Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO$_2$

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Abstract

Genetic variation in plant response to atmospheric carbon dioxide (CO$_2$) may have influenced paleo-vegetation dynamics and could determine how future elevated CO$_2$ drives plant evolution and ecosystem productivity. We established how levels of relatedness – the maternal family, population, and provenance – affect variation in the CO$_2$ 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 CO$_2$: 180, 270, 360, and 600 μL L^{-1} – representing Pleistocene through potential future conditions. We found that all levels of relatedness interacted with CO$_2$ to contribute to variation in response. Germination responses to CO$_2$ varied among families and populations, growth responses depended on families and regions of origin, and survivorship responses to CO$_2$ were particularly affected by regional identities. Differences among geographic regions accounted for 23% of the variation in biomass response to CO$_2$. If seeds produced under subambient CO$_2$ conditions behave similarly, our results suggest that *A. rubrum* may have experienced strong selection on seedling survivorship at Pleistocene CO$_2$ levels. Further, this species may evolve in response to globally rising CO$_2$ so as to increase productivity above that experimentally observed today. Species responses to future atmospheric CO$_2$ and the accompanying biotic effects on the global carbon cycle will vary among families, populations, and provenances.

Keywords: Acer rubrum, CO$_2$, evolutionary ecology, genetic variation, global change, red maple

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Introduction

Carbon dioxide (CO$_2$) 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 $\sim 370\,\mu$L 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 CO$_2$ (Beerling *et al.*, 1993; Woodward, 1993; Dippery *et al.*, 1995; Ward *et al.*, 2000; Sage & Coleman, 2001). Gene frequencies may change again with future CO$_2$ increases (Curtis *et al.*, 1994; Wayne & Bazzaz, 1995, 1997). Effects of rising atmospheric CO$_2$ 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 CO$_2$ on plant growth have reported a range of responses within individual species. For *Acer rubrum*, L. (red maple), approximately doubling modern CO$_2$ concentrations resulted in biomass increases ranging from 0%

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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. However, other studies have found differences in plant response to CO_2 due to genetic variation (Curtis et al., 1994; Wayne & Bazzaz, 1997; Ward et al., 2000). Unfortunately, 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 variation in germination, growth, biomass allocation, and survivorship of A. rubrum in response to four levels of CO_2: 180, 270, 360, and 600 $\mu L\,L^{-1}$. To our knowledge, this study is the first to examine genetic variation in tree responses to subambient CO_2 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 CO_2 response, we used a nested design with seeds collected from 26 maternal families, comprising 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 difference in germination of seeds from two families in response to elevated CO_2 (Grulke et al., 1993). The effects of increased CO_2 on plant survivorship are ambiguous, with studies utilizing ambient and elevated CO_2 concentrations finding both decreased (Bazzaz et al., 1995) and increased (Polley et al., 1999) survival. One study found no significant variation in CO_2 effects on survivorship among six families of honey mesquite (Prosopis glandulosa, Polley et al., 1999). Elevated CO_2 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 CO_2 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 CO_2 responses (DeLucia et al., 1994); variation among families was not considered.

Lack of regional differences in response to CO_2 is not surprising, as CO_2 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 congenic 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 germination, survivorship, root : shoot allocation, and growth responses to atmospheric CO_2 would be mostly contributed 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 CO_2.

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 increasingly abundant tree (Abrams, 1998) that may come to co-dominate eastern hardwood forests. Genetic variation 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*

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

*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.

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 $\sim 300\,\text{m}^2$ 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 *et al.*, 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 CO$_2$ concentrations was maintained in growth chambers at the Duke University Phytotron (Durham, NC, USA). Plants at each CO$_2$ treatment were grown in a single growth chamber; to minimize potential 'chamber effects', we rotated all plants and their respective CO$_2$ level every 10 (year 1) or 14 days (year 2) into new chambers. Excess CO$_2$ 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 CO$_2$ 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 $325\,\mu\text{mol m}^{-2}\,\text{s}^{-1}$ in year 1 and $750\,\mu\text{mol m}^{-2}\,\text{s}^{-1}$ 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 cm^2 germination trays. The seeds of each maternal family were randomly divided among the four CO$_2$ treatments of 180 µL L^{-1} (low periods of the Pleistocene), 270 µL L^{-1} (pre-Industrial Revolution), 360 µL L^{-1} (ambient CO$_2$ concentration at the initiation of the experiment), and 600 µL L^{-1} (potential future condition), and immediately transferred to the appropriate CO$_2$ 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 cm^3 '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 CO$_2$ 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 CO_2 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 CO_2 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 CO_2 level, were randomly selected and repotted for the second year of the experiment. Roots were not constricted around the sides of the cone-tainers and seedlings did not appear to be 'pot-bound' (Thomas & Strain, 1991). New pots were 7065 cm^3 sections of PVC piping with fiberglass screening fixed across the bottom. The trees were placed in larger growth chambers, each at the appropriate CO_2 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). CO_2 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 CO_2 and for region. CO_2 interaction terms were similarly tested against population interactions. To obtain the percent variation in response to CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2 concentrations from Pleistocene to projected future levels, CO_2 stimulated germination ($P < 0.0001$, Table 2 and Fig. 1). The impact of CO_2 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 CO_2 among the different regions ($P = 0.07$, Fig. 3). Differences among regions accounted for 4.7% of the variation in germination

Table 2 Results represent χ^2 tests from logistic regression analysis of the probability of germinating within 30 days*

$$\Pr(\text{Germinating}_{30\,\text{days}}) = (1 + e^X)^{-1}$$

$$X = \text{Region} + \text{Population(Region)} + \text{Family(Population)} + \text{CO}_2$$
$$+ [\text{CO}_2 \times \text{Region}] + [\text{CO}_2 \times \text{Population}] + [\text{CO}_2 \times \text{Family}]$$

Term	df	Pr(χ)
Region	2	**<0.0001**
Population (Region)	3	**<0.0001**
Family (Population)	20	**<0.0001**
CO$_2$	1	**<0.0001**
CO$_2$ × Region	2	0.07073
CO$_2$ × Population	3	**0.00065**
CO$_2$ × Family	20	**0.00731**

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.
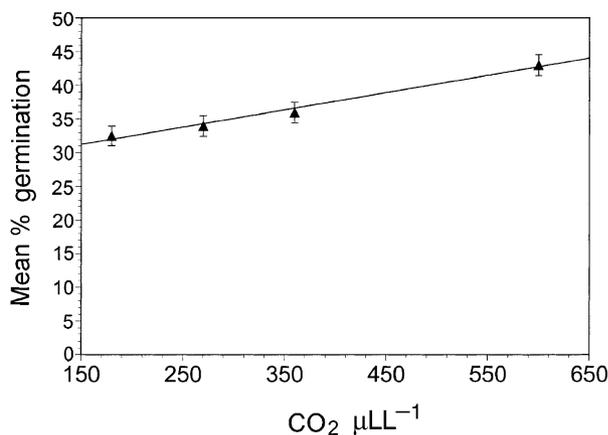
Fig. 1 Mean percentage of seeds germinated by 30 days at 180, 270, 360, and 600 $\mu\text{L}\,\text{L}^{-1}$ CO$_2$ concentrations. Error bars represent one standard error. $P = 0.006$ from logistic regression analysis of germination of individual seeds.

response to CO$_2$, 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

CO$_2$ 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 CO$_2$. Further, the effect of CO$_2$ on height

changed as the experiment progressed ($P = 0.0103$ for the CO$_2$ × Time interaction). In the first census, 1.3 months after planting, the 600 $\mu\text{L}\,\text{L}^{-1}$ trees were taller than those at other CO$_2$ levels ($P = 0.0002$). By the end of the second growing season, 14.5 months after planting, heights of the 600 $\mu\text{L}\,\text{L}^{-1}$ trees did not differ from individuals grown at 270 and 360 $\mu\text{L}\,\text{L}^{-1}$, but 180 $\mu\text{L}\,\text{L}^{-1}$ trees remained smaller than the rest ($P = 0.0010$; Fig. 4). Greater height of the elevated CO$_2$ trees was a short-term phenomenon in this experiment.

CO$_2$ × Region was the only CO$_2$ 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 biomass response to CO$_2$ (Table 3). Differences between populations within a region accounted for only 1.3% of the variation in response, and the CO$_2$ × 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 CO$_2$ was explained by differences among families; however, the CO$_2$ × Family interaction was not significant. Whereas both Coastal Plain and Mountain trees showed increasing biomass with CO$_2$ enrichment (Coastal Plain, $P < 0.0001$; Mountains, $P = 0.04$), trees from the Piedmont exhibited no effect of CO$_2$ on final biomass (Fig. 5). In contrast to the results for total biomass, only trees from the Piedmont region exhibited CO$_2$ impacts on root:shoot allocation, allotting more biomass towards roots as CO$_2$ 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
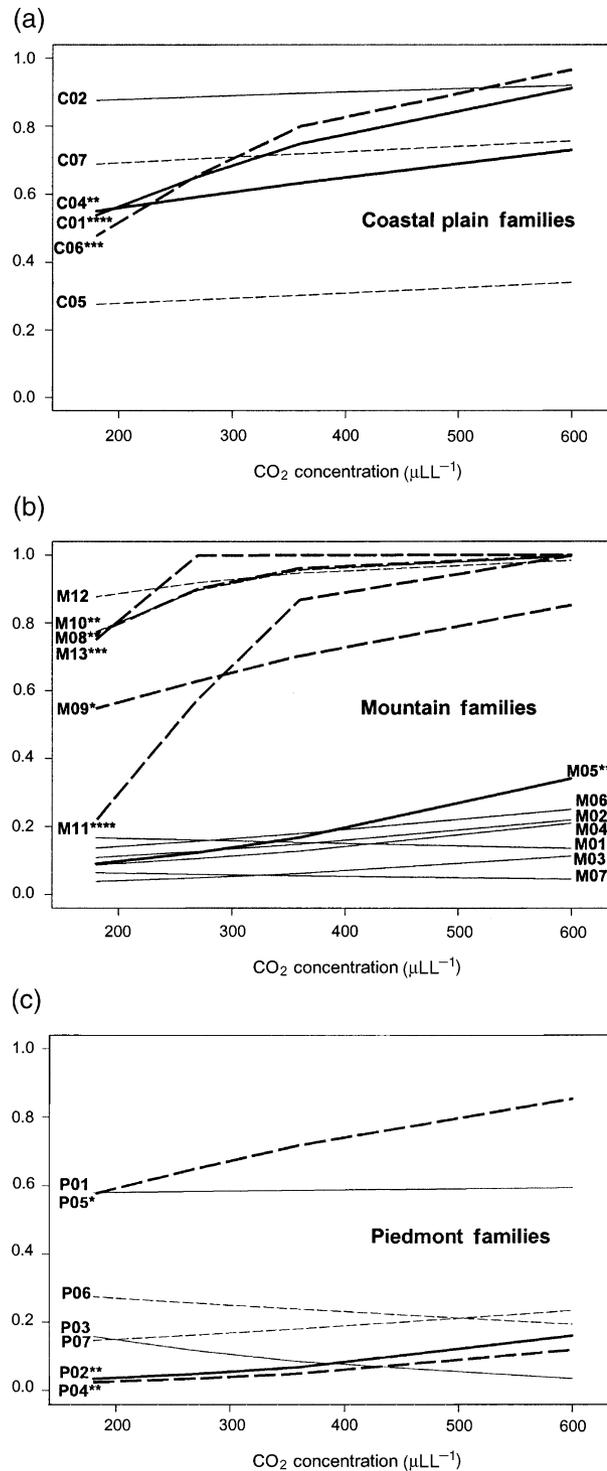
Fig. 2 (a–c) Logistic regression fits of the probability of germinating within 30 days (*y*-axis) as a function of atmospheric CO_2 carried out separately for each family originating from the (a) Coastal Plain (C01–C07), (b) Mountains (M01–M13), and (c) Piedmont (P01–P07). Dark lines depict families exhibiting significant responses to CO_2; 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 CO_2 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 CO_2.
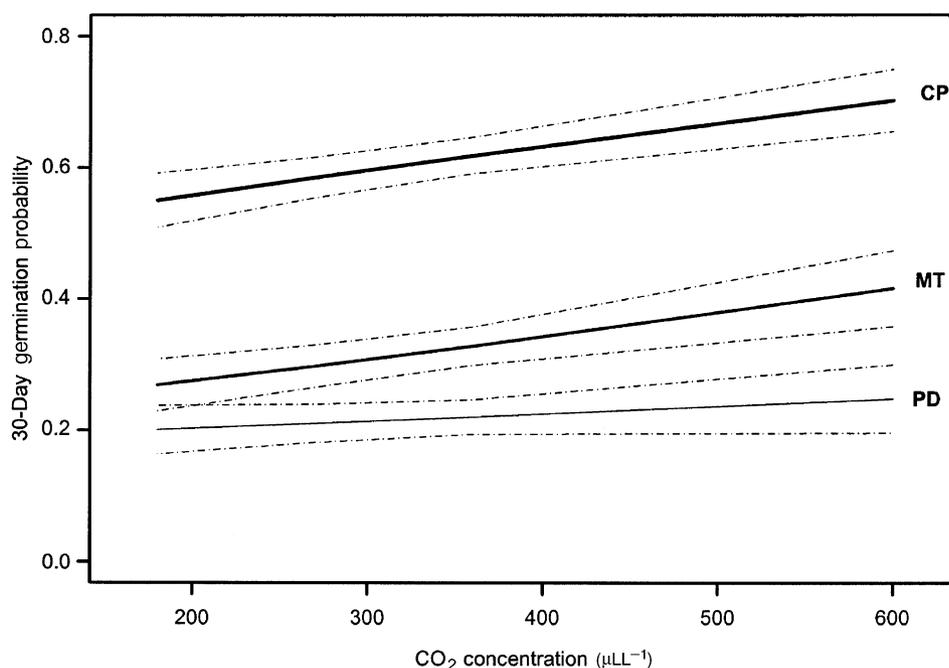
Fig. 3 Logistic regression fits (solid lines) and 95% confidence intervals (dashed lines) of the probability of germinating by 30 days as a function of CO$_2$ and Region of origin. Seeds collected from Coastal Plain ('CP') and Mountain ('MT') regions exhibit a positive effect of CO$_2$ on germination probability ($P < 0.0001$ and $P = 0.0002$, respectively), while seeds from the Piedmont ('PD') show no overall response to atmospheric CO$_2$.

Table 3 Percent of the variation in response to CO$_2$ that is explained by three levels of relatedness*

Response	Region (%)	Population (%)	Family (%)
Germination	4.7	11	39
Biomass	23	1.3	29
Mortality, year 1	23	13	58
Mortality, year 2	14	14	63

*The three percentages (Region, Population, and Family interactions with CO$_2$) for each response not summing to 100% reflects the variation due to the main effect of CO$_2$.

($P < 0.0001$, Table 5, Fig. 6a). After considering the effects of initial height, CO$_2$ had a negative impact on survivorship ($P = 0.07$) and all three levels of relatedness – regions, populations, and families – interacted with CO$_2$ and height to explain the probability of dying in the first year ($P = 0.0191$, 0.0013, and 0.0638, respectively; Fig. 6b). Differences among regions accounted for 23% of the variation in response to CO$_2$, 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 CO$_2$ depended on the families, populations, and regions of origin.

In the second year, CO$_2$ had an overall positive impact on survival (Fig. 6c) and the 40-day height remained a determinant of death ($P < 0.001$, Table 5). Small trees growing at 180–360 $\mu L\,L^{-1}$ exhibited declining mortality with increasing CO$_2$; this trend was reversed when comparing 360 and 600 $\mu L\,L^{-1}$ individuals (Fig. 6d). Initial seedling height also interacted with CO$_2$ 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 CO$_2$, 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 CO$_2$. 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 CO$_2$ differed by region, with 50% of Coastal Plain families showing positive CO$_2$ effects for two or more of these responses; Mountain families, 31%; and Piedmont

Table 4 Summary table of P-values from nested ANOVAs of growth responses to CO_2*

Independent variables in ANOVA	Response variables		
	Height	Final biomass	Final root : shoot ratio
ln final biomass	–	–	**<0.0001**
Region	0.5642	**0.0418**	0.3140
Population (within Region)	**0.0075**	**0.0091**	0.2751
Family (within Population)	0.0611	**0.0138**	0.3948
CO_2	**0.0002**	**0.0018**	**0.0008**
Time	**0.0002**	–	–
Region × Time	0.0522	–	–
Population (within Region) × Time	**0.0029**	–	–
Family (within Population) × Time	0.9473	–	–
CO_2 × Time	**0.0103**	–	–
CO_2 × Region	0.6005	**0.0125**	**0.0022**
CO_2 × Population (within Region)	**0.0260**	0.8617	0.9951
CO_2 × Family (within Population)	0.9861	0.9361	**<0.0001**
CO_2 × Region × Time	**0.0157**	–	–
CO_2 × Population (within Region) × Time	0.8307	–	–
CO_2 × Family (within Population) × Time	**0.0005**	–	–

*'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.
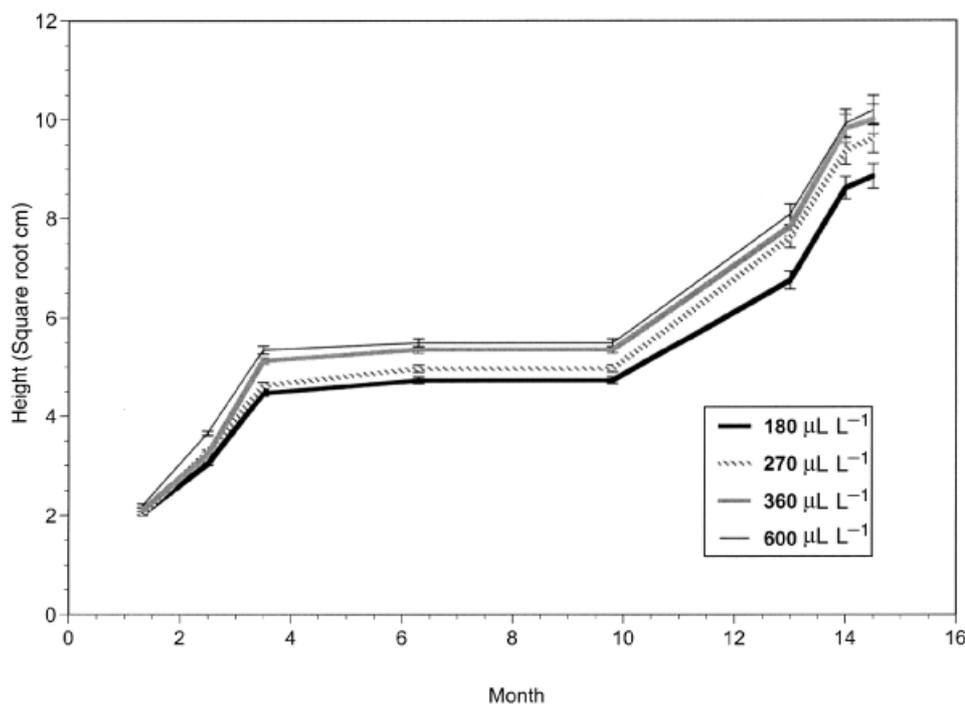
Fig. 4 CO_2 interacts significantly with Time to explain height growth in *A. rubrum* ($P = 0.01$ from repeated-measures nested ANOVA). The mean values of square-root transformed height for each CO_2 group at each of the eight height censes are shown. Error bars represent ± 1 SE. Note that the dormant measurements (months = 6.3 and 9.8) were not included in the analysis.
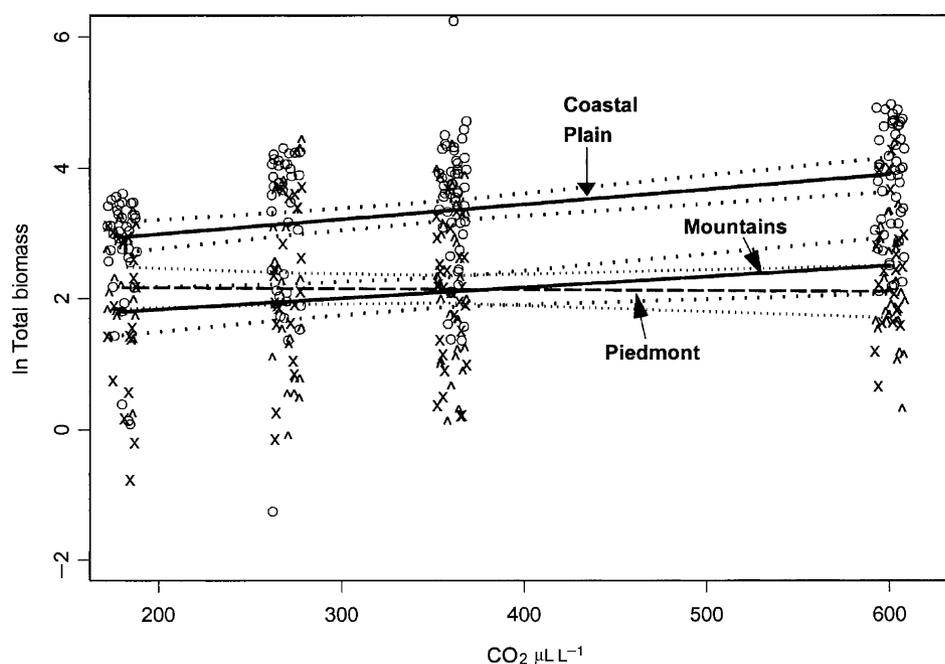
Fig. 5 Linear regression fits (solid lines for Coastal Plain and Mountains; dashed line for Piedmont) and 95% confidence intervals (dotted lines) for the ln-transformed total biomass of plants in each Region vs. atmospheric CO_2. Points represent individual trees. Coastal Plain ('o') and Mountain ('x') trees exhibit increasing biomass with increasing CO_2 (CP: $P < 0.001$ and $N = 168$, MT: $P = 0.035$ and $N = 87$). Piedmont trees ('^') show no effect of CO_2 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 $270 \, \mu L \, L^{-1}$ and $360 \, \mu L \, L^{-1}$, respectively, or the smallest Mountain tree at $180 \, \mu L \, L^{-1}$.

Table 5 Summary table presenting P-values from χ^2 tests in logistic regression analyses

	Mortality probability, year 1	Mortality probability, year 2
Initial height	**< 0.0001**	**< 0.0001**
Region	**0.0810**	**0.0032**
Population within Region	0.1595	0.7155
Family within Population	**0.0428**	0.4889
CO_2	0.0734	**0.0122**
$CO_2 \times$ Height	0.9363	0.3061
$CO_2 \times$ Region	0.2512	**0.0289**
$CO_2 \times$ Population	0.2307	**0.0122**
$CO_2 \times$ Family	0.5155	**0.0012**
Height $\times CO_2 \times$ Region	**0.0191**	**0.0012**
Height $\times CO_2 \times$ Population	**0.0013**	0.0660
Height $\times CO_2 \times$ Family	0.0638	0.1809

families, 14% (Table 6). As global CO_2 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 CO_2 response expressed within the different levels of relatedness could alter future plant responses to rising atmospheric CO_2, 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 CO_2 conditions will have greater opportunities to establish in favorable microsites (Harper, 1977; Clark *et al.*, 1999) and to exploit canopy gaps (Wayne &
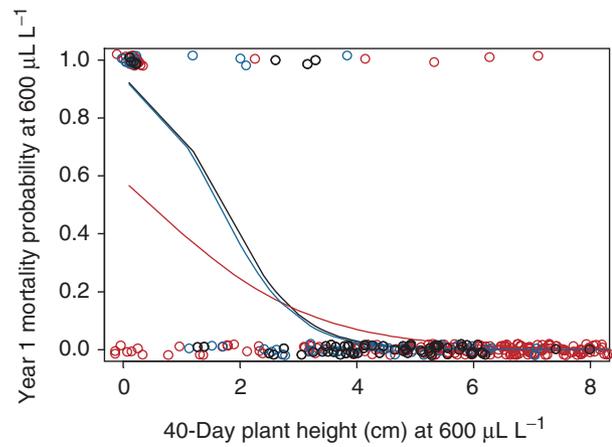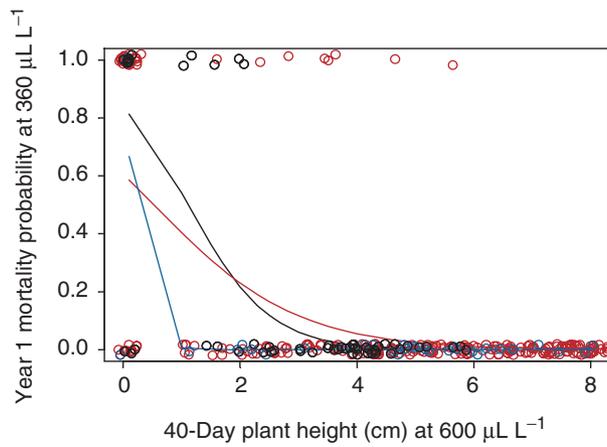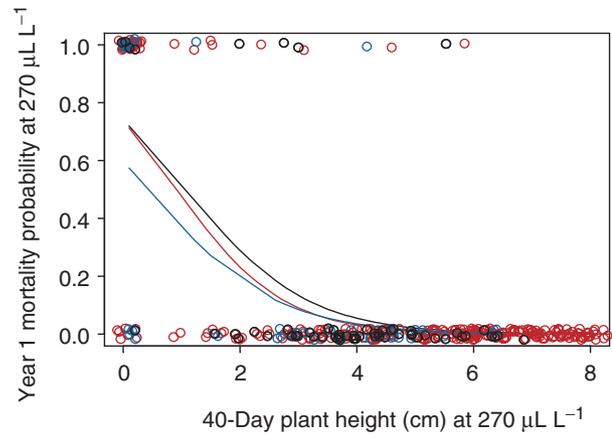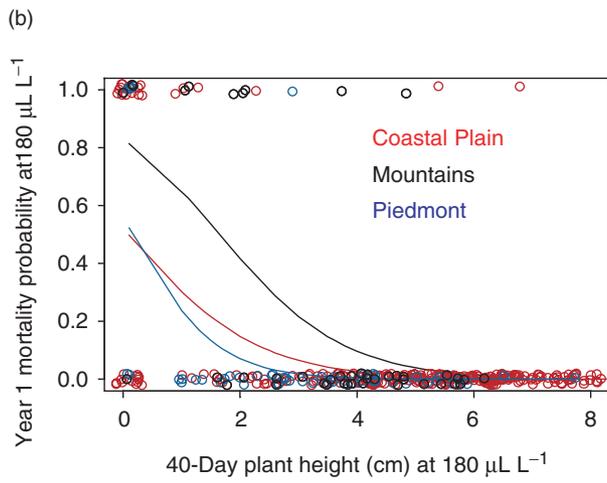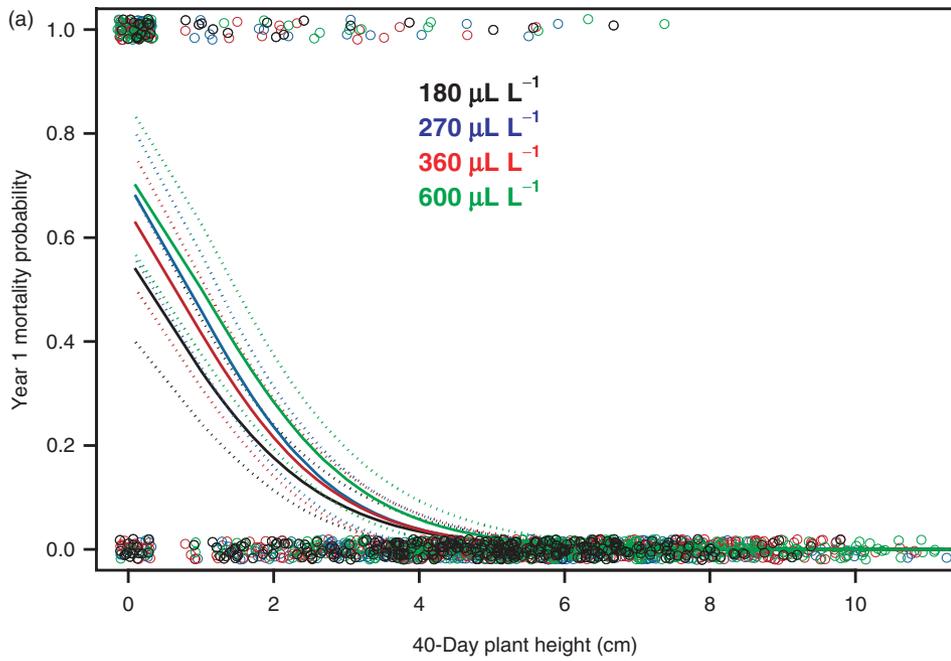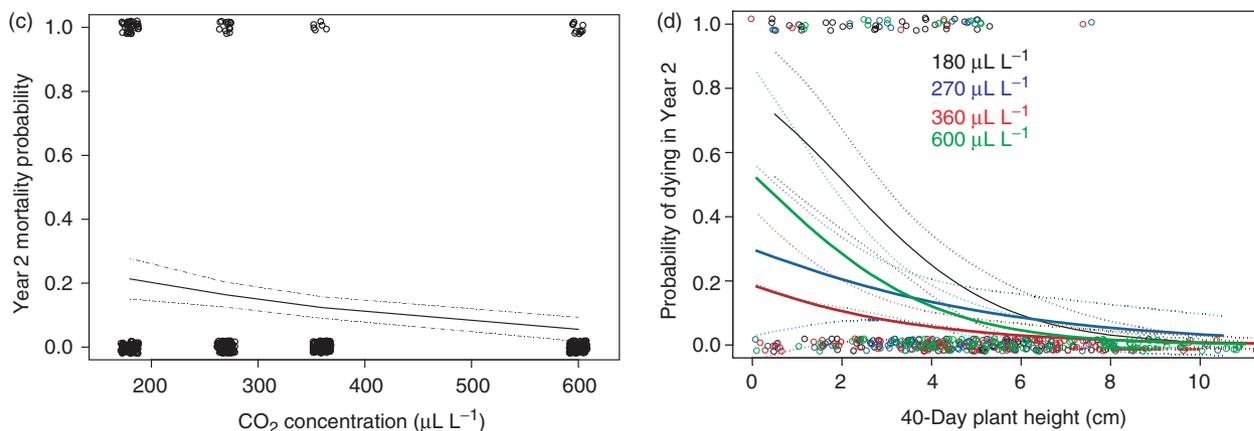
Fig. 6 (a) Logistic regression fits (solid lines) and 95% confidence intervals (dotted lines) of first-year mortality probabilities for trees growing at CO$_2$ concentrations of 180 μL L^{-1} (black), 270 μL L^{-1} (blue), 360 μL L^{-1} (red), and 600 μL L^{-1} (green). Points represent individual trees. Seeds with a live, emerged radicle but without an emerged shoot were analyzed with an initial height of zero. The binary mortality data equal either zero ('live') or one ('dead'), but are portrayed with a small amount of vertical and horizontal heterogeneity to depict individual points. (b) Logistic regression fits of first-year mortality probabilities plotted separately for 180, 270, 360, and 600 μL L^{-1} CO$_2$ treatments of trees originating from the Coastal Plain (red), Mountains (black), and the Piedmont (blue). Points represent individual trees. Seeds with a live, emerged radicle but without an emerged shoot were analyzed with an initial height of zero. The binary mortality data equal either zero ('live') or one ('dead'), but are portrayed with a small amount of vertical and horizontal heterogeneity to depict individual points. (c) Logistic regression fit (solid line) and 95% confidence intervals (dotted lines) for mortality probability in the second year as a function solely of atmospheric CO$_2$ ($P = 0.0003$). Points represent individual trees. The binary data equal either zero ('live') or one ('dead'), but are portrayed with a small amount of horizontal and vertical heterogeneity to depict individual points. Note that when subambient-CO$_2$ data are excluded from the analysis, there is no significant difference between 360 and 600 μL L^{-1} trees. (d) Logistic regression fits (solid lines) and 95% confidence intervals (dotted lines) of second-year mortality as a function of the 40-day ('initial') plant height, plotted separately for each CO$_2$ treatment. Seeds with a live, emerged radicle but without an emerged shoot were analyzed with an initial height of zero. Points represent individual trees. 180 μL L^{-1} trees (black) show the strongest response ($P = 0.000005$) followed by 600 μL L^{-1} trees (green, $P = 0.001$). Trees growing under 270 μL L^{-1} (blue) and 360 μL L^{-1} (red) exhibit only marginal responses ($P = 0.09$ and 0.07, respectively). The binary mortality data equal either zero ('live') or one ('dead'), but are portrayed with a small amount of horizontal and vertical heterogeneity to depict individual points.

Bazzaz, 1995, 1997). In accordance with our hypothesis, the impact of CO$_2$ 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 CO$_2$. Contrary to our expectation, seeds from different regions also had different germination responses to CO$_2$. While seeds collected from the Coastal Plain and Mountains showed higher germination probabilities with increasing CO$_2$ (Fig. 3), seeds from the Piedmont exhibited no overall effect of CO$_2$ on germination. Regional differences in the effect of CO$_2$ 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 CO$_2$ 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 CO$_2$ 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 CO$_2$ effects on percent germination or germination rate when comparing seeds at 350 and 700 μL L^{-1} (Grulke *et al.*, 1993). In the present study, high CO$_2$ had an overall positive impact on the germination of red maple. Increased CO$_2$ may interact with or even increase the production of ethylene, a plant growth regulator that stimulates germination (Kozlowski & Pallardy, 1997). The CO$_2$ 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 CO$_2$ level was below the CO$_2$ concentrations often measured in field soils, resulting from microbial decomposition of organic matter (Amundson & Davidson, 1990; Andrews &

Table 6 Families exhibiting positive germination, growth, and/or survivorship responses to CO_2 concentrations increasing from 180 to 600 $\mu L\,L^{-1\dagger}$

Family	Population	Germination	Height growth	Survivorship
C1	Coastal Plain 1	+ ****	+ ****	+ *
C2		–	+ ****	–
C4		+ **	+ ****	–
C5	Coastal Plain 2	–	+ ****	–
C6		+ ***	+ ****	+ *
C7		–	+ ***	–
M1	Mountain 1	–	–	–
M2		–	+ *	–
M3		–	–	–
M4		–	–	–
M5		+ **	–	–
M6		–	–	–
M7		–	–	–
M8	Mountain 2	+ **	+ ***	+ ***
M9		+ *	–	–
M10		+ **	–	+ **
M11		–	–	–
M12		–	+ *	+ *
M13		+ ***	+ ***	–
P1	Piedmont 1	–	–	–
P2		+ **	–	–
P3		–	–	–
P4	Piedmont 2	+ **	–	–
P5		+ *	–	+ **
P6		–	–	+ ***
P7		–	–	+ ***

*Indicates that CO_2 response has a $P < 0.10$,

**denotes $P < 0.05$,

***signifies $P < 0.01$, and

****means $P < 0.001$.

– indicates no significant CO_2 response.

†No families exhibited significant negative responses.

Schlesinger, 2001). It is possible that the positive CO_2 impacts on germination observed in this and in other studies are due to seeds not experiencing the high-CO_2 conditions of soils. The effects of CO_2 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 CO_2 (Table 4); these results agreed with our hypothesis and with previous studies (Conroy *et al.*, 1990; Grulke *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 CO_2 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 CO_2; previous studies did not lead us to expect this result. Future studies of height response to CO_2 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 CO_2 is also evident from the final biomass results, with the $CO_2 \times$ 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 CO_2 partial pressures (Billings *et al.*, 1961; Körner & Diemer, 1987), it might be anticipated

that Coastal Plain (7 m above-sea-level) and Mountain (>800 m asl) trees would respond differently to atmospheric CO$_2$, 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 CO$_2$. Regional differences also seem to be more than a simple maternal effect, in which only the largest seedlings benefit from high CO$_2$. 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 CO$_2$ response of the larger trees may have suppressed the response of smaller trees (McDonald et al., 2002). In nature, CO$_2$ 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 CO$_2$ plants), but this largely derives from the presence of Coastal Plain trees. Future dynamics of A. rubrum will be impacted by atmospheric CO$_2$ through differential effects on growth, and studies designed to understand growth responses to CO$_2$ 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, CO$_2$ 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} CO$_2$ ($P < 0.0001$). Previous work on herbaceous plants observed that the subambient CO$_2$ conditions of the Pleistocene resulted in high mortality of C$_3$ seedlings (Ward, 1997). The present study suggests that these low CO$_2$ 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 CO$_2$ continued through 360 μL L^{-1}, but maples with initially smaller heights had greater chances of dying at elevated than at ambient CO$_2$ ($P = 0.028$ for the CO$_2$ × 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-CO$_2$ world.

Implications for future competitiveness and range shifts

In addition to its direct impacts on fitness, elevated CO$_2$ 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 (Payette & Filion, 1985; Parmesan et al., 1999; Thomas & Lennon, 1999; Warren et al., 2001; Hill et al., 2002). Yet migration dynamics may be impacted by CO$_2$. We found that Coastal Plain trees from the warmest region in the study showed enhanced germination, growth, and relative survivorship under the high CO$_2$ conditions of the future. Conversely, Piedmont individuals adapted to warmer temperatures were relatively smaller than cooler-temperature Mountain seedlings under future CO$_2$ levels (Fig. 5), and no longer exhibited the survival advantage experienced under ambient CO$_2$ (Fig. 6b). If genotypes from warmer regions have reduced fitness under elevated CO$_2$, future migrations by plants adapted to higher temperatures may be delayed. Direct impacts of enhanced CO$_2$ 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 CO$_2$ 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 CO$_2$. Seeds from different populations within a physiographic province have different germination and survival responses to CO$_2$. Contrary to our expectation, seeds and seedlings originating from different regions also exhibit different germination, growth, and survivorship responses to CO$_2$.

In this study, red maple typically germinates better and grows larger with increasing atmospheric CO$_2$,

implying that future productivity may be higher than it is today. Genetic variation in CO_2 responses and the rapid increase in global CO_2 concentration suggest the potential for adaptive evolution of this species, which may further enhance productivity with CO_2 enrichment. Genetic variation in red maple's responses to CO_2 bears implications for the future competitiveness and invasive potential of this increasingly abundant tree.

Acknowledgements

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