Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO₂

JACQUELINE E. MOHAN*, JAMES S. CLARK*† and WILLIAM H. SCHLESINGER*†

*Graduate Program in Ecology, Department of Biology, Duke University, Durham, NC 27708, USA, †Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC 27708, USA

Abstract

Genetic variation in plant response to atmospheric carbon dioxide (CO₂) may have influenced paleo-vegetation dynamics and could determine how future elevated CO₂ drives plant evolution and ecosystem productivity. We established how levels of relatedness – the maternal family, population, and provenance – affect variation in the CO₂ response of a species. This 2-year growth chamber experiment focused on the germination, growth, biomass allocation, and survivorship responses of Acer rubrum to four concentrations of CO₂: 180, 270, 360, and 600 μL L⁻¹ – representing Pleistocene through potential future conditions. We found that all levels of relatedness interacted with CO₂ to contribute to variation in response. Germination responses to CO₂ varied among families and populations, growth responses depended on families and regions of origin, and survivorship responses to CO₂ were particularly affected by regional identities. Differences among geographic regions accounted for 23% of the variation in biomass response to CO₂. If seeds produced under subambient CO₂ conditions behave similarly, our results suggest that A. rubrum may have experienced strong selection on seedling survivorship at Pleistocene CO₂ levels. Further, this species may evolve in response to globally rising CO₂ so as to increase productivity above that experimentally observed today. Species responses to future atmospheric CO₂ and the accompanying biotic effects on the global carbon cycle will vary among families, populations, and provenances.

Keywords: Acer rubrum, CO₂, evolutionary ecology, genetic variation, global change, red maple

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Introduction

Carbon dioxide (CO₂) concentrations in the earth’s atmosphere have fluctuated widely over geological and paleo-ecological time scales (Petit et al., 1999; Retallack, 2001). During each of the Pleistocene glacial stages, levels dropped to below 200 μL L⁻¹ and rose again during subsequent interglacial events. Owing to fossil fuel emissions and tropical deforestation, the current concentration of ~370 μL L⁻¹ is expected to increase to 600 μL L⁻¹ by the end of this century (Prentice et al., 2001), representing the highest level in the past 420 000 years (Petit et al., 1999). The current rate of increase has never before been experienced by earth’s biota (Schlesinger, 1997).

Plant function has evolved in response to past changes in atmospheric CO₂ (Beerling et al., 1993; Woodward, 1993; Dippery et al., 1995; Ward et al., 2000; Sage & Coleman, 2001). Gene frequencies may change again with future CO₂ increases (Curtis et al., 1994; Wayne & Bazzaz, 1995, 1997). Effects of rising atmospheric CO₂ on plant fitness have implications for future net primary productivity and evolution (Schlesinger et al., 2001; Bazzaz et al., 1995).

Studies of the impacts of elevated atmospheric CO₂ on plant growth have reported a range of responses within individual species. For Acer rubrum, L. (red maple), approximately doubling modern CO₂ concentrations resulted in biomass increases ranging from 0%
to 70% (Bazzaz et al., 1990; Miao et al., 1992). Some of the
differences among studies can be explained by differing
experimental conditions, such as levels of light, soil
resources, plant densities, and rooting volumes. How-
ever, other studies have found differences in plant
response to CO2 due to genetic variation (Curtis et al.,
1994; Wayne & Bazzaz, 1997; Ward et al., 2000). Unfortu-
nately, many experiments use data collected from a limited number of individuals to characterize the
response of an entire species.

In this 2-year study, we examine intraspecific vari-
ation in germination, growth, biomass allocation, and
survivorship of A. rubrum in response to four levels of
CO2: 180, 270, 360, and 600 μL L⁻¹. To our knowledge,
this study is the first to examine genetic variation in tree
responses to subambient CO2 concentrations, and the
only study to consider multiple levels of relatedness. To
determine which level of intraspecific organization – the
maternal family, the population, or the geographic
region of origin (provenance) – explains most of the
variation in CO2 response, we used a nested design
with seeds collected from 26 maternal families, com-
prising six populations from three regions of this
species’ range (Table 1).

Several studies provide a confusing array of potential
genetic interactions. One experiment found no differ-
ence in germination of seeds from two families in
response to elevated CO2 (Grulke et al., 1993). The
effects of increased CO2 on plant survivorship are
ambiguous, with studies utilizing ambient and elevated
CO2 concentrations finding both decreased (Bazzaz
et al., 1995) and increased (Polley et al., 1999) survival.
One study found no significant variation in CO2 effects
on survivorship among six families of honey mesquite
(Prosopis glandulosa, Polley et al., 1999). Elevated CO2
may increase, decrease, or have no effect upon root:
shoot allocation (Bazzaz et al., 1990; Curtis & Wang,
1998; Bernacchi et al., 2000), but we are aware of only
one study addressing genetic variation in allocation
response (Veteli et al., 2002). Studies examining ambient
vs. elevated CO2 responses have found variation in the
growth of trees from different families within a
population (Conroy et al., 1990; Grulke et al., 1993;
Wayne & Bazzaz, 1995, 1997) and from different
populations within a region (Callaway et al., 1994;
DeLucia et al., 1994). The only study to examine variation
among populations as well as variation between
regions found that trees from two distinct regions had similar CO2 responses (DeLucia et al.,
1994); variation among families was not considered.

Lack of regional differences in response to CO2 is not
surprising, as CO2 is well-mixed in the troposphere and
lacks the broad geographic gradients typical of most
environmental variables (Schlesinger, 1997). One study
of DNA markers in congeneric Acer saccharum found that
families within a forest stand accounted for most of the
genetic variation, with less than 2% of the variation
determined by regional differences (Gunter et al.,
2000). Accordingly, we hypothesized that variation in germi-
nation, survivorship, root:shoot allocation, and growth
responses to atmospheric CO2 would be mostly con-
tributed by the maternal family. This variation, as well
as the variation among populations, would overwhelm
variation among regions. We hypothesized that A. rubrum trees originating from different provenances
within this species’ range would respond similarly to CO2.

Red maple is one of the most abundant tree species in
eastern North America and occurs across a wide range
of soil types, texture, moisture, pH, and elevation
(Walters & Yawney, 1990). The regions used in the
present study occur near the center of A. rubrum’s
distribution and encompass a variety of habitats and
elevation extremes. Red maple is becoming an increas-
ingly abundant tree (Abrams, 1998) that may come to
co-dominate eastern hardwood forests. Genetic varia-
tion in the response of this nearly ubiquitous species
may be an important determinant of global change
impacts on eastern forests.

Table 1 Site descriptions of the three regions and six populations used as sources of Acer rubrum seeds*

<table>
<thead>
<tr>
<th>Region</th>
<th>Population</th>
<th>Elevation above sea level (m)</th>
<th>Mean January temperature 1997–1999 (°C)</th>
<th>Mean July temperature 1997–1999 (°C)</th>
<th>Longitude and latitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal Plain, NC (CP)</td>
<td>CP1</td>
<td>7</td>
<td>8.1</td>
<td>26.9</td>
<td>36°11'N, 76°37'W</td>
</tr>
<tr>
<td></td>
<td>CP2</td>
<td>7.5</td>
<td>8.1</td>
<td>26.9</td>
<td>36°11'N, 76°37'W</td>
</tr>
<tr>
<td>Piedmont, NC (PD)</td>
<td>PD1</td>
<td>140</td>
<td>5.7</td>
<td>26.3</td>
<td>36°18'N, 78°37'W</td>
</tr>
<tr>
<td></td>
<td>PD2</td>
<td>170</td>
<td>5.9</td>
<td>26.5</td>
<td>35°55'N, 79°05'W</td>
</tr>
<tr>
<td>Blue Ridge Mountains,</td>
<td>MT1</td>
<td>1067</td>
<td>4.7</td>
<td>23.0</td>
<td>35°04'N, 83°26'W</td>
</tr>
<tr>
<td>NC (MT)</td>
<td>MT2</td>
<td>820</td>
<td>4.7</td>
<td>23.0</td>
<td>35°04'N, 83°26'W</td>
</tr>
</tbody>
</table>

*County-based temperature, longitude, and latitude data obtained from the National Oceanic and Atmospheric Administration (NOAA) via the National Climate Data Center (NCDC) at http://cdo.ncdc.noaa.gov.

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Materials and methods

Seed collection

We collected seeds from 26 *A. rubrum* var. *rubrum* (trees were keyed to var. *rubrum* status using the flora developed by A. S. Weakley and available online from the University of North Carolina Herbarium at: http://www.herbarium.unc.edu/weakley_flora/) trees, from within six populations in three regions. Seeds derived from the three physiographic provinces of North Carolina (the Blue Ridge Mountains, the Piedmont, and the Coastal Plain, Table 1). Within each region, populations were described by groups of three to seven seed-producing trees occurring within a ~ 300 m² area. In principle, it would be better to have a larger sample size, but the number of trees from which seeds were collected was constrained by the number of trees bearing attainable seeds and occurring within an area small enough that pollen transport and genetic exchange were likely. Populations were separated from other collection sites by at least 2 km, a distance greater than the transport of predominantly insect-pollinated *Acer* pollen (Calcote, 1995; Jackson & Kearsley, 1998) and seeds (Clark, 1998) observed in other studies. Seeds were collected from 13 trees in the Mountains, originating from two populations; from seven trees in the Piedmont, consisting of two populations; and from six trees in the Coastal Plain, comprising two populations.

*A. rubrum* is mono-dioecious; seeds borne by maternal trees may result from pollen dispersed from different paternal trees. Seeds collected from a given tree are at least half-sibs, representing a specific maternal family. When possible, seeds were shaken from tree branches. When no fecund branches were within reach, seeds were collected near the base of the tree. In these cases, only maternal trees that were separated by more than 15 m from other seed trees were used to avoid familial contamination. Seeds were stored at 4 °C between collection and planting.

Germination and growing conditions

A range of atmospheric CO₂ concentrations was maintained in growth chambers at the Duke University Phytotron (Durham, NC, USA). Plants at each CO₂ treatment were grown in a single growth chamber; to minimize potential ‘chamber effects’, we rotated all plants and their respective CO₂ level every 10 (year 1) or 14 days (year 2) into new chambers. Excess CO₂ was removed from the 180 and 270 μL L⁻¹ chambers by passing the chamber air over a hydrated lime/vermiculite mixture. Distilled water was applied three times daily, including a morning fertilization with half-strength Hoagland’s fertilizer (Downs & Hellmers, 1978). Watering was reduced to twice daily after seedlings were transplanted for growth and survivorship experiments; fertilizer applications continued each morning. Seedlings were randomly arranged within populations, and populations were grouped according to region; regional groups were randomly rearranged within each CO₂ level every 10 (year 1) or 14 days (year 2). In the first year, seedlings grew in a 1:2:1 mixture of sand, vermiculite, and gravel. In the second year, Metro-Mix 200 (vermiculite, sphagnum, and perlite; The Scotts Company, Marysville, OH, USA) was used as a non-nutritive growing medium. Daytime photosynthetically active radiation (PAR) was 325 μmol m⁻² s⁻¹ in year 1 and 750 μmol m⁻² s⁻¹ in year 2, and humidity was maintained at ambient levels in year 1 and at 70% relative humidity during year 2. Annual differences in PAR and humidity were consistent across treatments. During periods of growth (non-dormancy), daytime and night-time temperatures were 25 °C and 20 °C, respectively, and the photo- and thermoperiods were 14 h per day.

Germination experiment

In June 1997, 3179 seeds were placed on damp sand in 640 cm² germination trays. The seeds of each maternal family were randomly divided among the four CO₂ treatments of 180 μL L⁻¹ (low periods of the Pleistocene), 270 μL L⁻¹ (pre-Industrial Revolution), 360 μL L⁻¹ (ambient CO₂ concentration at the initiation of the experiment), and 600 μL L⁻¹ (potential future condition), and immediately transferred to the appropriate CO₂ environment. The sample size of each family depended on the number of seeds collected and ranged from 44 to 256. This unequal number leads to unbalanced data, which is discussed in the Analysis section but did not have a significant impact on our results. Germination, defined as the emergence of a radicle, was censused 10, 20, and 30 days postplanting.

Growth and survivorship – year 1

Thirty days after seeds were planted into germination trays, all live seedlings (1485 total seedlings) were transplanted individually into 226 cm³ ‘cone-tainers’ (Stuewe and Sons, Inc., Corvallis, OR, USA). Thus seedlings did not compete for soil resources, but, as they grew taller, did compete for light. Sample sizes varied from 380 to 392 seedlings per CO₂ treatment, with a mean of 57 ± 16 seedlings per family.

Survival and height were determined 40, 74, and 106 days after the initiation of the experiment. After 112...
days, we began to induce dormancy by reducing the photo- and thermoperiods to 11 h and decreasing day and night temperatures to 20°C and 15°C, respectively. These conditions severely reduced seedling growth, but did not stimulate leaf senescence. At day 128, photo- and thermoperiods were further decreased to 8-h days/16-h nights. Day and night-time temperatures were gradually reduced to 11°C and 5°C, respectively, by day 142. In addition, the red/far red light ratio was increased to simulate light conditions in forest understories after autumn canopy senescence. By day 184 (December 1997), all leaves had senesced, and the majority had abscised. The dormant plants were moved into cold, dark chambers (4°C) to simulate winter conditions. Plants were watered twice weekly, and the experimental CO2 levels were not maintained during this time. Since plants were not photosynthesizing and would have had low respiration rates at this temperature, it is unlikely that the cessation of CO2 treatments during dormancy impacted the experimental results. Heights of the dormant plants were recorded again in January 1998.

Growth and survivorship – year 2

In May 1998, 425 live seedlings, representing each family at each CO2 level, were randomly selected and repotted for the second year of the experiment. Roots were not constricted around the sides of the containers and seedlings did not appear to be ‘pot-bound’ (Thomas & Strain, 1991). New pots were 7065 cm3 sections of PVC piping with fiberglass screening fixed across the bottom. The trees were placed in larger growth chambers, each at the appropriate CO2 level. Height and survivorship were censused 13, 14, and 14.5 months after initial planting. The final harvest was completed in late August 1998. Trees were again inspected and did not appear to be pot-bound. Stems were clipped at the soil surface, and the aboveground portions were oven dried at 67°C for at least 7 days before weighing. The pots containing the roots were maintained at 4°C until the roots could be washed free of substrate. Root washing was completed within 10 days of harvest. The cleaned roots were placed into the 67°C oven, where they dried for at least 7 days before weighing.

Analysis

Owing to the inherently ‘nested’ nature of the three levels of relatedness, we used nested analyses of variance (ANOVA; SAS Institute, 1990; Sokal & Rohlf, 1995). CO2 and, where appropriate, time were crossed with the nested region, population within region, and family within population terms. Family mean squares were used as error terms for populations, and population mean squares were used for CO2 and for region. CO2 interaction terms were similarly tested against population interactions. To obtain the percent variation in response to CO2 that was explained by a specific term, we divided the type I sum of squares of the term by the total sum of squares for all CO2 variables (the main effect and all interactions).

Height data were square-root-transformed and final biomass was natural-log transformed to meet assumptions of normality and homoscedasticity (Sokal & Rohlf, 1995). Using repeated-measures ANOVA, we analyzed the height of each seedling over the 2 years of growth (six measurements). We included the identity of each plant in this analysis to account for the non-independence of repeated measurements on the same individual. Final biomass and root:shoot allocation were one-time measurements and accordingly analyzed with a simple nested ANOVA model. We included the final biomass in the ANOVA for root:shoot allocation, as previous studies have found that differences in growth rates and final biomass may account for apparent allocation responses to CO2 enrichment (Tissue et al., 1997; Bernacchi et al., 2000). Logistic regression analyses were used to analyze the binary germination and survivorship data (Venables & Ripley, 1997). We additionally used ANOVAs to obtain sums of squares for these variables to establish the percent variation explained by the three levels of relatedness. The unequal number of seeds and seedlings representing specific groups leads to unbalanced data, so the sums of squares attributed to independent variables is order dependent (Everitt, 2002); however, this sequence dependence is also typical of the nested and logistic regression models we use to analyze the data. Further, we changed the order of CO2 and the nested relatedness terms in the analyses and found that the order of the variables had negligible impacts on the results.

Results

Germination

Over a range of CO2 concentrations from Pleistocene to projected future levels, CO2 stimulated germination (P < 0.0001, Table 2 and Fig. 1). The impact of CO2 on the 30-day germination probability further depended on the population from which seeds originated and on the maternal family within each population (P = 0.0007 and 0.007, respectively; Fig. 2a–c). We also observed a difference in the response to CO2 among the different regions (P = 0.07, Fig. 3). Differences among regions accounted for 4.7% of the variation in germination.
response to CO2, differences between populations within a region accounted for 11%, while differences among families within a population explained 39% of the variation (Table 3). The relatively large amount of variation explained by differences among families attests to the distinctiveness of different maternal trees within a forest stand.

Growth

CO2 and time (in months) interacted with both the region and family levels of relatedness to determine the height of A. rubrum seedlings ($P = 0.0157$ and 0.0005, respectively; Table 4). Hence seedlings from different maternal families within a population and from different regions exhibited different growth responses to increasing CO2. Further, the effect of CO2 on height changed as the experiment progressed ($P = 0.0103$ for the CO2 x Time interaction). In the first census, 1.3 months after planting, the 600 μL L$^{-1}$ trees were taller than those at other CO2 levels ($P = 0.0002$). By the end of the second growing season, 14.5 months after planting, heights of the 600 μL L$^{-1}$ trees did not differ from individuals grown at 270 and 360 μL L$^{-1}$, but 180 μL L$^{-1}$ trees remained smaller than the rest ($P = 0.0010$; Fig. 4). Greater height of the elevated CO2 trees was a short-term phenomenon in this experiment.

CO2 x Region was the only CO2 interaction accounting for variation in the final biomass of A. rubrum ($P = 0.0125$, Table 4), with differences among regions responsible for 23% of the variation in response to CO2 (Table 3). Differences between populations within a region accounted for only 1.3% of the variation, and the CO2 x Population interaction was not significant. Owing to the relatively large number of families and accompanying degrees of freedom, 29% of the variation in biomass response to CO2 was explained by differences among families; however, the CO2 x Family interaction was not significant. Whereas both Coastal Plain and Mountain trees showed increasing biomass with CO2 enrichment (Coastal Plain, $P < 0.0001$; Mountains, $P = 0.04$), trees from the Piedmont exhibited no effect of CO2 on final biomass (Fig. 5). In contrast to the results for total biomass, only trees from the Piedmont region exhibited CO2 impacts on root:shoot allocation, allotting more biomass towards roots as CO2 increased ($P < 0.0001$ from linear regression, data not shown).

Mortality

In the first year of the experiment, the 40-day height of a seedling was an important predictor of mortality.

Table 2

<table>
<thead>
<tr>
<th>Term</th>
<th>df</th>
<th>$Pr(\chi^2)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td>2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Population (Region)</td>
<td>3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family (Population)</td>
<td>20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CO2</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CO2 x Region</td>
<td>2</td>
<td>0.07073</td>
</tr>
<tr>
<td>CO2 x Population</td>
<td>3</td>
<td>0.0065</td>
</tr>
<tr>
<td>CO2 x Family</td>
<td>20</td>
<td>0.00731</td>
</tr>
</tbody>
</table>

*Germination is defined as the emergence of a radicle. The asterisk indicates interactions between two terms; interaction terms are enclosed within brackets in the model. Parentheses indicate that a term is nested within a higher-level term. Bold terms are significant at the $P < 0.05$ level.
Fig. 2 (a–c) Logistic regression fits of the probability of germinating within 30 days (y-axis) as a function of atmospheric CO2 carried out separately for each family originating from the (a) Coastal Plain (C01–C07), (b) Mountains (M01–M13), and (c) Piedmont (P01–P07). Dark lines depict families exhibiting significant responses to CO2; pale lines denote no significant response. For each region, solid lines represent fits for families from Population 1, and dashed lines denote families from Population 2. *Refers to CO2 parameters in logistic regressions having $P < 0.10$, **signifies $P < 0.05$, ***refers to $P < 0.01$, and ****denotes $P < 0.001$. Lack of asterisks indicates that there was no significant germination response to CO2.
After considering the effects of initial height, CO₂ had a negative impact on survivorship \((P < 0.07)\) and all three levels of relatedness – regions, populations, and families – interacted with CO₂ and height to explain the probability of dying in the first year \((P = 0.0191, 0.0013, \text{and } 0.0638, \text{respectively}; \text{Fig. 6b})\). Differences among regions accounted for 23% of the variation in response to CO₂, differences between populations accounted for 13%, and differences among families within a population accounted for 58% of the variation (Table 3). The first-year mortality responses of seedlings with a given initial size to atmospheric CO₂ depended on the families, populations, and regions of origin.

In the second year, CO₂ had an overall positive impact on survival (Fig. 6c) and the 40-day height remained a determinant of death \((P < 0.001, \text{Table 5})\). Small trees growing at 180–360 μL L⁻¹ exhibited declining mortality with increasing CO₂; this trend was reversed when comparing 360 and 600 μL L⁻¹ individuals (Fig. 6d). Initial seedling height also interacted with CO₂ and the region of origin to influence mortality \((P = 0.001)\). Thus, seedlings of a given initial height continued to have different mortality responses to CO₂, depending upon the region from which they had originated.

**Discussion**

*A. rubrum* demonstrated intraspecific variation in germination, growth, root: shoot allocation, and survivorship responses to atmospheric CO₂. This variation was expressed by seeds and seedlings originating from different maternal families within a population, from different populations within a provenance, and from different physiographic regions within this species’ range. The degree of congruency among germination, growth, and survivorship responses to CO₂ differed by region, with 50% of Coastal Plain families showing positive CO₂ effects for two or more of these responses; Mountain families, 31%; and Piedmont families, 23%.

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**Table 3** Percent of the variation in response to CO₂ that is explained by three levels of relatedness

<table>
<thead>
<tr>
<th>Response</th>
<th>Region (%)</th>
<th>Population (%)</th>
<th>Family (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination</td>
<td>4.7</td>
<td>11</td>
<td>39</td>
</tr>
<tr>
<td>Biomass</td>
<td>23</td>
<td>1.3</td>
<td>29</td>
</tr>
<tr>
<td>Mortality, year 1</td>
<td>23</td>
<td>13</td>
<td>58</td>
</tr>
<tr>
<td>Mortality, year 2</td>
<td>14</td>
<td>14</td>
<td>63</td>
</tr>
</tbody>
</table>

*The three percentages (Region, Population, and Family interactions with CO₂) for each response not summing to 100% reflects the variation due to the main effect of CO₂.*
Table 4  Summary table of P-values from nested ANOVAs of growth responses to CO₂*  

<table>
<thead>
<tr>
<th>Independent variables in ANOVA</th>
<th>Height</th>
<th>Final biomass</th>
<th>Final root : shoot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>In final biomass</td>
<td>–</td>
<td>–</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Region</td>
<td>0.5642</td>
<td>0.0418</td>
<td>0.3140</td>
</tr>
<tr>
<td>Population (within Region)</td>
<td>0.0075</td>
<td>0.0091</td>
<td>0.2751</td>
</tr>
<tr>
<td>Family (within Population)</td>
<td>0.0611</td>
<td>0.0138</td>
<td>0.3948</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.0002</td>
<td>0.0018</td>
<td>0.0008</td>
</tr>
<tr>
<td>Time</td>
<td>0.0002</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Region × Time</td>
<td>0.0522</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Population (within Region) × Time</td>
<td>0.0029</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Family (within Population) × Time</td>
<td>0.9473</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CO₂ × Time</td>
<td>0.0103</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CO₂ × Region</td>
<td>0.6005</td>
<td>0.0125</td>
<td>0.0022</td>
</tr>
<tr>
<td>CO₂ × Population (within Region)</td>
<td>0.0260</td>
<td>0.8617</td>
<td>0.9951</td>
</tr>
<tr>
<td>CO₂ × Family (within Population)</td>
<td>0.9861</td>
<td>0.9361</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CO₂ × Region × Time</td>
<td>0.0157</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CO₂ × Population (within Region) × Time</td>
<td>0.8307</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CO₂ × Family (within Population) × Time</td>
<td>0.0005</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Time’ is measured in units of months (for height). Height measurements are repeated measures with temporal interactions (the seedling identity term used in the repeated-measures height analysis is not included in this table). Parentheses indicate that a term is nested within a higher-level term. Final biomass and root:shoot are one-time only measurements and therefore have no interactions with time; these terms are designated with ‘–’ in the table. To account for the potential dependence of root:shoot biomass ratio on the total biomass of a plant, ln(Biomass) was included in the root:shoot ANOVA. Height data were square-root transformed; biomass and biomass ratio data were natural log transformed.

Fig. 4  CO₂ interacts significantly with Time to explain height growth in A. rubrum (P = 0.01 from repeated-measures nested ANOVA). The mean values of square-root transformed height for each CO₂ group at each of the eight height censes are shown. Error bars represent ±1 SE. Note that the dormant measurements (months = 6.3 and 9.8) were not included in the analysis.
families, 14% (Table 6). As global CO₂ rises, responsive families may have an advantage over less-responsive lineages, and the range in congruency among regions suggests that Coastal Plain maples in particular may become even more competitive. The variation in CO₂ response expressed within the different levels of relatedness could alter future plant responses to rising atmospheric CO₂, and likely impacted selection processes during the Pleistocene.

Germination

Like many temperate forest trees, *A. rubrum* is currently recruitment limited, in part due to low seedling establishment success (Clark *et al.*, 1998, 1999). Families with seeds that germinate better under projected future CO₂ conditions will have greater opportunities to establish in favorable microsites (Harper, 1977; Clark *et al.*, 1999) and to exploit canopy gaps (Wayne &

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**Table 5** Summary table presenting *P*-values from *χ²* tests in logistic regression analyses

<table>
<thead>
<tr>
<th></th>
<th>Mortality probability, year 1</th>
<th>Mortality probability, year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial height</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Region</td>
<td>0.0810</td>
<td>0.0032</td>
</tr>
<tr>
<td>Population within Region</td>
<td>0.1595</td>
<td>0.7155</td>
</tr>
<tr>
<td>Family within Population</td>
<td>0.0428</td>
<td>0.4889</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.0734</td>
<td>0.0122</td>
</tr>
<tr>
<td>CO₂ × Height</td>
<td>0.9363</td>
<td>0.3061</td>
</tr>
<tr>
<td>CO₂ × Region</td>
<td>0.2512</td>
<td>0.0289</td>
</tr>
<tr>
<td>CO₂ × Population</td>
<td>0.2307</td>
<td>0.0122</td>
</tr>
<tr>
<td>CO₂ × Family</td>
<td>0.5155</td>
<td>0.0012</td>
</tr>
<tr>
<td>Height × CO₂ × Region</td>
<td>0.0191</td>
<td>0.0012</td>
</tr>
<tr>
<td>Height × CO₂ × Population</td>
<td>0.0013</td>
<td>0.0660</td>
</tr>
<tr>
<td>Height × CO₂ × Family</td>
<td>0.0638</td>
<td>0.1809</td>
</tr>
</tbody>
</table>

---

**Fig. 5** Linear regression fits (solid lines for Coastal Plain and Mountains; dashed line for Piedmont) and 95% confidence intervals (dotted lines) for the ln-transformed total biomass of plants in each Region vs. atmospheric CO₂. Points represent individual trees. Coastal Plain (‘o’) and Mountain (‘x’) trees exhibit increasing biomass with increasing CO₂ (CP: *P* < 0.001 and *N* = 168, MT: *P* = 0.035 and *N* = 87). Piedmont trees (‘^’) show no effect of CO₂ on total biomass (*P* = 0.8 and *N* = 101). Note that the results do not change with the exclusion of the small and large Coastal Plain trees at 270 μL L⁻¹ and 360 μL L⁻¹, respectively, or the smallest Mountain tree at 180 μL L⁻¹.
In accordance with our hypothesis, the impact of CO2 on seed germination largely depended on the maternal origin (39% of the variation; Fig. 2a–c) and population (11% of the variation), suggesting that natural populations of A. rubrum may undergo selection for enhanced germination with increasing CO2. Contrary to our expectation, seeds from different regions also had different germination responses to CO2. While seeds collected from the Coastal Plain and Mountains showed higher germination probabilities with increasing CO2 (Fig. 3), seeds from the Piedmont exhibited no overall effect of CO2 on germination. Regional differences in the effect of CO2 on germination have not been observed before, and may affect future expansions of red maple genotypes by stimulating germination of seeds native to some regions and increasing the success of long-distance dispersal events. Future atmospheric CO2 will likely impact the evolution and ecology of A. rubrum through differential effects on germination within and among populations as well as among regions.

In studies of herbaceous plants, elevated CO2 has been shown to enhance (Esashi et al., 1989; Ziska & Bunce, 1993), to have no effect upon (Garbutt et al., 1990; Ward, 1997), and to inhibit (Andalo et al., 1996) germination. One previous study examining a tree species found no CO2 effects on percent germination or germination rate when comparing seeds at 350 and 700 µL L⁻¹ (Gruulke et al., 1993). In the present study, high CO2 had an overall positive impact on the germination of red maple. Increased CO2 may interact with or even increase the production of ethylene, a plant growth regulator that stimulates germination (Kozlowski & Pallardy, 1997). The CO2 effect appears to involve a conformational switch in phytochrome (Esashi et al., 1987), a pigment that mediates germination responses to light environments (Casal & Sanchez, 1998; Shinomura et al., 1998).

Finally, even the highest experimental CO2 level was below the CO2 concentrations often measured in field soils, resulting from microbial decomposition of organic matter (Amundson & Davidson, 1990; Andrews &
It is possible that the positive CO₂ impacts on germination observed in this and in other studies are due to seeds not experiencing the high-CO₂ conditions of soils. The effects of CO₂ on germination, and potential genetic variation in these effects, needs to be examined under field conditions.

**Growth**

Different maternal families within a population exhibited different height growth responses to CO₂ (Table 4); these results agreed with our hypothesis and with previous studies (Conroy et al., 1990; Grukle et al., 1993; Wayne & Bazzaz, 1995, 1997). Owing to asymmetric size-based competition for light in forest understories and canopy gaps, future increases in atmospheric CO₂ will likely augment selection within populations in favor of those genotypes capable of rapid height growth. However, trees originating from different regions also had different height responses to CO₂; previous studies did not lead us to expect this result. Future studies of height response to CO₂ should use seedlings from several maternal families and include seeds collected from different parts of a species’ range.

The importance of the region in determining a tree’s response to CO₂ is also evident from the final biomass results, with the CO₂ × Region interaction explaining 23% of the variation in response. Differences among regions are especially noteworthy, given the relatively restricted area of our study compared with the full extent of red maple’s range. Based on previous work that found that plants from high altitudes are better adapted to low CO₂ partial pressures (Billings et al., 1961; Körner & Diemer, 1987), it might be anticipated...

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**Table 6 Families exhibiting positive germination, growth, and/or survivorship responses to CO₂ concentrations increasing from 180 to 600 μL.L⁻¹**

<table>
<thead>
<tr>
<th>Family</th>
<th>Population</th>
<th>Germination</th>
<th>Height growth</th>
<th>Survivorship</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Coastal Plain 1</td>
<td>+ ****</td>
<td>+ ****</td>
<td>+ *</td>
</tr>
<tr>
<td>C2</td>
<td></td>
<td></td>
<td>+ ****</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td></td>
<td>+ **</td>
<td>+ ****</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>Coastal Plain 2</td>
<td></td>
<td>+ ****</td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td></td>
<td>+ ****</td>
<td>+ ****</td>
<td>+ *</td>
</tr>
<tr>
<td>C7</td>
<td></td>
<td></td>
<td>+ ***</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>Mountain 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td></td>
<td></td>
<td>+ *</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M5</td>
<td></td>
<td>+ **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M7</td>
<td></td>
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</tr>
<tr>
<td>M8</td>
<td>Mountain 2</td>
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<tr>
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<td></td>
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<tr>
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<td></td>
<td>+ **</td>
<td></td>
<td>+ *</td>
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<td></td>
<td></td>
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<tr>
<td>M12</td>
<td></td>
<td></td>
<td>+ *</td>
<td>+ *</td>
</tr>
<tr>
<td>M13</td>
<td></td>
<td>+ ***</td>
<td>+ ***</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>Piedmont 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td></td>
<td>+ **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>Piedmont 2</td>
<td>+ **</td>
<td></td>
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<tr>
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<td>+ *</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Indicates that CO₂ response has a $P < 0.10$,

**denotes $P < 0.05$,

***signifies $P < 0.01$, and

****means $P < 0.001$.

– indicates no significant CO₂ response.

No families exhibited significant negative responses.
that Coastal Plain (7 m above-sea-level) and Mountain (>800 m asl) trees would respond differently to atmospheric CO₂, and that Piedmont trees (~155 m asl) would exhibit an intermediate response. Instead, we found that trees from both the highest and lowest elevations exhibited positive biomass responses to CO₂. Regional differences also seem to be more than a simple maternal effect, in which only the largest seedlings benefit from high CO₂. Rather, seedlings from the Coastal Plain and the Mountains included some of the largest as well as some of the smallest trees. The percent increase in biomass of Coastal Plain trees growing at 600 vs. 180 μL·L⁻¹, however, was greater than that of the Mountain trees (193 ± 9.59% vs. 132 ± 5.67%, respectively), suggesting that the stronger CO₂ response of the larger trees may have suppressed the response of smaller trees (McDonald et al., 2002). In nature, CO₂ enrichment may act to favor the growth of larger plants preferentially, thereby increasing productivity enhancements over time. Initially, taller plants were also more likely to have a larger final biomass ($R^2 = 0.52$, $P < 0.0001$); these individuals may have an advantage over smaller trees when competing for light, further biasing gene pools in favor of larger, responsive genotypes. Interestingly, the 50% decline in biomass of herbaceous plants grown under 180 vs. 360 μL·L⁻¹ reported by Sage & Coleman (2001) compares with the present experiment (mean biomass of 180 μL·L⁻¹ trees was 43.8% less than ambient CO₂ plants), but this largely derives from the presence of Coastal Plain trees. Future dynamics of A. rubrum will be impacted by atmospheric CO₂ through differential effects on growth, and studies designed to understand growth responses to CO₂ should examine plants from several families within a population and from several regions within a species' range.

**Mortality**

The initial height of a tree seedling significantly affected its survival probability in both years of this experiment. It is likely that early seedling height is a function of seed size and, thus, a maternal effect (Harper, 1977; Miao, 1995). However, even after initial size was accounted for, CO₂ affected survival probabilities particularly in the second year (Fig. 6d). Decreasing mortality probabilities with increasing initial height were especially evident for maple trees growing at 180 μL·L⁻¹ CO₂ ($P < 0.0001$). Previous work on herbaceous plants observed that the subambient CO₂ conditions of the Pleistocene resulted in high mortality of C₃ seedlings (Ward, 1997). The present study suggests that these low CO₂ conditions were particularly stressful for small seedlings, likely due to a combination of light competition and a suboptimal carbon resource, and probably imposed strong selection pressure on tree seedling survival. Declining mortality probabilities with increasing CO₂ continued through 360 μL·L⁻¹, but maples with initially smaller heights had greater chances of dying at elevated than at ambient CO₂ ($P = 0.028$ for the CO₂ × Height interaction of ambient and elevated trees; Fig. 6d). As suggested in previous work (Bazzaz et al., 1995), potential increases in light competition and self-thinning imply that smaller seedlings may be more likely to die in a future elevated-CO₂ world.

**Implications for future competitiveness and range shifts**

In addition to its direct impacts on fitness, elevated CO₂ is increasing mean global temperatures. The rate of expected climate change may be the most rapid in the last 2.4 million years (Huntley, 1995) and is already causing changes in species' ranges (Fayette & Filion, 1985; Parmesan et al., 1999; Thomas & Lennon, 1999; Warren et al., 2001; Hill et al., 2002). Yet migration dynamics may be impacted by CO₂. We found that Coastal Plain trees from the warmest region in the study showed enhanced germination, growth, and relative survivorship under the high CO₂ conditions of the future. Conversely, Piedmont individuals adapted to warmer temperatures were relatively smaller than cooler-temperature Mountain seedlings under future CO₂ levels (Fig. 5), and no longer exhibited the survival advantage experienced under ambient CO₂ (Fig. 6b). If genotypes from warmer regions have reduced fitness under elevated CO₂, future migrations by plants adapted to higher temperatures may be delayed. Direct impacts of enhanced CO₂ may amplify or diminish the effects of elevated temperature on plant fitness and might influence migration responses to global warming.

**Conclusions**

The responses of A. rubrum to atmospheric CO₂ depend on the regional, population, and familial identities of individual trees. Seeds collected from different families within a population exhibit different germination responses, and growth and survival of the resulting seedlings are differentially affected by CO₂. Seeds from different populations within a physiographic province have different germination and survival responses to CO₂. Contrary to our expectation, seeds and seedlings originating from different regions also exhibit different germination, growth, and survivorship responses to CO₂.

In this study, red maple typically germinates better and grows larger with increasing atmospheric CO₂,
implying that future productivity may be higher than it is today. Genetic variation in CO₂ responses and the rapid increase in global CO₂ concentration suggest the potential for adaptive evolution of this species, which may further enhance productivity with CO₂ enrichment. Genetic variation in red maple’s responses to CO₂ bears implications for the future competitiveness and invasive potential of this increasingly abundant tree.

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