural trade, one for its sensitivity, the other for its insensitivity to heat (Sibley et al. 1995*a*, 1995*b*, J. Ruter, University of Georgia, pers. comm.). We tested the hypothesis that the heat-insensitive Florida genotype has higher rates of net photosynthesis than the heat-sensitive Minnesota genotype at moderately elevated temperatures.

Materials and methods

Plant material and treatments

Dormant rooted cuttings of red maple were transplanted to 3-l pots containing a 1:2:1 (v/v) mix of sand:peat:silt loam and fertilized twice weekly with soluble fertilizer (20,10,20 N,P,K plus micronutrients) at the Clemson University Biosystems Research Complex (Clemson, SC). The study was conducted from June to early November 2002 and replicated during the same period in 2003. We used two genotypes of red maple, known in the horticultural trade as 'Northwood' and 'Florida Flame', which were originally collected from indigenous populations in Minnesota (46°55′44″ N, 92°55′10″ W) and Florida (30°33′34″ N, 84°19′33″ W), respectively.

To control temperature, four Mylar chambers (4 m × 1 m × 1.5 m) were constructed within a Biosystems Research Complex greenhouse. A Campbell 21X data logger (Campbell Scientific, Logan, UT) equipped with type T thermocouples monitored and controlled chamber temperature by a heat exchange pump (Model YSO9, Friedrich Inc., San Antonio, TX). Photoperiod and light quantity were similar to natural local conditions (34°41′0″ N, 82°50′15″ W) for the duration of the measurement period (June–September). To minimize potential chamber effects, plants were alternated among chambers on a weekly basis. After three weeks at a day/night temperature of 27/25 °C, plants were randomly assigned to treatment (33/25 °C) or control (27/25 °C) temperatures, with ambient humidity and light.

Gas exchange

Net assimilation (A_{net}) versus intercellular CO_2 (C_i) response curves were constructed between June 14 and September 24. Calculations of gas exchange parameters were performed according to Farquhar et al. (1980), and derivation of net photosynthetic parameters was as described by Bauerle et al. (2003). To ensure steady-state activation of Rubisco before measurement with a Ciras-1 gas analyzer (PP systems, Haverhill, MA), the leaf in the cuvette was acclimated to a CO_2 concentration of 360 μmol mol^{-1} and a saturating net photosynthetic photon flux (PPF; 1200 μmol m^{-2} s^{-1}) for 10–15 minutes. To determine maximal rate of Rubisco carboxylation (V_{cmax}) and the CO_2 compensation point (Γ*), the CO_2 limiting linear phase of the response curve was constructed by lowering the cuvette atmospheric CO_2 concentration (C_a) from 200 μmol mol^{-1} in four steps to 150, 100, 75, and 50 μmol mol^{-1}. At the end of the measurements, C_a was stabilized at 360 μmol mol^{-1} and A_{net} recorded. This procedure allowed us to compare post- and pre- A_{net} values at 360 μmol mol^{-1} to ensure open stomata and verify the stability of the photosynthetic apparatus. Lastly, the light source was turned off and the cuvette shrouded by a black cloth, and leaf dark respiration (R_d) measured. Response curves were obtained in 21 and 2% O_2 at a vapor pressure deficit (VPD) of 1.2–1.7 kPa.

With a parallel Ciras-1 gas analyzer, light response curves were generated on the leaf opposite to that measured for the A_{net} versus C_i response curves. Cuvette CO_2 concentration was held at 360 μmol mol^{-1} and VPD and O_2 concentrations were the same as in the A_{net} versus C_i experiment. Light response curves were used to estimate quantum yield of CO_2 (ϕ_{CO_2}), which can be easy to underestimate (Singsaas et al. 2001).

Therefore, several precautions were taken, including the use of 21% (photorespiratory conditions) and 2% (non-photorespiratory conditions) O_2 concentrations (see Singsass et al. 2001). With 21% O_2, C_a was adjusted to maintain a C_i of 23 to 25 Pa to compensate for errors incurred by photorespiration, and at least three data points were collected below 100 μmol m^{-2} s^{-1} PPF to avoid the respiratory incorporation of nonlinear data points (Singsaas et al. 2001).

Chlorophyll fluorescence

Chlorophyll a fluorescence parameters were measured with an OS-500 pulse amplitude modulated fluorometer (Opti-Science, Westand, MA). The fiber optic cable from the fluorometer was coupled to the Ciras-1 gas exchange system by a specially constructed light source, PLC 5(B). Actinic light was provided by the integrated Ciras-1 leaf chamber. To estimate F_o' (minimal fluorescence) under fully oxidized conditions (Q_A), the actinic light was terminated and a far-red pulse was generated from the fluorometer for 5 s to fully oxidize Q_A. Steady-state fluorescence (F_s) was measured after excitation by a weak modulated red light and maximum fluorescence (F_m') was determined after a saturating pulse (8000 μmol m^{-2} s^{-1} for 1 s) of white light. To ensure a fully reduced Q_A, maximal photosynthetic efficiency was determined at predawn and directly before the response curve by using dark adaptation clips. Results indicated that 20 min of dark adaptation produced readings comparable with predawn measurements.

Light absorbtance

Leaf light absorbtance (α) was estimated as described by Bauerle et al. (2004), where readings from a Minolta SPAD 502 meter (Spectrum Technologies, Plainfield, IL) were nonlinearly correlated to red maple light absorbtance, reflectance and transmittance.

Statistics

A randomized complete block design with sampling was used to test temperature effects on net photosynthesis. Specifically, 10 plants of each genotype were randomly selected and placed in Mylar chambers set at 27/25 °C or 33/25 °C. Two chambers were set at 27/25 °C and an additional two chambers were set at 33/25 °C. Each week throughout the study, plants were transferred within chambers to minimize chamber effects. The actual number of plants sampled varied according to the parameter tested and is indicated in the relevant figure caption. For each photosynthetic parameter reported in Table 2, a 2-way ANOVA was performed with genotype and temperature as independent variables. Error terms were pooled and reported as standard error of the difference of the means.

Results

Growth characteristics

After 42 days in a greenhouse at a day/night temperature of 33/25 °C, the Minnesota genotype failed to show measurable increases in stem height or leaf or branch number (Table 1), whereas the Florida genotype showed increases in all measured parameters. Under common garden conditions (at Clemson University, South Carolina, USA), both genotypes showed increased stem height from May until September; however, the increase was 46% greater in the Florida genotype than in the Minnesota genotype (Table 1).

Temperature response in 21% oxygen and 27/25°C

The intrinsic photosynthetic response of the Minnesota and Florida genotypes of red maple to elevated temperature was assessed by simultaneous measurements of gas exchange and chlorophyll a fluorescence. Leaf net photosynthetic characteristics differed between genotypes and in response to temperature. In the 27/25 °C treatment, Florida plants exhibited higher net CO_2 assimilation rates than Minnesota plants (Figure 1a). Stomatal conductance was higher for Florida plants and displayed a small increase in response to increasing measurement temperature up to 30 °C, before declining as temperature increased further. Unlike the Florida plants, the Minnesota plants did not show an initial rise in stomatal conductance with increasing temperature, but rather maintained a declining trend across the range of measurement temperatures (Figure 1b). Mean intercellular CO_2 partial pressure was higher in Florida plants than in Minnesota plants at temperatures below 42 °C. Above this temperature, intercellular CO_2 partial pressure rose sharply in both genotypes subsequent to reduced net photosynthesis, with the magnitude of the response being greatest in Minnesota plants (Figure 1c).

Quantum yield of PSII (ϕ_{PSII}) provided an estimate of the proportion of electrons passing though PSII per quantum absorbed and was higher for the Florida genotype than for the Minnesota genotype at all measurement temperatures (Figure 2a). The temperature optimum was broader for ϕ_{PSII} than for net CO_2 assimilation, with declining rates not apparent until ~37 °C. The proportion of open and undamaged PSII reaction centers, as measured by photochemical quenching (qP), was higher for Florida plants than for Minnesota plants at temperatures above 25 °C (Figure 2b). Non-photochemical quenching (qN), which is an indication of the amount of absorbed energy dissipated as heat, was similar between geno-

Table 1. Growth characteristics of *Acer rubrum* genotypes Florida (FL) and Minnesota (MN). Plants were grown at a day/night temperature of 33/25 °C for 42 days in growth chambers or in a common garden at Clemson University, Clemson, SC, USA from May until September. Plants under both environmental conditions were well-watered and provided with supplemental nutrition. Abbreviation: ND = not detectable. Values are means (± standard errors) of the growth increase per genotype for the common garden and growth chamber study (n = 8 per genotype per environment).

Increase	Growth chamber		Common garden	
	FL	MN	FL	MN
Stem height (mm)	20.0 (5.0)	ND	180 (18.0)	100 (21.0)
Branch no.	0.5 (0.2)	ND		
Leaf no.	1.5 (0.3)	ND		

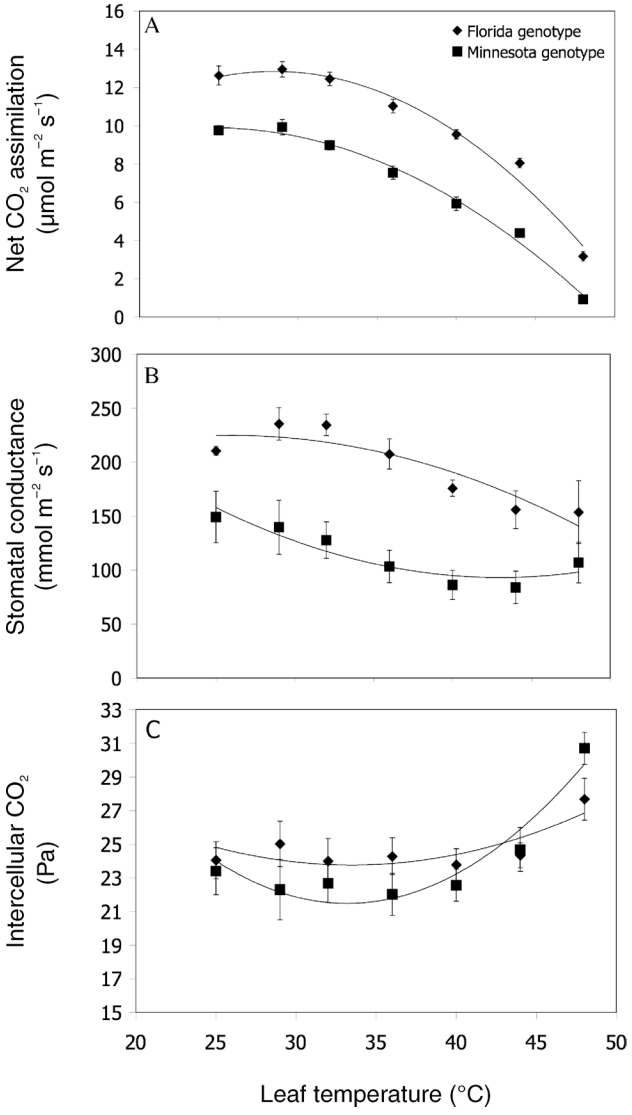

Figure 1. Temperature responses of net CO_2 assimilation rate (A), stomatal conductance (B), and intercellular CO_2 partial pressure (C) in Florida (◆) and Minnesota (■) genotypes of *Acer rubrum*. Photosynthesis was measured at a photosynthetic photon flux of 500 μmol m^{-2} s^{-1}, 360 ppm CO_2 and 21% O_2. Each value represents a mean (± SE) where $n = 5$.

types regardless of temperature (Figure 2c).

The ratio of ϕ_{PSII} to ϕ_{CO_2} provides an estimate of the moles of electrons passing though PSII needed to reduce one mole of CO_2. When measured under photorespiratory conditions (21% O_2), this estimate also includes electrons contributing to the photorespiratory pathway. We used this parameter as a proxy to estimate changes in carboxylation efficiency in response to temperature (Figure 3). Under photorespiratory conditions, the quantum requirement of PSII per mole of CO_2 (ϕ_{PSII}) was higher for the Minnesota genotype than for the Florida genotype at all measurement temperatures (Figure 3). The rate of increase in ϕ_{PSII}/ϕ_{CO_2} as a function of temperature was 51% higher for the Minnesota genotype, as measured by difference in slope, than for the Florida genotype (Figure 3).

Temperature response of warm-acclimated plants in 21% and 2% oxygen

The photosynthetic response of red maple to prolonged exposure to elevated temperature was investigated by acclimating plants to moderately elevated (33/25 °C, day/night) and ambient (27/25 °C) temperatures for 21 days. Under photorespiratory conditions (21% O_2), maximum net photosynthesis ($A_{max21\%}$) decreased significantly for both genotypes when ac-

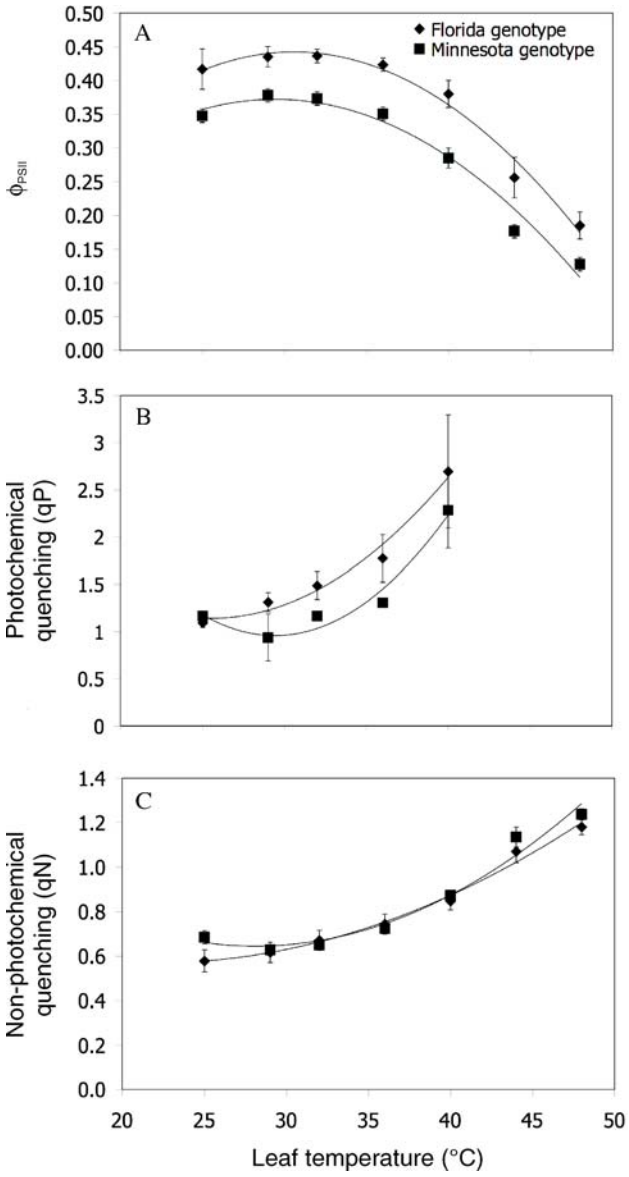

Figure 2. Temperature responses of the quantum yield of PSII (A), photochemical quenching (B), and non-photochemical quenching (C) in Florida (◆) and Minnesota (■) genotypes of *Acer rubrum*. Measurements were made at a photosynthetic photon flux of 500 μmol m^{-2} s^{-1}, 360 ppm CO_2 and 21% O_2. Each value represents a mean (± SE) where $n = 5$.

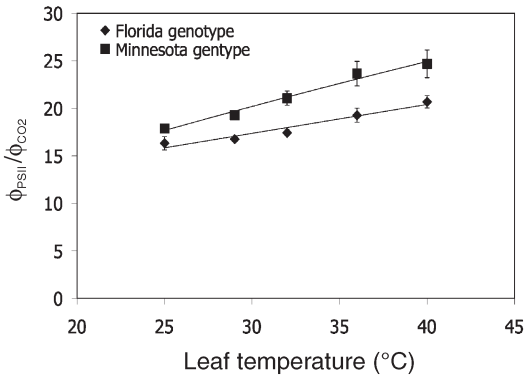

Figure 3. Temperature response of the quantum requirement of PSII per mole CO_2 (ϕ_{PSII}/ϕ_{CO_2}) fixed in Florida (◆) and Minnesota (■) genotypes of *Acer rubrum*. Measurements were made at a photosynthetic photon flux of 500 μmol m^{-2} s^{-1}, 360 ppm CO_2 and 21% O_2. Each value represents the mean (± SE), where $n = 5$.

climated at 33/25 °C and measured at 33 °C (Table 2). Specifically, the mean difference in $A_{max21\%}$ from ambient to warm temperatures decreased by 11 and 25% in the Florida and Minnesota genotypes, respectively. However, when warm-acclimated plants were analyzed under non-photorespiratory conditions ($A_{max2\%}$), net photosynthesis again decreased, but the magnitude of the decline was less severe at 9 and 18% in the Florida and Minnesota genotypes, respectively.

Under photorespiratory conditions, V_{cmax} decreased in Florida and Minnesota plants acclimated at 33/25 °C and measured at 33 °C by 26 and 34%, respectively (Table 2, Figure 4). The percent decrease was significant for both genotype and temperature (Table 2). To investigate the intrinsic response of V_{cmax} to heat, plants acclimated to both ambient and warm conditions were measured at 33 °C and compared with plants acclimated and measured at ambient temperature. As depicted in Figure 4, the decline in A_{net}, as representative of a change in

Table 2. Means (± standard errors) for gas exchange and fluorescence parameters of leaves of *Acer rubrum* genotypes Florida and Minnesota ($n = 6$ plants per genotype). Symbols: A_{max}, maximum CO_2 assimilation; V_{cmax}, maximal rate of Rubisco carboxylation; R_d, leaf dark respiration; ϕ_{CO_2}, quantum yield of CO_2; ϕ_{PSII}/ϕ_{CO_2}, quantum yield of photosystem II per mol of CO_2; Γ*, CO_2 compensation point; α, leaf light absorptance; 21%, 21% oxygen; 2%, 2% oxygen; G, genotype; T, temperature; and ns, not significant. Plants were acclimated to day/night temperatures of 27/25 °C or 33/25 °C for 21 days and measured at the acclimation day temperature. The *P* values are for the 2-way ANOVA with genotype and temperature as independent variables.

Parameter	Florida genotype		Minnesota genotype		*P* value
	27/25 °C	33/25 °C	27/25 °C	33/25 °C	
$A_{max21\%}$ (μmol CO_2 m^{-2} s^{-1})	10.6 (0.4)	9.5 (0.4)	8.7 (0.4)	6.5 (0.4)	Genotype, $P < 0.0001$ Temperature, $P = 0.0005$ G × T, ns
V_{cmax} (μmol CO_2 m^{-2} s^{-1})	79.8 (3.2)	58.7 (3.2)	72.5 (3.2)	47.9 (3.2)	Genotype, $P < 0.0227$ Temperature, $P = 0.0001$ G × T, ns
$A_{max2\%}$ (μmol CO_2 m^{-2} s^{-1})	16.3 (0.6)	14.9 (0.6)	14.7 (0.6)	12.1 (0.6)	Genotype, $P < 0.0029$ Temperature, $P = 0.0061$ G × T, ns
R_d (μmol CO_2 m^{-2} s^{-1})	0.3 (0.1)	0.5 (0.1)	0.4 (0.1)	0.6 (0.1)	Genotype, $P < 0.0001$ Temperature, $P = 0.043$ G × T, ns
$\phi_{CO_2\,2\%}$	0.10 (0.01)	0.07 (0.01)	0.08 (0.01)	0.06 (0.01)	Genotype, ns Temperature, $P = 0.0002$ G × T, $P = 0.033$
$\phi_{PSII}/\phi_{CO_2\,21\%}$	6.5 (0.25)	7.7 (0.25)	6.6 (0.25)	8.8 (0.25)	Genotype, $P = 0.0312$ Temperature, $P < 0.0001$ G × T, ns
$\phi_{PSII}/\phi_{CO_2\,2\%}$	4.8 (0.15)	5.1 (0.15)	4.5 (0.15)	5.3 (0.15)	Genotype, ns Temperature, $P = 0.0025$ G × T, ns
Γ* (μmol mol^{-1})	60.5 (1.8)	79.1 (1.8)	64.5 (1.8)	82.4 (1.8)	Genotype, ns Temperature, $P = 0.0001$ G × T, ns
α	84.0 (0.47)	82.0 (0.47)	84.4 (0.47)	83.3 (0.47)	Genotype, na Temperature, $P = 0.0058$ G × T, ns

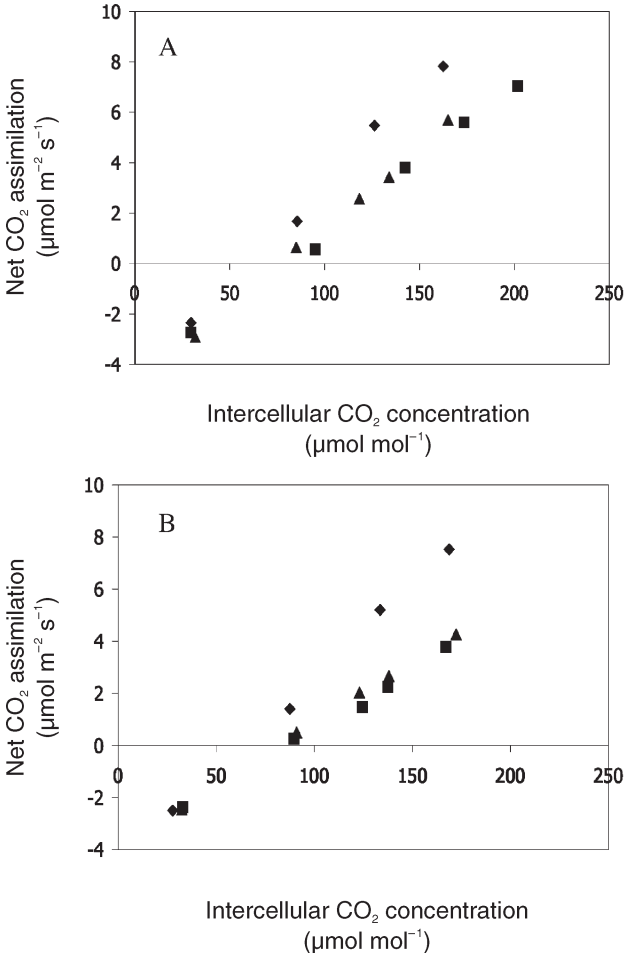

Figure 4. Net carbon dioxide assimilation rate as a function of intercellular CO_2 concentration for leaves of Florida (A) and Minnesota (B) genotypes of *Acer rubrum* acclimated and measured at day/night temperatures of 27/25 °C (◆) or 33/25 °C (▲), or acclimated to 27/25 °C and measured at 33/25 °C (■). Measurements were made at a photosynthetic photon flux of 1100 µmol $m^{-2}\ s^{-1}$, 360 ppm CO_2 and 21% O_2. Each value represents a mean (± SE) where n = 7–10.

V_{cmax}, was similar for both ambient- and warm-acclimated plants when measured at 33 °C.

The quantum yield of CO_2 measured in 2% O_2 did not differ significantly between genotypes, and was similar to values previously reported for unstressed C_3 plants (Long et al. 1993, Table 2). Photosynthetic efficiency was investigated by comparing the quantum yield of PSII with that of CO_2 in ambient- and warm-acclimated plants under both photorespiratory and non-photorespiratory conditions (Table 2). In 2% O_2, only a slight increase in ϕ_{PSII}/ϕ_{CO_2} was noted and was significant for temperature only, indicating minor partitioning of electrons to alternative electron sinks such as the Mehler and nitrogen pathway. By measuring and comparing photosynthetic efficiency (ϕ_{PSII}/ϕ_{CO_2}) under both photorespiratory and non-photorespiratory conditions, the proportion of electrons contributing to alternative sinks and that of photorespiration can be estimated (Long and Bernacchi 2003). Using this approach, ϕ_{PSII}/ϕ_{CO_2} measured in 21% O_2 revealed significant increases of 25 and 16% for Minnesota and Florida plants, respectively (Table 2). Taken together, these results indicate considerable partitioning of electrons to the photorespiratory pathway, particularly for the Minnesota genotype.

Effects of a transient heat stress on net photosynthesis and fluorescence parameters of warm-acclimated plants

To further investigate the mechanistic properties of the photosynthetic apparatus, Florida and Minnesota genotypes acclimated to moderately elevated temperatures were subjected to a 10 min heat stress of 42 °C and allowed to recover. Net photosynthesis and fluorescence parameters were measured before, during and after the transient heat treatment (Figure 5). To compensate for genotypic differences in intercellular CO_2 partial pressure (C_i), at high temperature (cf. Figure 1c), the experiment was conducted at a high CO_2 concentration (1000 ppm) and 2% oxygen. Net photosynthesis declined for both genotypes immediately following heat stress induction (Figure 5D). However, the magnitude of inhibition was greater in Minnesota plants than in Florida plants. Photosynthetic recovery approached pre-heat treatment values for both genotypes. There was a reduction in the quanta passing through PSII (ϕ_{PSII}) as a consequence of the heat treatment, which was followed by a recovery to near pre-treatment values, similar to the recovery in net photosynthesis (Figure 5C). In contrast to the photosynthetic response, the decline in ϕ_{PSII} in the Minnesota genotype was comparable with that observed in the Florida plants. Non-photochemical quenching (qN) increased in both genotypes following heat induction (Figure 5A); however, qN of Minnesota plants drifted up markedly during the heat treatment, which was seen in all three replicate curves (n = 3). Minimal fluorescence measured in the light, F_o', increased in plants of both genotypes following heat induction and the increase was greater in Minnesota plants than in Florida plants (Figure 5b).

Discussion

The inability of leaf-level physiology to explain the range expansion of red maple to diverse habitats has puzzled biologists and has led to the term "red maple paradox" (Abrams 1998). Understanding the mechanisms underlying this range expansion has relevance beyond that of forest management, and may even reveal key heritable traits for warm habitats. We found several within-species characteristics at the leaf level that differed between a heat-sensitive and a heat-insensitive genotype. Notably, the higher growth rate observed for the heat insensitive Florida genotype was associated with greater net photosynthesis, a higher V_{cmax} and a more efficient quantum requirement of PSII per mole of CO_2.

Although shifts in temperature optimum in relation to growth condition were not measured in this study, the failure of either genotype to maintain maximal rates of net photosynthesis under warm acclimation and measurement conditions indicates that complete photosynthetic acclimation did not oc-

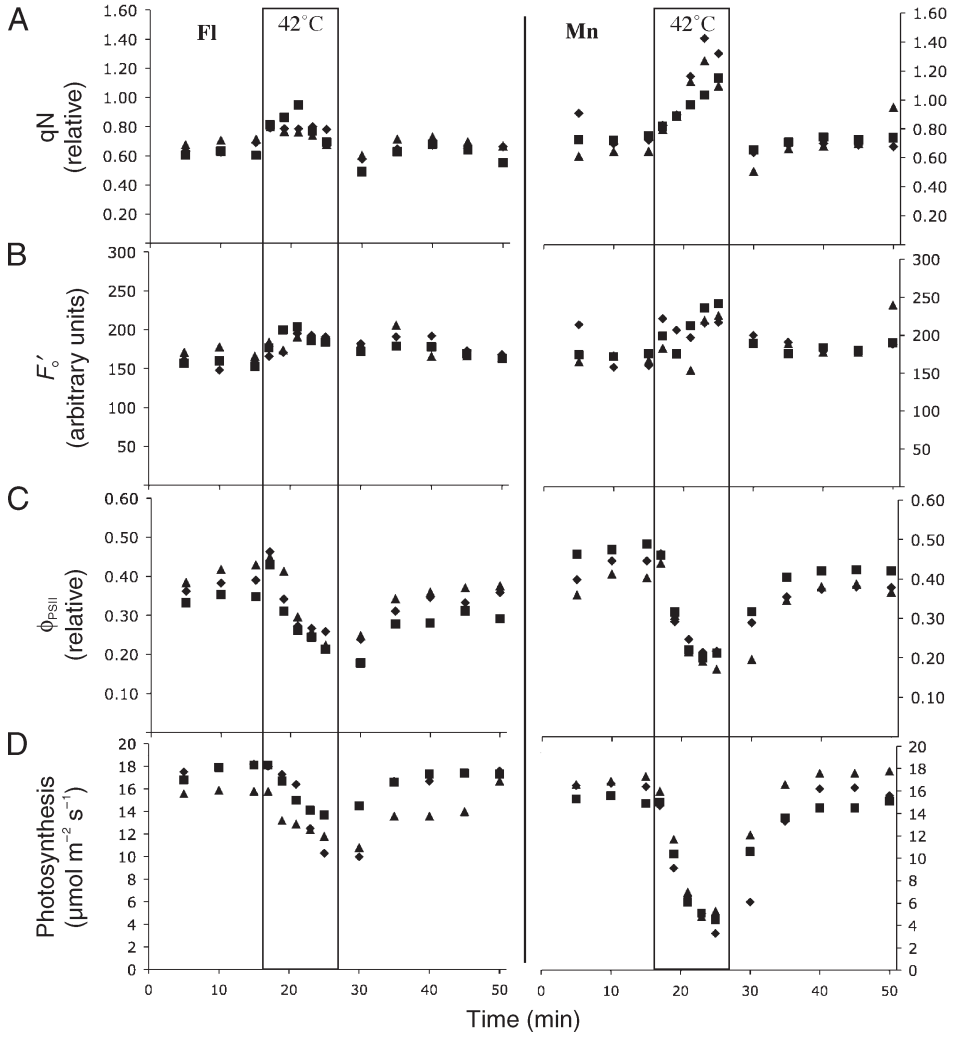

Figure 5. Fluorescence (A and B) and photosynthetic (C and D) parameters of Florida (Fl) and Minnesota (Mn) genotypes of *Acer rubrum* before, during and after a 10-minute heat treatment at 42 °C. Plants had been acclimated to a day/night temperature of 33/25 °C. Measurements were made at a photosynthetic photon flux of 500 μmol m^{-2} s^{-1} at 1000 ppm CO_2 and 2% O_2. Results from three replicate experiments (n = 3) are plotted. Abbreviations: qN, non-photochemical quenching; F_o', minimal fluorescence; and ϕ_{PSII}, quantum yield of photosystem II.

cur in our study. In addition, the nearly identical decline in V_{cmax} for both ambient- and warm-acclimated plants measured at a moderately elevated temperature suggests that thermal acclimation of photosynthesis was incomplete, and may even be of minor importance for the performance of these genotypes under moderately elevated temperatures. As reviewed by Berry and Björkman (1980), the potential for physiological acclimation of photosynthesis to temperature is highly variable, ranging from marked shifts in temperature optimum to no change, or even negative adjustments. Our data are similar to those reported for populations of *Eucalyptus pauciflora*, Sieb. ex Spreng., where peak assimilation rates correlated with endemic habitat and were possibly reflective of past ecotypic adaptation (Slatyer 1977*a*, 1977*b*, Ferrar et al. 1989). These data contrast with findings in *Acer saccharum* Marsh. (sugar maple), *Eucalyptus globulus* Labill., *Eucalyptus camaldulensis* Dehn. and *Eucalyptus nitens* (Deane and Maiden) Maiden, where populations exhibited more evidence of physiological adjustments than of ecotypic adaptation (Ferrar et al. 1989, Battaglia et al. 1996, Gunderson et al. 2000).

We found inherent differences between the genotypes grown under ambient temperature conditions. Specifically, A_{max} was substantially higher in the Florida genotype than in the Minnesota genotype across the range of measurement temperatures and was accompanied by higher stomatal conductance, more open PSII reaction centers, a greater PSII quantum yield and a lower quantum requirement of PSII per mole of CO_2. When plants were acclimated to and measured at a moderately elevated temperature, A_{max} declined for both genotypes; however, the decline was more severe for the Minnesota genotype (Table 2). Taken together, these results indicate that, compared with the Minnesota genotype, the Florida genotype has an enhanced intrinsic ability for higher CO_2 assimilation as well as higher growth temperature tolerance.

To investigate the decline in A_{max} in response to heat in more detail, we partitioned electron flow between photorespiration and alternative sinks, such as Mehler, nitrogen and the ascorbate-malate shuttle. Under non-photorespiratory conditions, a theoretical minimum of four electrons and eight photons are needed to fix one molecule of CO_2. Therefore, at 2% oxygen (non-photorespiratory conditions), electrons in excess of four are assumed to contribute to alternative electron sinks. We

found that the $\phi_{PSII} / \phi_{CO_2}$ for both genotypes under non-photorespiratory conditions increased minutely in response to heat, which is in accordance with other studies reporting that < 10% of electrons participate in alternative sinks (Ruuska et al. 2000), even at high-temperatures (Laisk et al. 1998). By performing the same measurements under photorespiratory conditions (21% oxygen), contributions to photorespiration and alternative sinks can be determined. Thus, photorespiration can be estimated by comparing the difference between $\phi_{PSII} / \phi_{CO_2}$ at photorespiratory and non-photorespiratory conditions (Long and Bernacchi 2003). In this way, we found that the Minnesota genotype partitioned two extra photons per mole of CO_2 fixed compared with the Florida genotype at elevated acclimation and measurement temperatures, which is most likely the result of increased photorespiration rather than increased respiration (Table 2). A similar response has been reported previously for C_3 plants that was also attributed to photorespiration (Oberhuber and Edwards 1993). Because we did not measure liquid phase diffusive resistance, it is unclear if the increase in photorespiration is in response to decreased internal CO_2 concentrations (C_i) alone or to a combination of decreased C_i and increased temperature.

The Minnesota genotype had markedly lower stomatal conductance compared with the Florida genotype. This was reflected in a small but noticeable difference in internal CO_2 concentration, which would promote photorespiration. However, the heat-induced decline in A_{max} that we observed was not completely alleviated when measured at 2% oxygen. Thus, there appear to be additional limitations to photosynthesis at moderately high temperatures.

Another possible contributing factor to the heat-induced reduction in net photosynthesis is V_{cmax}, which decreased in heat-acclimated plants of both genotypes, with greater reductions observed in the Minnesota genotype. In addition, F_o' increased in response to transient heat stress more in the Minnesota genotype than in the Florida genotype. This parameter has been used previously to separate the effects of heat between electron transport and stromal-based processes for Rubisco-activase-deficient mutants (Sharkey et al. 2001). The marked rise in F_o' in the Minnesota genotype is strikingly similar to that reported for Rubisco-activase-deficient tobacco mutants (Sharkey et al. 2001). A decrease in Rubisco activation in response to moderate heat has been widely reported (Feller et al. 1998, Laisk et al. 1998, Crafts-Brandner and Law 2000, Crafts-Brandner and Salvucci 2000, Salvucci et al. 2001, Salvucci and Crafts-Brandner 2004*b*, 2004*c*), and has been attributed to the heat sensitivity of the protein responsible for Rubisco activation, Rubisco activase (see reviews by Portis 2002, Salvucci and Crafts-Brandner 2004*b*).

The difference in growth between genotypes under warm acclimation conditions (Table 1) was dramatic and could complicate interpretation of the data because photosynthesis is highly regulated and can be influenced by internal factors (e.g., sink demand; Thomas and Strain 1991, Maier and Teskey 1992) as well as temperature. The general consensus is that an increase in sink demand is positively correlated with an increase in photosynthesis (e.g., Myers et al. 1999, Lavigne et al. 2001, Vaast et al. 2005). Thus, it is possible that some of the photosynthetic processes that we observed are not direct responses to heat, but are indirect consequences of the effects of heat on internal factors. However, data from the transient heat stress experiment (Figure 5)—which likely reflect direct responses to heat and not indirect responses mediated by internal factors—indicate that the Florida genotype is more thermotolerant than the Minnesota genotype. For example, the marked rise in F_o' is an indication of PSII acceptor side limitation and is known to decrease upon nonphotochemical quenching. However, Minnesota plants were unable to quench F_o' to the values observed in Florida plants, even with higher relative nonphotochemical quenching. This response was subsequently followed by lower net photosynthesis and PSII quantum yield compared with the Florida genotype. These results demonstrate a greater thermotolerance of the photosynthetic apparatus in the Florida genotype compared with the Minnesota genotype, and suggest that growth differences were not a critical factor in the transient heat stress experiment.

Several mechanisms have been proposed to explain the expansion in the range and dominance of red maple in North America over the last 100 years, including adjustments to variable soil water conditions (Bauerle et al. 2003), dark respiration rates (Turnbull et al. 2001), shoot damage recovery (Sipe and Bazzaz 2001), seedling recruitment (Lambers and Clark 2005), herbivore tolerance (e.g., gypsy moths, Jedlicka et al. 2004) and low resource requirement for leaf biomass (Nagel et al. 2002). However, gas exchange measurements from northeastern forest canopies revealed that red maple has a relatively low maximum photosynthetic rate (Jurik et al. 1988, Kloeppel et al. 1993, Kubiske and Pregitzer 1996, Turnbull et al. 2002). Our data, particularly the findings for the heat-sensitive Minnesota genotype, support these previous observations of a low maximum photosynthetic rate in this species. However, in contrast to previous studies, we found that the heat-insensitive Florida genotype had a relatively high photosynthetic rate. This may be a reflection of the high genetic diversity of red maple that has resulted in various taxonomic varieties or subspecies including *A. rubrum*, *A. rubrum* var. *trilobum* Torr. and Gray ex K. Koch, and *A. rubrum* var. *drummondii* (Hook. and Arn. ex Nutt.) Sarg. (Flora of North America Project, http://hua.huh.harvard.edu/FNA/index.html). The Florida genotype we studied has the morphological characteristics of *A. rubrum* var. *drummondii*, which is endemic to the southeastern USA, perhaps explaining its greater growth and photosynthetic capacity in moderately elevated temperatures. Although our study of two genoptypes precludes generalizations to subspecies, future research should consider comparisons between and among taxonomic varieties and populations of widespread species, such as red maple, in response to temperature. Such studies are necessary to reveal intraspecific variation in response to a changing climate, the consequences of which could affect predictions of climate-carbon feedbacks (King et al. 2006), species range models (Helman et al., unpublished data) and the microevolutionary consequences of climate change (Rice and Emery 2003).

In conclusion, we demonstrated intraspecific variation in

red maple photosynthetic response to heat at the leaf level which may, in part, explain the success of this species in thermally contrasting habitats. Most notably, the heat-insensitive genotype has an enhanced intrinsic ability to maintain high net photosynthetic rates in response to elevated temperature. Our results suggest that the maintenance of higher net photosynthesis was the result of multiple factors including a higher V_{cmax} and a more efficient quantum requirement of PSII per mole of CO_2, which is likely a subsequent response to lower photorespiration.

Acknowledgments

We gratefully acknowledge Stan Wullschleger, Brandon Moore and Gerry Edwards for helpful comments on this manuscript.

References

Abrams, M.D. 1998. The red maple paradox. BioScience 48: 355–364.

Abrams, M.D. and M.E. Kubiske. 1990. Photosynthesis and water relations during drought in *Acer rubrum* genotypes from contrasting sites in central Pennsylvania. Funct. Ecol. 4:727–733.

Battaglia, M., C. Beadle and S. Loughhead. 1996. Photosynthetic temperature responses of *Eucalyptus globules* and *Eucalyptus nitens*. Tree Physiol. 16:81–89.

Bauerle, W.L., T.H. Whitlow, T.L. Setter, T.L. Bauerle and F.M. Vermeylen. 2003. Ecophysiology of *Acer rubrum* L. seedlings from contrasting hydrologic habitats: growth, gas exchange, tissue water relations, abscisic acid and carbon isotope discrimination. Tree Physiol. 23:841–850.

Bauerle, W.L., D.J. Weston, J.D. Bowden, J.B. Dudley and J.E. Toler. 2004. Leaf absorptance of photosynthetically active radiation in relation to chlorophyll meter estimates among woody plant species. Sci. Hortic. 101:169–178.

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

Bukov, N.G., C. Wiese, S. Neimanis and U. Heber. 1999. Heat sensitivity of chloroplasts and leaves: leakage of protons from thylakoids and reversible activation of cyclic electron transport. Photosynth. Res. 59:81–93.

Burns, R.M. and B.H. Honkala. 1990. Silvics of North America. Agricultural Handbook No. 654, USDA, Washington, DC, 877 p.

Crafts–Brandner, S.J. and R.D. Law. 2000. Effect of heat stress on the inhibition and recovery of the ribulose-1,5-bisphosphate carboxylase/oxygenase activation state. Planta 212:67–74.

Crafts-Brandner, S.J. and M.E. Salvucci. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proc. Natl. Acad. Sci. USA 97:13430–13435.

Easterling, D.R., G.A. Meehl, C. Parmesan, S.A. Changnon, T.R. Karl and L.O. Mearns. 2000. Climate extremes: observations, modeling, and impacts. Science 289:2068–2074.

Eckardt, N.A. and A.R. Portis, Jr. 1997. Heat denaturation profiles of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and Rubisco activase and the inability of Rubisco activase to restore activity of heat-denatured Rubisco. Plant Physiol. 113:243–248.

Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90.

Feller, U., S.J. Crafts-Brandner and M.E. Salvucci. 1998. Moderately high temperatures inhibit ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. Plant Physiol. 116:539–46.

Ferrar, P.J., R.O. Slayter and J.A. Vranjic. 1989. Photosynthetic temperature acclimation in *Eucalyptus* species from diverse habitats, and a comparison with *Nerium oleander.* Aust. J. Plant Physiol. 16:199–217.

Gunderson, C.A., R.J. Norby and S.D. Wullschleger. 2000. Acclimation of photosynthesis and respiration to simulated climatic warming in northern and southern populations of *Acer saccharum*: laboratory and field evidence. Tree Physiol. 20:87–96.

Houghton, J.T., Y. Ding and D.J. Griggs. 2001. Climate change 2001. *In* The Scientific Basis: Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Eds. K. Maskell and C.A. Johnson. Cambridge University Press, Cambridge, U.K., 944 p.

Jedlicka, J., J. Vandermeer, K. Aviles-Vazquez, O. Barros and I. Perfecto. 2004. Gypsy moth defoliation of oak trees and a positive response of red maple and black cherry: an example of indirect interaction. Am. Mid. Nat. 152:231–236.

Jurik, T.W., J.A. Weber and D.M. Gates. 1988. Effects of temperature and light on photosynthesis of dominant tree species of a northern hardwood forest. Bot. Gaz. 149:203–208.

Kim, K. and A.R. Portis, Jr. 2005. Temperature dependence of photosynthesis in *Arabidopsis* plants with modifications in Rubisco activase and membrane fluidity. Plant Cell Physiol. 46:522–30.

King, A.W., C.A. Gunderson, W.M. Post, D.J. Weston and S.D. Wullschleger. 2006. Plant respiration in a warmer world. Science 312:536–537.

Kloeppel, B.D., M.D. Abrams and M.E. Kubiske. 1993. Seasonal ecophysiology and leaf morphology of four successional Pennsylvania barrens species in open versus understory environments. Can. J. For. Res. 23:181–189.

Kubiske, M.E. and K.S. Pregitzer. 1996. Effects of elevated CO_2 and light availability on the photosynthetic light response of trees of contrasting shade tolerance. Tree Physiol. 16:351–358.

Laisk, A., B.H. Rasulov and F. Loreto. 1998. Thermoinhibition of photosynthesis as analyzed by gas exchange and chlorophyll fluorescence. Russ. J. Plant Physiol. 45:412–421.

Lambers, J.H.R. and J.S. Clark. 2005. The benefits of seed banking for red maple (*Acer rubrum*): maximizing seedling recruitment. Can. J. For. Res. 35:806–813.

Lavigne, M.B., C.H.A. Little and J.E. Major. 2001. Increasing the sink:source balance enhances photosynthetic rate of 1-year-old balsam fir foliage by increasing allocation of mineral nutrients. Tree Physiol. 21:417–426.

Long, S.P. and C.J. Bernacchi. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. J. Exp. Bot. 54: 2393–2401.

Long, S.P., W.F. Postl and H.R. Bolhar-Nordenkampf. 1993. Quantum yields for uptake of carbon dioxide in C_3 vascular plants of contrasting habitats and taxonomic groupings. Planta 189: 226–234.

Maier, C.A. and R.O. Teskey. 1992. Internal and external control of photosynthesis and stomatal conductance of mature eastern white pine (*Pinus strobus*). Tree Physiol. 22:1387–1394.

Myers, D.A., R.B. Thomas and E.H. DeLucia. 1999. Photosynthetic responses of loblolly pine (*Pinus taeda*) needles to experimental reduction in sink demand. Tree Physiol. 19:235–242.

Nagel, J.M., K.L. Griffin, W.S.F. Schuster, D.T. Tissue, M.H. Turnbull, K.J. Brown and D. Whitehead. 2002. Energy investment in leaves of red maple and co-occurring oaks within a forested watershed. Tree Physiol. 22:859–867.

Oberhuber, W. and G.E. Edwards. 1993. Temperature dependence of the linkage of quantum yield of photosystem II to CO_2 fixation in C_4 and C_3 Plants. Plant Physiol. 101:507–512.

Portis, Jr, A.R. 2002. The Rubisco activase-Rubisco system: an ATPase-dependent association that regulates photosynthesis. *In* Protein–Protein Interactions in Plant Biology. Eds. M.T. McManus, W.L. Laing and A.C. Allen. Sheffield Academic Press, Sheffield, U.K., pp 30–52.

Rice, K.J. and N.C. Emery. 2003. Managing microevolution: restoration in the face of global change. Front. Ecol. Environ. 1:469–478.

Robinson, S.P. and A.R. Portis, Jr. 1989. Adenosine triphosphate hydrolysis by purified Rubisco activase. Arch. Biochem. Biophys. 268:93–99.

Ruuska, S.A., M.R. Badger, T.J. Andrews and S. von Caemmerer. 2000. Photosynthetic electron sinks in transgenic tobacco with reduced amounts of Rubisco: little evidence for significant Mehler reaction. J. Exp. Bot. 51:357–68.

Salvucci, M.E. and S.J. Crafts-Brandner. 2004*a*. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiol. Plant 120:179–186.

Salvucci, M.E. and S.J. Crafts-Brandner. 2004*b*. Mechanism for deactivation of Rubisco under moderate heat stress. Physiol. Plant 122:513–519.

Salvucci, M.E. and S.J. Crafts-Brandner. 2004*c*. Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco activase in plants from contrasting thermal environments. Plant Physiol. 134:1460–70.

Salvucci, M.E., K.W. Osteryoung, S.J. Crafts-Brandner and E. Vierling. 2001. Exceptional sensitivity of Rubisco activase to thermal denaturation in vitro and in vivo. Plant Physiol. 127: 1053–1064.

Schrader, S.M., R.R. Wise, W.F. Wacholtz, D.R. Ort and T.D. Sharkey. 2004. Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. Plant Cell Environ. 27: 725–735.

Sharkey, T.D., M.R. Badger, S. von Caemmerer and T. J. Andrews. 2001. Increased heat sensitivity of photosynthesis in tobacco plants with reduced Rubisco activase. Photosynth. Res. 67:147–156.

Sibley, J.L., D.J. Eakes, C.H. Gilliam, G.J. Keever and W.A. Dozier. 1995*a*. Gas exchange rates of selected red maple cultivars grown in Alabama. J. Environ. Hort. 13:30–32.

Sibley, J.L., D.J. Eakes, C.H. Gilliam, G.J. Keever and W.A. Dozier. 1995*b*. Growth and fall color of red maple selections in the Southeastern United States. J. Environ. Hort. 13:51–53.

Singsaas, E., D.R. Ort and E.H. DeLucia. 2001. Variation in measured values of photosynthetic quantum yield in ecophysiological studies. Oecologia 128:15–23.

Sipe, T.W. and F.A. Bazzaz. 2001. Shoot damage effects on regeneration of maples (*Acer*) across an understorey-gap microenvironmental gradient. J. Ecol. 89:761–773.

Slayter, R.O. 1977*a*. Altitudinal variation in the photosynthetic characteristics of snow gum, *Eucalyptus pauciflora* Sieb. ex Spreng. VI. Comparison of field and phytotron responses to growth temperature. Aust. J. Plant Physiol. 4:583–594.

Slayter, R.O. 1977*b*. Altitudinal variation in the photosynthetic characteristics of snow gum, *Eucalyptus pouciflora* Sieb. ex Spreng. III. Temperature response of material grown in contrasting thermal environments. Aust. J. Plant Physiol. 4:301–312.

Thomas, R.B. and B.R. Strain. 1991. Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. Plant Physiol. 96:627–634.

Thomas, C.D., A. Cameron, R.E. Green et al. 2004. Extinction risk from climate change. Nature 427:145–148.

Turnbull, M.H., D. Whitehead, D.T. Tissue, W.S.F. Schuster, K.J. Brown and K.L. Griffin. 2001. Responses of leaf respiration to temperature and leaf characteristics in three deciduous tree species vary with site water availability. Tree Physiol. 21:571–578.

Turnbull, M.H., D. Whitehead, D.T. Tissue, W.S.F. Schuster, K.J. Brown, V.C. Engel and K.L. Griffin. 2002. Photosynthetic characteristics in canopies of *Quercus rubra*, *Quercus prinus* and *Acer rubrum* differ in response to soil water availability. Oecologia 130:515–524.

Vaast, P., J. Angrand, F. Nicolas, J. Dauzat and M. Génard. 2005. Fruit load and branch ring-barking affect carbon allocation and photosynthesis of leaf and fruit of *Coffea arabica* in the field. Tree Physiol. 25:753–760.

Wise, R.R., A.J. Olson, S.M. Schrader and T.D. Sharkey. 2004. Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. Plant Cell Environ. 27:717–724.