Leaf respiratory acclimation to climate: comparisons among boreal and temperate tree species along a latitudinal transect

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In common gardens along an ~900 km latitudinal transect through Wisconsin and Illinois, USA, tree species typical of boreal and temperate forests were compared with respect to the nature and magnitude of leaf respiratory acclimation to contrasting climates. The boreal representatives were trembling aspen (Populus tremuloides Michx.) and paper birch (Betula papyrifera Marsh.), while the temperate species were eastern cottonwood (Populus deltoides Bartr ex. Marsh var. deltoides) and sweetgum (Liquidambar styraciflua L.). Assessments were conducted on seedlings grown from seed sources collected near southern and northern range boundaries, respectively. Nighttime rates of leaf dark respiration (RD) at common temperatures, as well as RD’s short-term temperature sensitivity (energy of activation, Eo), were assessed for all species and gardens twice during a growing season. Little evidence of RD thermal acclimation was observed, despite a 12 °C range in average air temperature across gardens. Instead, RD variation at warm temperatures was linked most closely with prior leaf photosynthetic performance, while RD variation at cooler temperatures was most strongly related to leaf nitrogen concentration. Moreover, Eo differences across species and gardens appeared to stem from the somewhat independent limitations on warm versus cool RD. Based on this, an empirical model relying on RD estimates from leaf photosynthesis and nitrogen concentration explained 55% of the observed Eo variation.

Keywords: boreal, climate change, leaf respiration, nitrogen, temperate, temperature, thermal acclimation.
a narrow definition of acclimation (Atkin and Tjoelker 2003)—an adjustment of metabolic rate that compensates for a change in growth temperature, potentially resulting in metabolic homeostasis (i.e., identical metabolic rates in contrasting thermal regimes, when measured in situ). A general hypothesis underlying our research is that, relative to their temperate counterparts, boreal tree species are less capable of acclimating metabolically to increasingly warm environments, and this lack of plasticity compromises their ability to compete and persist in temperate climates.

One of our foci along the latitudinal transect has been tissue dark respiration ($R_d$), which provides energy and metabolites for myriad biosynthetic, maintenance and transport processes in plants (Amthor 2000), but also results in losses of assimilated carbon sometimes exceeding 50% of gross photosynthesis (Ryan 1991). Hence, climate can exert a strong influence on plant carbon balance, as, at any given point in time, $R_d$ is generally quite sensitive to short-term variation in tissue temperature (Dewar et al. 1999). The response of $R_d$ to temperature is commonly characterized using either of two related metrics—a $Q_{10}$ quantifying the relative rise in $R_d$ with a 10 °C increase in temperature, or an energy of activation ($E_o$) calculated using a modified Arrhenius equation. (Here we adopt the recommendation by Kavanau (1951) and Lloyd and Taylor (1994) to use $E_o$ rather than the traditional $E$ to denote activation energy calculated using the Arrhenius equation (Arrhenius 1889). The rationale for a change in subscript is that, in the case of respiration, activation energy can only be calculated for an overall process composed of multiple enzymatic reactions. Thus, the implications of $E_o$ differ from those of $E$, which pertains to a single reaction.) Although $Q_{10}$ and $E_o$ vary widely across plant tissues, species and environments, they are frequently of sufficient magnitude to bring about at least a doubling of $R_d$ with a 10 °C increase in tissue temperature (Atkin et al. 2005).

Following longer-term exposure (e.g., lasting days to weeks) to increased growth temperature, respiratory metabolism is often adjusted in a manner that minimizes a potentially detrimental acceleration of carbon loss (Atkin and Tjoelker 2003). The converse is also observed when plants are exposed to decreasing temperatures, presumably to maintain adequate rates of key plant processes in cool environments (e.g., Armstrong et al. 2006a, 2006b). The nature and extent of $R_d$ thermal acclimation appears to vary considerably among species and growth environments (e.g., Ziska and Bunce 1994, Larigauderie and Korner 1995, Arnone and Korner 1997, Tjoelker et al. 1999a, Atkin et al. 2006, Wright et al. 2006). While homeostasis has been observed in certain instances (e.g., Bruhn et al. 2008), an absence of thermal acclimation has been reported in others (e.g., Frantz et al. 2004). When observed, thermal acclimation may be manifested by changes in $R_d$ at some base temperature (e.g., Tjoelker et al. 1999a, Bruhn et al. 2008), or $E_o$ (e.g., Atkin et al. 2006) or both (e.g., Loveys et al. 2003, Ow et al. 2010). In an effort to make sense of this, Atkin and Tjoelker (2003) posit that there are two modes of $R_d$ thermal acclimation: Type I, in which $Q_{10}$ (or $E_o$) decreases with increasing growth temperature (with no change in cool or base $R_d$); and Type II—which is thought to typify plant tissues that fully develop in a particular climate—where cool or base $R_d$ declines as growth temperature increases (with no change in $Q_{10}$, or $E_o$).

At the moment, there is no consensus as to whether species adapted to cooler environments acclimate differently than those from warmer climates (e.g., Atkin et al. 2006, Yamori et al. 2008). Collectively, however, data from several studies (Arnone and Korner 1997, Tjoelker et al. 1999a, Xiong et al. 2000, Atkin et al. 2006) hint at a possible tendency for species from higher latitudes and elevations to exhibit Type II acclimation, whereas Type I is more frequently observed among species from lower latitudes and elevations. Causes for observed differences in the mode and extent of thermal $R_d$ acclimation are often unclear, largely because the underlying mechanisms are not yet fully resolved. Results of many studies support a strong link between a tissue’s temperature-adjusted $R_d$ and its nitrogen concentration (e.g., Ryan 1991, Tjoelker et al. 2001, Wright et al. 2006). Tissue nitrogen status (concentration or content) has also been found to explain significant variation in $E_o$ (or $Q_{10}$) (e.g., Griffin et al. 2002, Turnbull et al. 2003). However, there is increasing evidence that the factors controlling $R_d$ differ at warm versus cool temperatures and, correspondingly, that $E_o$ is in large part a function of the somewhat different behaviors of warm and cool $R_d$ (Atkin and Tjoelker 2003, Atkin et al. 2005).

Here, we assess the degree to which observed variation in leaf respiratory traits along our latitudinal transect has been consistent with the following hypotheses: (i) the $R_d$ of foliage at a given temperature declines with increases in average growth temperature; (ii) the mode and extent of leaf $R_d$ acclimation to contrasting growth temperatures differs between selected provenances of temperate and boreal species—with the former exhibiting pronounced Type I acclimation, and the latter, to a lesser degree, displaying Type II adjustments; and (iii) key acclimatory responses of respiratory metabolism to growth temperature closely mirror temperature-mediated trends in leaf nitrogen concentration.

Materials and methods

Plant material

The four North American tree species included in this study—trembling aspen, paper birch, eastern cottonwood and sweetgum—were chosen based on their ecological attributes and native geographical ranges. Aspen and birch are
chiefly boreal species (but have ranges that extend southward at higher elevations in the West), while cottonwood and sweetgum are temperate species (Burns and Honkala 1990). All are early successional, shade intolerant and capable of rapid juvenile growth.

Owing primarily to concerns about the time required to travel among gardens and measure leaf nighttime respiration, we confined our assessment to one provenance per species. For our boreal and temperate species, respectively, we chose provenances near the southern and northern range limits, because, relative to populations near range centers, they are more likely to (i) possess traits that are adaptive for warmer or cooler climates, respectively, and (ii) shape the genome of propagules whose fates largely determine species demographics in nearby range margins (Davis and Shaw 2001). Hence, aspen and cottonwood seeds were collected from southern Wisconsin (43°N 89°W), sweetgum seeds were collected from central Kentucky (38°N 84°W), and birch seeds were collected from northern Wisconsin (45°N 89°W). We recognize that this approach necessarily restricts the study’s scope of inference to the particular provenances we used.

Seeds were germinated in a greenhouse on the University of Wisconsin-Madison campus in April and May of 2007. Air temperature in the greenhouse was maintained at 23°C (±3°C) both day and night. Germinants were transplanted into 0.5-l pots containing, on a relative volume basis, two parts peat, one part sand and one part field soil (silt loam). All seedlings were grown in the greenhouse to a height of 10–20 cm, at which time (late May) they were out-planted to field gardens (see below). Out-planting was completed by June 15.

Garden descriptions

Three common gardens were established along an ~900 km latitudinal transect from northern Wisconsin to southern Illinois (Table 1). Gardens were located (north to south) on the University of Wisconsin Rhinelander Agriculture Experiment Station (43°N 89°W), University of Wisconsin Arlington Agriculture Experiment Station (45°N 89°W) and University of Illinois Dixon Springs Agriculture Research Station (37°N 88°W). Hereafter, the gardens are referred to as northern Wisconsin, southern Wisconsin and Illinois, respectively. All gardens were located in former agricultural fields. The Illinois garden, underlain by a silt loam, was maintained in fescue prior to the study. The southern Wisconsin garden, also underlain by a silt loam, was previously maintained as a mixture of native grasses and forbs. The northern Wisconsin garden, underlain by a sandy loam, had been maintained as a mixture of clover and winter wheat.

In early spring of 2007, all gardens were treated with herbicide to eliminate extant vegetation, disked and tilled. Landscape cloth was installed to inhibit weed growth, and seedlings were planted through the cloth. Each garden was fenced with poultry netting (1 m height) and multi-strand electric fencing (2 m height) to prevent mammal herbivory. All seedlings were watered twice each week throughout the study, and were top-dressed initially with a slow-release fertilizer (Osmocote, 15-9-12, Scott’s, Marysville, OH, USA) to minimize potential garden differences in soil fertility. Gardens were hand-weeded periodically. Beginning in early July, air temperature ($T_{air}$) data were collected at each garden using shielded thermocouples located 1 m above the soil surface and attached to a CR-10 data logger (Campbell Scientific Inc., Logan, UT, USA), with instantaneous measurements logged every 10 min throughout the study. A continuous record of air temperature throughout the study period is provided for each garden in Dillaway and Kruger (2010).

### Measures of leaf dark respiration

Leaf $R_d$ and its response to temperature were assessed at night, for each species at all gardens, in July (July 14–22) and again in August (August 10–24) of 2007. That timing allowed trees to acclimate to their respective growth environments for at least 6 weeks after out-planting to minimize possible legacies from the greenhouse. Trees grew rapidly during that period, and, by the first round of $R_d$ measurements, <20% of the tree crown was composed of foliage that had developed in

<table>
<thead>
<tr>
<th>Interval</th>
<th>Average air temperature (°C)</th>
<th>Illinois</th>
<th>Southern WI</th>
<th>Northern WI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>August</td>
<td>July</td>
</tr>
<tr>
<td>5-Day diel</td>
<td></td>
<td>27.5</td>
<td>30.6</td>
<td>20.1</td>
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<tr>
<td>5-Day diurnal</td>
<td></td>
<td>30.5</td>
<td>32.1</td>
<td>25.5</td>
</tr>
<tr>
<td>5-Day nocturnal</td>
<td></td>
<td>26.1</td>
<td>27.4</td>
<td>13.4</td>
</tr>
<tr>
<td>Entire study</td>
<td></td>
<td>28.5</td>
<td>22.2</td>
<td>19.3</td>
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</tbody>
</table>

Table 1. Average air temperatures observed at the three gardens along our latitudinal transect during the study period in 2007. July and August averages were based on instantaneous measures, using a shielded thermocouple located 1 m above the soil surface, during the 5 days prior to each respiration measurement campaign. Also included is the average of temperature measurements throughout the entire study period (from the beginning of July through the final day of respiration measurements at each garden in August).
the greenhouse. Leaf gas exchange was measured in situ using a LI-6400 portable photosynthesis system (Li-Cor Biosciences, Lincoln, NE, USA). Dark respiration rate \( (R_d) \) was determined on a recently mature, fully expanded leaf (leaf plastochron index = 5–8) from six trees per species per garden, at two cuvette temperatures (20 and 30 °C). Measured leaves were of similar age in both campaigns, as trees of all species exhibited indeterminate shoot growth throughout the study period. The LI-6400 \( CO_2 \) mixer was used to control cuvette \( pCO_2 \), which was set to 40 Pa (in a reference chamber) in order to approximate the average nocturnal \( pCO_2 \) observed (before midnight) across gardens.

The two cuvette temperatures, 20 and 30 °C, were selected to (i) bracket the range of nighttime \( T_w \), most commonly observed across the three gardens at the outset of the study (Table 1), and (ii) afford the largest feasible span in leaf measurement temperature \( (T_{\text{leaf}}) \), given the limitations in extent of thermal regulation of the gas exchange cuvette in the field. If either of the target cuvette temperatures could not be achieved, the other was adjusted accordingly to maintain a 10 °C measurement span. We also chose to use two temperatures (as opposed to three or more) in order to maximize replication and minimize the nightly measurement duration (and thus the potentially confounding influence of nocturnal \( R_d \) dynamics). During a pilot study along our transect in 2006, we compared the implications of different measurement protocols (i.e., two versus three measurement temperatures) for estimation of respiration parameters (see descriptions below). Leaf respiration data collected from seedlings of the study species \( (n = 36 \text{ leaves}) \) revealed that (i) \( R_d \) consistently exhibited an exponential response to temperature throughout a broad \( T_{\text{leaf}} \) range (19.5–34.8 °C), and (ii) relaxion on two temperatures rather than three (i.e., 20 and 30 °C instead of 20, 25 and 30 °C) introduced no discernible bias in estimates of \( R_d \) at a common temperature or \( E_o \). Specifically, the slope and intercept did not differ significantly from 1 and 0, respectively, in regressions of respiration variables estimated from two versus three measurement temperatures (for all regressions, \( 0.97 < \text{slope} < 1.03,\ r^2 \geq 0.95,\ P < 0.001,\ \text{data not shown} \)). Furthermore, the two protocols yielded estimates with very similar variance structures (e.g., similar standard deviations for species and garden means).

Measurements of \( R_d \) began at dusk \(( \sim 2100 \text{ CDT} ) \) and were concluded by midnight. Based on regression analysis, the time of night at which a leaf was measured (between 2100 and 2400 CDT) bore no significant influence on estimated \( R_d \) at a given \( T_{\text{leaf}} \) (i.e., \( R_{20} \) or \( R_{30} \), \( P > 0.58, \text{data not shown} \)). Leaves were allowed to equilibrate inside the cuvette until \( R_d \) was steady \(( \sim 3–4 \text{ minutes} ) \). All leaves at a garden were measured on the same night. At the conclusion of respiration measurements each night, all measured leaves were harvested and stored on ice until their projected areas were determined using a LI-3100 leaf area meter (Li-Cor Biosciences). Leaves were then dried to a constant mass at 70 °C and weighed. The area and dry mass of each leaf were then used to calculate its specific leaf area (SLA). \( R_d \) was converted to a mass basis \(( \text{nmol g}^{-1} \text{ s}^{-1} ) \) by multiplying the area-based rate with SLA.

Recent studies of leaf gas exchange (e.g., Ow et al. 2008) have included a correction factor recommended by Pons and Welschen (2002) in order to account for the ‘gasket effect’ of commercially available photosynthesis systems (e.g., the LI-6400). This correction adds half the area of the cuvette gasket \(( \sim 3 \text{ cm}^2 ) \) to the leaf area in the sample chamber \(( 6 \text{ cm}^2 \) for a LI-6400) to account for diffusion of \( CO_2 \) from the gasket into the cuvette. In our case, this adjustment led to a 33% reduction in \( R_d \) across all species, gardens and campaigns.

**Calculating temperature responses of respiration**

The response of \( R_d \) to \( T_{\text{leaf}} \) was estimated using a modified Arrhenius equation (Lloyd and Taylor 1994, Turnbull et al. 2001, Griffin et al. 2002):

\[
R_o = R_d e^{[E_o / R_o (T_{\text{leaf}} - T_1)]}
\]

where \( R_o \) and \( R_d \) are respiration rates at different leaf temperatures \( (T_o \text{ and } T_{\text{leaf}} \text{, respectively, in K} ) \). \( E_o \) (kJ mol\(^{-1}\) K\(^{-1}\)) is a fitted value for the energy of activation (similar to a \( Q_{10} \)). \( R_o \) is the ideal gas constant \((8.314 \text{ J mol}^{-1} \text{ K}^{-1}) \). In the present study, \( T_o \) and \( T_{\text{leaf}} \) were leaf temperatures observed when the cuvette temperature was set to 20 and 30 °C, respectively. At these cuvette temperatures, the recorded range in \( T_{\text{leaf}} \) was 16.6–22.4 and 27.0–31.3 °C, respectively. We employed the Arrhenius equation, as opposed to a \( Q_{10} \)-based approach, primarily because, from a mathematical standpoint, \( E_o \) does not vary as a function of measurement temperature (Lloyd and Taylor 1994). Accordingly, use of the Arrhenius equation allowed us to estimate \( R_o \) for a particular leaf (with its unique \( E_o \) and temperature-adjusted \( R_d \)) at any \( T_{\text{leaf}} \) of interest. Respiration responses to growth environment were examined using several estimated parameters. The \( R_o \) of individual leaves was calculated at two common temperatures \((20 \text{ and } 30 \text{ °C}) \) using Eq. (1), and these temperature-adjusted rates \((R_{20} \text{ and } R_{30}) \) respectively, along with \( E_o \), were compared across gardens and measurement campaigns for each species.

**Leaf nitrogen analyses**

Nitrogen concentration was determined on ground samples of all leaves measured for \( R_d \) using an Elemental Vario Macro CHN analyzer (Elementar Analysysysteme GmbH, Hanau, Germany).

**Study of potential soil influences on respiration**

In order to assess the degree to which observed variation in leaf \( R_d \) along the latitudinal transect might have been
attributable to differences in garden edaphic conditions, a pot study was conducted near Madison, WI, using soil collected from each of the three gardens. Eastern cottonwood seed from southern Wisconsin was sown in flats and transplanted into 5-l pots filled with garden soils. Trees were top-dressed with a controlled-release fertilizer similar to those in the common gardens, and were irrigated as needed to keep the soil near field capacity. Pots were located in full sun and trees were allowed to grow in the different soils until they had increased in size by at least 10-fold. At that time (~45 days after transplanting), $R_d$ was measured in the manner described above on a recently mature, fully expanded leaf from five trees in each soil, at two temperatures (20 and 30 °C). Measurements began at dusk and were concluded before midnight. These data allowed us to compare temperature-adjusted $R_d$ and $E_d$ among the three garden soils. Again, these data were adjusted by 33%, as suggested by Pons and Welschen (2002).

### Statistical analyses

For a given species, the significance of variation in leaf properties across gardens and measurement campaigns was determined with analysis of variance (procedure GLM, SAS Institute Inc., Cary, NC, USA, 1998), treating individual leaves as experimental units. Effects were deemed significant when $P < 0.05$. Linear regression was employed (again with procedure GLM in SAS) to examine relationships between leaf properties and garden climate. The climate variable we used was air temperature at the time of leaf measurement, within and across species, gardens and campaigns. We did not detect a significant influence of ambient $T_{air}$ on $R_{d20}$ for any species ($P \geq 0.24$, data not shown). Furthermore, across gardens, large differences in $R_{d20}$ were observed when air temperatures during $R_d$ measurements were similar (i.e., when garden differences were <2 °C).

### Results

#### Air temperature variation along the latitudinal transect

Temporal dynamics in garden environments during the growing season of 2007 are detailed in Dillaway and Kruger (2010), and here we summarize trends in air temperature ($T_{air}$) along the transect (Table 1). $T_{air}$ varied considerably among gardens during July and August. For example, average diel $T_{air}$ across the 5 days prior to each leaf measurement campaign (i.e., ‘growth temperature’) differed between the coolest and warmest garden (northern Wisconsin and Illinois, respectively) by 9 °C in July and nearly 12 °C in August.

#### Comparisons of leaf respiration at common temperatures

On the whole, estimates of $R_d$ at a common temperature ($R_{d20}$ and $R_{d30}$) tended to decrease with increasing latitude, particularly in July (Table 2). In neither campaign, however, was $R_{d30}$ or $R_{d20}$ significantly related to growth temperature (Figure 1), even after accounting for variation between groups (boreal versus temperate) and species (nested within groups). Across gardens, $R_{d30}$ and $R_{d20}$ were generally higher in July than in August (Table 2), and the difference, which was most evident for $R_{d20}$, coincided with an opposing change in growth temperature (Table 1). Yet, when this link was examined further, there was no significant correlation, within or across boreal and temperate groups, between the relative size of the $R_d$ deflection and the corresponding difference in growth temperature (Figure 2).
Table 2. Respiration parameters for foliage of the four study species grown in each of the three gardens along our latitudinal transect, based on measurements in July and August. Parameters include dark respiration estimated at leaf temperatures of 20 °C ($R_{n6}$) and 30 °C ($R_{n30}$), as well as respiration’s temperature sensitivity ($E_o$). Means and standard errors are based on $n = 6$ leaves per species and campaign. For a given parameter and species, the corresponding $P$ value indicates the significance of differences among means for garden ($P_{\text{garden}}$), campaign ($P_{\text{campaign}}$), and the interaction between garden and campaign ($P_{\text{GxC}}$).

<table>
<thead>
<tr>
<th>Trait/species</th>
<th>July</th>
<th>August</th>
<th>Southern WI</th>
<th>Northern WI</th>
<th>P values</th>
<th>P values</th>
<th>P values</th>
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<tr>
<td></td>
<td>July</td>
<td>August</td>
<td>July</td>
<td>August</td>
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<tr>
<td>$R_{n6}$ (nmol g$^{-1}$ s$^{-1}$)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspen</td>
<td>37.7 (1.7)</td>
<td>32.6 (2.7)</td>
<td>23.7 (1.5)</td>
<td>23.6 (0.2)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>Birch</td>
<td>27.7 (1.3)</td>
<td>24.9 (1.4)</td>
<td>24.8 (1.4)</td>
<td>21.8 (1.5)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.282</td>
</tr>
<tr>
<td>Cottonwood</td>
<td>42.0 (4.0)</td>
<td>29.5 (0.4)</td>
<td>34.6 (1.7)</td>
<td>36.8 (3.8)</td>
<td>0.334</td>
<td>0.285</td>
<td>0.107</td>
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<tr>
<td>Sweetgum</td>
<td>22.9 (1.5)</td>
<td>21.1 (1.4)</td>
<td>20.1 (1.2)</td>
<td>22.1 (2.2)</td>
<td>&lt;0.001</td>
<td>0.268</td>
<td>0.007</td>
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<td>$R_{n30}$ (nmol g$^{-1}$ s$^{-1}$)</td>
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<tr>
<td>Aspen</td>
<td>25.3 (2.3)</td>
<td>12.9 (0.9)</td>
<td>18.4 (0.8)</td>
<td>10.0 (0.3)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.009</td>
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<tr>
<td>Birch</td>
<td>17.2 (1.6)</td>
<td>10.0 (0.2)</td>
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<td>11.5 (0.7)</td>
<td>0.043</td>
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<td>16.5 (1.1)</td>
<td>0.140</td>
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<td>Sweetgum</td>
<td>16.5 (2.1)</td>
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<td>11.6 (0.8)</td>
<td>0.002</td>
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<td>$E_o$ (K mol$^{-1}$ K$^{-1}$)</td>
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<tr>
<td>Aspen</td>
<td>30.2 (4.9)</td>
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<td>18.3 (2.5)</td>
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<td>Birch</td>
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<tr>
<td>Sweetgum</td>
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<td>54.7 (8.3)</td>
<td>172 (1.8)</td>
<td>463 (5.5)</td>
<td>0.401</td>
<td>&lt;0.001</td>
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</table>

In an effort to explain observed variation in $E_o$ across species and gardens, we adopted the construct proposed by Akin and iOSelker (2003). Specifically, $E_o$ is primarily a function of transitions in the temperature sensitivity of dark respiration ($R_{n6}$) and light respiration ($R_{n30}$), and the positive relationships between $E_o$ and $R_{n30}$ were not significantly different between species or gardens. The slope of the relationship between $E_o$ and $R_{n30}$ was different between species and gardens, and the relationship between $E_o$ and $R_{n6}$ was not significantly different between species or gardens.
and August campaigns. Temperature-normalized values for parameters such as the maximum rate of ribulose bisphosphate carboxylation ($V_{\text{cmax}}$) and regeneration ($J_{\text{max}}$), along with each variable's estimated temperature sensitivity, were used to calibrate a biochemical model of photosynthesis (Farquhar et al. 1980, with refinements by Ethier and Livingston 2004).

Photosynthesis was modeled using instantaneous measures of light and temperature collected at each garden every 10 min throughout the study. While use of the Farquhar model usually requires an estimate of intercellular $p$CO$_2$ ($C_i$), we adopted an alternative approach (Chen et al. 1999) in which photosynthesis ($A$) was calculated using quadratic reformulations of the model.

Figure 1. Relationships between leaf properties and the diel average air temperature recorded during the 5-day period prior to each measurement campaign (referred to as growth temperature) at a given garden. Data for each campaign (July and August) are presented separately. Properties include rates of dark respiration at leaf temperatures of 30 °C ($R_{30}$) and 20 °C ($R_{20}$), sensitivity of respiration to instantaneous changes in leaf temperature ($E_o$) and leaf nitrogen concentration ($N_{\text{mass}}$). In the absence of true replication at a particular growth temperature (for a given species), garden × species means ($n = 12$ per campaign) are used as experimental units for these analyses. Species are denoted with the following symbols: trembling aspen (open circle), paper birch (open triangle), eastern cottonwood (filled square) and sweetgum (filled diamond). Solid lines indicate global relationships ($P < 0.05$) between the leaf trait and growth temperature that do not differ significantly between boreal and temperate groups in either slope or intercept. Dashed or dotted lines indicate significant trends only for the boreal or temperate group, respectively, in the absence of a global relationship.
components relying instead on an ambient pCO$_2$ (C$_a$, 37 Pa in our case) and stomatal conductance ($g_s$), where the expression $C_a$=1.6A/$g_s$ was substituted for $C_i$. For each species within a garden, $g_s$ was modeled based on its responses to variation in $T_{leaf}$ and photosynthetic photon flux (PPF) observed on seedlings along the same transect in 2006 as well as 2007. Specifically, the relative response of $g_s$ to PPF was characterized from light-response curves measured (at ambient pCO$_2$) with the LI-6400 in 2006 (data not shown). These relative trends were then combined with those generated in 2007 on the response of $g_s$ to $T_{leaf}$ (at high PPF and ambient pCO$_2$). Our resulting $g_s$ models assumed that $T_{leaf}$ = $T_{air}$ (based on measures of $T_{leaf}$ on all species at the southern WI garden), and that there was no PPF $\times$ $T_{leaf}$ interaction. We did not include other environmental variables such as vapor pressure deficit (VPD) in our $g_s$ model because in no case did they explain a significant amount of additional $g_s$ variation.

Across species, gardens and campaigns, $R_{30}$ was positively related ($r^2 = 0.61, P < 0.001$) to the average rate of photosynthesis per unit leaf mass ($A_{mass}$) during the photoperiod prior to respiration measurements (Figure 4). Based on this finding, we modeled $E_o$ of individual leaves using photoperiod average $A_{mass}$ as a predictor of $R_{30}$, and employing $N_{mass}$—a potentially effective correlate of respiratory enzyme activity or capacity (Tjoelker et al. 2008)—to predict $R_{10}$. We developed models based on estimated $R_{10}$ (derived with the Arrhenius equation), as opposed to $R_{20}$, simply because use of the former resulted in a more accurate and precise $E_o$ prediction. Here it was necessary to incorporate a separate $R_{10}$-$N_{mass}$ model for each campaign, owing to marked differences between July and August relationships (see Figure 5 caption for $R_{10}$-$N_{mass}$ models). The effort yielded an $E_o$ prediction that was linearly related ($r^2 = 0.55, P < 0.001$) to the observed $E_o$ (Figure 5), with no significant bias in slope or intercept.

Figure 2. Relationships between relative differences in leaf properties across measurement campaigns and corresponding (absolute) differences in the diel average air temperature recorded during the 5-day period prior to each campaign. Campaign differences (calculated as Trait$_{Aug}$/Trait$_{July}$ − 1) were analyzed in the following leaf traits: rates of dark respiration at leaf temperatures of 30 °C ($R_{30}$) and 20 °C ($R_{20}$), sensitivity of respiration to instantaneous changes in leaf temperature ($E_o$) and leaf nitrogen concentration ($N_{mass}$). In the absence of true replication for a particular difference in average growth temperature, garden $\times$ species means ($n = 12$ per campaign) are used as experimental units in these analyses. Species are denoted with the following symbols: trembling aspen (open circle), paper birch (open triangle), eastern cottonwood (filled square) and sweetgum (filled diamond). Solid lines indicate global relationships ($P < 0.05$) between the leaf trait and growth temperature that do not differ significantly between boreal and temperate groups in either slope or intercept. Dashed or dotted lines indicate significant trends only for the boreal or temperate group, respectively, in the absence of a global relationship.
Table 3. Leaf traits of the four study species grown at each of the three gardens along our latitudinal transect, based on measurements in July and August. Parameters include specific leaf area (SLA), leaf nitrogen concentration (N mass), and averages for photosynthesis per unit leaf mass during the photoperiod prior to respiration measurements. In the July campaign, photosynthesis data were not acquired for aspen in northern Wisconsin or for birch at any garden. Means and standard errors are based on 3–4 leaves per species and campaign. For a given parameter and species, the corresponding P value indicates the significance of differences among means for garden (Pgarden), campaign (Pcampaign) and the interaction between garden and campaign (P × C).

Table 4. Leaf respiration parameters for potted cottonwood trees grown in soils collected from each of the three study gardens along our latitudinal transect. Parameters include dark respiration estimated at leaf temperatures of 20 °C (R30), as well as respiration temperature sensitivity (Eo). Trees were grown in a common garden near Madison, WI, for 45 days prior to measurement. Means and standard errors are based on n = 5 leaves per soil type. For a given variable, the corresponding P value indicates the significance of differences among soil means.

Reexamining species relationships between respiration and growth temperature

In light of the trend in Figure 4, we reexamined relationships between R30 and growth temperature after normalizing the former, through analysis of covariance, for its hypothetical dependence on Amass (Figure 6). Normalization decreased R30 variation among species and campaigns (cf. Figure 1), and, largely owing to the trend for aspen, normalized R30 was positively related to growth temperature ($r^2 = 0.58, P = 0.029$) in the boreal group.

Discussion


Along our latitudinal transect, however, there was little evidence for foliar thermal acclimation in any of the estimated respiration parameters. On the contrary, in all species, temperature-adjusted R30 and Eo tended to rise, rather than fall, with increasing growth temperature. Thus, our study is one of a growing number in which acclimation was not observed for at least one species, population or phenotype (e.g., Larigauderie and Korner 1995, Arnone and Korner 1997, Mitchell et al. 1999, Bolstad et al. 1999, Tjoelker et al. 1999, Gunderson et al. 2000, Loveys et al. 2003, Frantz et al. 2004, Wright et al. 2006, Atkin et al. 2006, Bruhn et al. 2008, Yamori et al. 2008).

Notably, our study also joins a smaller set documenting metabolic adjustments (e.g., R30 responses) that would appear to amplify, rather than minimize, divergence in ambient respiration rates across a climatic gradient (e.g., Larigauderie and Korner 1995, Mitchell et al. 1999, Bolstad et al. 1999, Loveys et al. ...
Figure 3. Relationships between respiration parameters and leaf nitrogen concentration ($N_{mass}$) within each measurement campaign, based on data from individual leaves pooled across all species. Respiration parameters include rates of dark respiration at leaf temperatures of 30 °C ($R_{30}$) and 20 °C ($R_{20}$), and the sensitivity of respiration to instantaneous changes in leaf temperature ($E_0$). Filled symbols denote July campaign data, and open symbols denote August data. Solid lines represent significant ($P < 0.05$) correlations for July and August data, respectively. For $R_{30}$, the July model was $Y = 5.08X + 5.11$ ($r^2 = 0.25$, $P < 0.001$, $n = 63$), and the August model was $Y = 7.57X - 0.66$ ($r^2 = 0.44$, $P < 0.001$, $n = 63$). For $R_{20}$, the July model was $Y = 4.29X + 1.54$ ($r^2 = 0.44$, $P < 0.001$, $n = 63$), and the August model was $Y = 2.9X + 0.5$ ($r^2 = 0.43$, $P < 0.001$, $n = 70$). For both $R_{30}$ and $R_{20}$, campaign relationships differed with respect to intercept ($P = 0.002$) but not slope ($P > 0.08$). Neither of the campaign trends was significant in the case of $E_0$.

Figure 4. Relationship between leaf dark respiration, estimated at a leaf temperature of 30 °C ($R_{30}$), and the average rate of photosynthesis per unit leaf mass ($A_{mass}$) during the photoperiod prior to each respiration measurement. Leaf $A_{mass}$ data were acquired from ancillary gas exchange assessments on a subset of the same leaves measured for $R_d$ (Dillaway and Kruger 2010). During the July campaign, photosynthesis data were not acquired for aspen in northern Wisconsin or for birch at any garden. The regression model, based on individual leaf data pooled across gardens, species and campaigns, was $Y = 0.13X - 0.0001X^2 + 1.3$ ($r^2 = 0.61$, $P < 0.001$, $n = 78$).

Figure 5. Relationship between observed values of $E_0$ and those generated, with a modified Arrhenius equation (see methods), using values of $R_{30}$ and $R_{10}$ predicted from other leaf properties, where $R_{30}$ was estimated with the $A_{mass}$-based model in Figure 4, and $R_{10}$ was estimated with campaign-specific $N_{mass}$-based models (July: $Y = 2.54X + 2.6$, $r^2 = 0.27$, $P < 0.001$, $n = 63$; August: $Y = 1.53X - 0.5$, $r^2 = 0.43$, $P < 0.001$, $n = 70$). The $E_0$ regression model, based on individual leaf data from all gardens, species and campaigns, was $Y = 0.96X - 2$ ($r^2 = 0.55$, $P < 0.001$, $n = 75$). The slope and intercept of this relationship did not differ significantly from 1 ($P = 0.13$) and 0 ($P = 0.39$), respectively.
In at least one respect, the $R_d$ variation we observed resembled temperature responses reported for some of our target tree species in controlled-environment studies. Namely, while Tjoelker et al. (1999a) found that shoot $R_d$ of birch seedlings acclimated, through changes in temperature-adjusted rates (as opposed to $Q_{10}$), across a 12 °C range in growth temperature, shoot $R_d$ of aspen failed to do so. In a less direct comparison, Ow et al. (2008) found that, in foliage of a $P. \ deltoides \times \ nigra$ hybrid, temperature-adjusted $R_d$, but not $E_d$, acclimated across a 10 °C range in growth temperature. In both experiments, as in ours, measured tissues had developed fully in their respective temperature regimes, although we note that regimes were constant in the controlled environments and quite variable at each of our gardens (Dillaway and Kruger 2010).

Regarding a particular species, discrepancies in results among studies may be attributable in part to contrasts among populations in the ability to adjust metabolism to different thermal regimes (Matyas 2006). To date, however, results have been mixed with respect to the magnitude of phenotypic plasticity among populations from different positions along latitudinal or other environmental gradients. Several common-garden assessments with angiosperms have found only modest variation among accessions in $R_d$ and its temperature responses (e.g., Chapin and Oechel 1983, Gunderson et al. 2000, Bolstad et al. 2003, Lee et al. 2005), whereas others focusing on conifer species have reported clear distinctions across populations (e.g., Reich et al. 1996, Oleksyn et al. 1998, Tjoelker et al. 2008). In our case, the temperature responses of aspen from southern Wisconsin were similar overall to those reported by Tjoelker et al. (1999a) for a population from northern Minnesota (~4 ° difference in latitude), whereas cross-study disparities in birch behavior occurred between two populations from more similar latitudes (northern Wisconsin in our study and northern Minnesota in Tjoelker et al. 1999a). In any case, variation in leaf respiratory metabolism, and its response to temperature, did not appear to be associated with the 7 ° range in latitude of origin across the four provenances we used in the present study.

Among our hypotheses, the third was supported most by our results, as $N_{mass}$ explained a significant amount of variation in temperature-adjusted cool $R_d$—although we could not reconcile the difference between relationships in July and August. Nevertheless, our data corroborate the well-established link between respiration and nitrogen status in leaves (e.g., Ryan 1995, Reich et al. 1996, 2008, Tjoelker et al. 1999b, Turnbull et al. 2003, Lee et al. 2005, Wright et al. 2006). At the same time, they reaffirm that $N_{mass}$ (or N content, g N m$^{-2}$ leaf area) alone is often an inadequate predictive trait owing to $R_d$'s dependence on variables potentially unrelated to nitrogen status, such as substrate availability (e.g., Azcon-Bieto et al. 1983, Atkin et al. 2000, Tjoelker et al. 1999b, 2008, Atkin and Tjoelker 2003, Lee et al. 2005) or mitochondrial characteristics (Armstrong et al. 2006a, 2006b). Perhaps the most poignant illustration of this limitation in the present study was the absence of a direct link between $E_d$ and $N_{mass}$ (Figure 3) or leaf N content ($P \geq 0.11$, data not shown). This contrasts with the findings of Turnbull et al. (2003), who observed that variation across a similar range in $E_d$ was positively related to leaf N content, and with the data of Griffin et al. (2002), wherein there was a positive correspondence between $E_d$ and $N_{mass}$.

Our study also provides additional support for the intuitive notion that the $R_d$ of a leaf is closely coupled with its prior photosynthetic performance (e.g., Loveys et al. 2003, Whitehead et al. 2004, Wright et al. 2006, Hartley et al. 2006, Bunce 2007, Werton and Teskey 2010, Zaragoza-Castells et al. 2008, Maseyk et al. 2008), in the present case seemingly regardless of a leaf’s thermal ‘preconditioning.’ Although we are unable to compare many published relationships directly, owing to differences in reported photosynthetic traits (e.g., area- versus mass-based averages of light-saturated versus ambient rates), the trend we observed in Figure 4 was similar to that found by Loveys et al. (2003), except that ours was curvilinear, like that reported for Glycine max by Bunce (2007).

A curvilinear relation between warm $R_d$ and average ambient $A_{mass}$ may reflect substrate saturation of respiratory metabolism at high photosynthetic rates, and this implies that $R_d$ variation will not always appear to closely track photosynthetic dynamics, especially if photosynthetic rates remain high overall. Moreover, an inherently nonlinear relationship would likely contribute to

Figure 6. Normalized values of leaf dark respiration at 30 °C ($R_{30}$) plotted against growth temperature, based on data pooled across campaigns. Here $R_{30}$ was adjusted, through analysis of covariance, for the hypothetical influence of prior leaf photosynthetic performance (average $A_{mass}$ during the previous photoperiod), based on the relationship in Figure 4. Values are adjusted means for each species × garden × campaign, when photosynthesis data were available (see Table 3). Species are denoted with the following symbols: trembling aspen (open circle), eastern cottonwood (filled square), paper birch (open triangle) and sweetgum (filled diamond). Only the boreal group exhibited a significant trend (dashed line, $Y = 0.45X + 17.7, r^2 = 0.58$, $P = 0.029$).

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temporal dynamics and treatment-induced variation in the balance between ambient rates of leaf dark respiration and photosynthesis (e.g., Zaragoza-Castells et al. 2008).

The hypothetical, substrate-mediated dependence of leaf $R_d$ (and to some extent $E_o$) on prior photosynthetic performance might help to explain some of the apparent inconsistencies reported in the literature regarding respiratory thermal acclimation. Namely, apparent acclimatory responses, with respect to either temperature-adjusted $R_d$ or $R_d$’s temperature sensitivity, may in some instances reflect nothing other than a decline in photosynthetic performance with increasing growth temperature. On the other hand, a decoupling of $R_d$ and photosynthesis (e.g., decreases in $R_d$ at a given average $A_{max}$ in warm climates) may constitute some of the most compelling evidence of $R_d$ thermal acclimation. We note that this sort of behavior was not observed among any of the species in our study (Figure 6).

To date, the causes underlying $E_o$ (or $Q_{10}$) variation have remained elusive (e.g., Loveys et al. 2003, Atkin et al. 2006, Zaragoza-Castells et al. 2007, Bruhn et al. 2008). At least two lines of evidence in our study support the argument that $E_o$ is largely a culmination of the somewhat independent limitations imposed on warm and cool $R_d$ (Atkin and Tjoelker 2003, Atkin et al. 2005). Most importantly, an empirical model based on leaf photosynthetic performance (for warm $R_d$) and nitrogen status (for cool $R_d$) was fairly effective in predicting the $E_o$ variation we observed (Figure 5). Additionally, the marked $E_o$ increase from July to August coincided primarily with a sizable decrease in $R_{20}$, as opposed, for instance, to a large rise in $R_{30}$. We acknowledge that this behavior does not fully align with the theoretical construct developed by Atkin and Tjoelker (2003) and widely embraced (e.g., Hartley et al. 2006, Zaragoza-Castells et al. 2007, Ow et al. 2008), where $R_d$ variation across different temperature regimes can result from either of two modes of thermal acclimation, known as Type I and Type II. Neither of these modes was evident along our transect.

The question remains as to why, contrary to the outcomes of other studies, none of our target tree species exhibited behaviors consistent with thermal $R_d$ acclimation along the latitudinal transect. Of course, we must consider the possibility that influences of a large climate gradient on tree metabolism might have been obscured or otherwise confounded by other biotic or abiotic factors, such as garden differences in edaphic characteristics, pathogen and herbivorous insect pressures, and photoperiod length. We can address some of these issues. For example, we doubt that our findings were confounded to any appreciable degree by garden edaphic differences, as our ancillary assessment failed to detect soil-mediated variation in leaf $R_d$ or its temperature sensitivity (Table 4). Additionally, we observed minimal insect or pathogen damage to any of our target species during the study period. On the other hand, we cannot speculate on the potentially confounding role of garden differences in photoperiod (e.g., photoperiod length was, on average, ~45 minutes shorter in southern Illinois than in northern Wisconsin during the study). Very little is known about the implications of modest variation in photoperiod for respiratory metabolism. In growth chambers, Oleksyn et al. (1992) mimicked the photoperiods corresponding to latitudes of 50 and 60 ° (peak difference ~2.5 h during the growing season), and found that needle $R_d$ of Pinus sylvestris was similar in the two light regimes.

Finally, while a particular definition of thermal acclimation underpinned our study, it seems equally appropriate to evaluate the patterns of leaf metabolism we observed in the context of a different definition—namely one in which metabolic adjustments to climate allow plants to maintain homeostasis in the balance between organ (or plant) $R_d$ and leaf (or crown) photosynthesis (i.e., $R/P$ ratio). Dewar et al. (1999) proposed a respiration modeling strategy centered on long-term homeostasis in $R/P$, and others have assessed its constancy across different temperature regimes (e.g., Loveys et al. 2003, Atkin et al. 2006, 2007, Zaragoza-Castells et al. 2008). Results to date have been mixed, with some studies reporting homeostasis (e.g., Loveys et al. 2003, Yamori et al. 2008) and others reporting considerable treatment variation (e.g., Atkin et al. 2006, 2007). The positive relation between $A_{max,norm}$-normalized $R_{30}$ and growth temperature for the selected provenances of aspen and birch in Figure 6 implied that their $R/P$ might increase with growth temperature (especially for aspen). In any case, a shift in $R/P$ would be attributable in part to a lack of photosynthetic thermal acclimation, observed in aspen as well as the other species, across our gardens (Dillaway and Kruger 2010). In a forthcoming manuscript, we assess the consequences of these and associated adjustments for integrated ambient $R_d$, carbon-use efficiency and growth of the four species along our latitudinal (and climatic) transect.

Acknowledgments

We would like to thank Bronwyn Aly (University of Illinois) for help with the southern Illinois garden and Bryan Bowen (University of Wisconsin-Madison) for help with the northern Wisconsin garden.

Funding

This study was supported by the National Science Foundation (award #0802729) and by McIntire-Stennis formula funding (project #WIS01227).

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