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Genotypic variation in response of quaking aspen (*Populus tremuloides*) to atmospheric CO₂ enrichment

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Abstract Enriched atmospheric CO₂ alters the quantity and quality of plant production, but how such effects vary among plant genotypes is poorly known. We evaluated the independent and interactive effects of CO₂ and nutrient availability on growth, allocation and phytochemistry of six aspen (*Populus tremuloides* Michx.) genotypes. One-year-old trees, propagated from root cuttings, were grown in CO₂-controlled glasshouses for 64 days, then harvested. Foliage was analyzed for levels of water, nitrogen, starch, phenolic glycosides and condensed tannins. Of seven plant growth/allocation variables measured, four (biomass production, stem growth, relative growth rate and root:shoot ratio) exhibited marginally to highly significant CO₂ × genotype interactions. CO₂ enrichment stimulated growth of some genotypes more than others, and this interaction was itself influenced by soil nutrient availability. In addition, enriched CO₂ increased the magnitude of the among-genotype variance for four of the growth/allocation variables. Of six foliar chemical constituents analyzed, CO₂-mediated responses of two (the phenolic glycoside tremulacin and condensed tannins) varied among genotypes. Moreover, enriched CO₂ increased the magnitude of among-genotype variance for four of the chemical variables. Given the importance of these growth and chemical characteristics to the biological fitness of aspen, this research suggests that projected atmospheric CO₂ increases are likely to alter the genetic structures and evolutionary trajectories of aspen populations.

Keywords Aspen · Elevated CO₂ · Genetic variation · *Populus tremuloides* · Primary production

Introduction

Over the last decade, research on the impact of enriched atmospheric CO₂ on plants has expanded at an exponential rate. Such work has emphasized evaluation of the effects of CO₂ at the extremes of the ecological continuum. At the physiological level, research has focused on metabolic processes and growth of individual plants; at the ecosystem level, research has focused on carbon cycling and sequestration. Comparatively less attention has been directed toward the effects of CO₂ enrichment on population- and community-level processes and organization.

This is particularly true of evolutionary responses of plants to elevated CO₂ (Geber and Dawson 1993; Bazzaz et al. 1995; Ward and Strain 1999). Evolutionary effects will be determined by the magnitude of genetic variation in a population, and by the response of fitness-related traits to environmental change (i.e., strength of the selective force). Shifts in the genetic structure of populations may arise directly, due to differential effects of CO₂ on plant productivity. Enriched CO₂ tends to enhance plant growth and reproduction (Kimball 1983; Ceulemans and Mousseau 1994; Saxe et al. 1998), although little is known about intraspecific variation in such responses (Curtis et al. 1994). Alternatively, shifts in genetic structure may arise indirectly, due to differential effects of CO₂ on exploitative or competitive interactions (Bazzaz et al. 1995). Enriched CO₂ can alter the defensive (Lindroth 1996; Peñuelas and Estiarte 1998) or competitive (Owensby et al. 1993; Potvin and Vasseur 1997) capacities of plants, but again, little information exists with respect to potential intraspecific variation in responses.

An important, albeit preliminary, step toward ascertaining the potential for evolutionary adaptation is to evaluate genetic variation in plant responses to enriched CO₂. Quaking aspen (*Populus tremuloides*) is an ideal

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experimental organism for such research. This clonal species is arguably the most genetically variable plant species known to science (Mitton and Grant 1996). Striking variation exists among clones with respect to growth rates and foliar secondary chemistry (Lindroth and Hwang 1996a, b; Hwang and Lindroth 1997; Osier and Lindroth, unpublished data). Moreover, CO₂ enrichment can accelerate the growth of aspen saplings (Lindroth et al. 1993; Kinney and Lindroth 1997a) and alter the concentrations of various foliar phenolics (Lindroth et al. 1993; Roth and Lindroth 1995; Roth et al. 1998). Several of these phenolic constituents play pivotal roles in mediating interactions between aspen and its herbivores and pathogens (Lindroth and Hwang 1996a). CO₂-mediated changes in the foliar chemistry of early- to mid-season foliage are of particular interest, as this is the period of feeding by major outbreak folivores, such as gypsy moths (*Lymantria dispar*) and forest tent caterpillars (*Malacosoma disstria*).

The purpose of the research reported here was to address two complementary aspects of genetic variation in responses of aspen saplings to elevated atmospheric CO₂. To add to the generality of our results, we conducted our experiment with two levels of nutrient availability, as soil fertility is known to modulate the effects of CO₂ on aspen (Kinney and Lindroth 1997; Kinney et al. 1997; Kubiske et al. 1998). Accordingly, our first objective was to assess whether various genotypes respond differently to CO₂ enrichment (i.e., do genotype × CO₂ interactions occur?). Our second objective was to determine whether the magnitude of among-genotype variance differs between CO₂ environments. We predicted that for growth, variation among genotypes would be higher in enriched CO₂ environments, especially under conditions of non-limiting nutrient availability. We also predicted that for accumulation of carbon-based storage and defense compounds, concentrations as well as variation among genotypes would be greater under enriched CO₂, especially under conditions of low nutrient availability. The latter prediction derives from carbon-nutrient balance theory (Bryant et al. 1983), which postulates that levels of carbon-based storage and secondary compounds increase in plants under environmental conditions in which growth is inhibited more than is photosynthesis.

Materials and methods

Experimental design and set-up

This research was conducted at the University of Wisconsin Biotron from April to July 1997. Due to the complexity of the study, our interest in mid-season foliar quality, and the availability of growth rooms, we were constrained to an experimental period of several months. Given, however, that aspen exhibits 3- to 15-fold growth during this period (depending on genotype and resource availability), and that phytochemical responses to CO₂ are similar to those of trees grown under CO₂ for several seasons (Roth et al. 1998), we deemed this of sufficient duration to detect potential CO₂ × genotype interactions.

We used a split-split-plot experimental design, with CO₂ level (ambient and elevated) as the whole plot treatment, soil nutrient level (low and high) as the subplot treatment, and aspen genotype as the sub-subplot treatment. CO₂ concentrations were monitored in eight 4×5 m (floor area) glasshouse rooms, with four rooms set at ambient concentration (measured as 393 ppm during the study) and four rooms at an elevated concentration (700 ppm). Within each room, one-half the area was randomly selected for placement of non-fertilized trees, and the remaining half for fertilized trees. Within each half of each room, we grew 2–3 trees of each of six aspen genotypes.

Glasshouse thermal regimes were set on a 15:9 h schedule, with temperatures of 25°C and 18°C for day and night, respectively. Artificial lighting (high pressure sodium vapor lamps) was added to supplement natural lighting (photophase only) during the first 28 days of the experiment. Average light level (photosynthetically active radiation) at solar noon was 963±98 (SE) μmol m⁻² s⁻¹, at 20 cm above the plant canopy. From day 29 onward, rooms received only natural light.

The aspen genotypes selected for this study had previously been shown to exhibit substantial variation in allocation of carbon to growth versus chemical defense (Hwang and Lindroth 1997). Six genotypes were vegetatively propagated from root stock in spring 1996. Source root material came from trees grown in a common garden; these trees had been propagated several years earlier from field-collected root stock (five from aspen clones growing in south-central Wisconsin and one from a clone in north-central Colorado, USA). After 6–9 months of growth, trees were allowed to go dormant and placed in cold storage (bare-rooted in sphagnum moss) for the winter. In April 1997, trees were planted in 16 l pots containing a mixture of 70% sand and 30% silt-loam topsoil. Slow-release fertilizer (5.3 g/l of 19:6:12 NPK, 3–4 months release rate) was added to half of the pots, and the trees distributed among glasshouse rooms. Trees were watered with an automatic irrigation system at the beginning, and 4 h before the end, of the photophase.

Growth measurements

Prior to planting, we recorded stem length (root collar to apical bud) and mass of each tree. A subsample (8–10) of trees from each clone was then oven-dried (60°C) and weighed, for determination of fresh:dry mass ratios. These ratios were then used to calculate initial dry masses of experimental trees. Remaining trees were divided randomly among the CO₂ and nutrient treatments, and planted as described previously. Day 0 was determined as the date when 50% of the trees had broken bud. Budbreak was synchronous among clones and experimental treatments.

Leaves used for measurements of physical and chemical characteristics were harvested on days 61–62 of the study. We collected 15 leaves randomly from the canopy of each tree. Leaves were weighed, and leaf areas measured with a Li-Cor 3100 leaf area meter. Leaves were then flash-frozen in liquid nitrogen, freeze-dried, ground (40 mesh), and stored at –20°C until chemically analyzed.

Whole trees were harvested on day 64 of the study. We recorded stem height and total (fresh) leaf mass and number. Roots were washed, then all components were oven-dried (60°C) for 10 days and weighed. Relative growth rate (RGR) was calculated for individual trees as $[\ln(\text{harvest dry mass}) - \ln(\text{initial dry mass})] \times (64 \text{ days})^{-1}$. Leaf mass ratio was calculated as $(\text{dry leaf mass}) \times (\text{total dry biomass})^{-1}$. Root:shoot ratio was calculated as $(\text{root dry mass}) \times (\text{leaf dry mass} + \text{stem dry mass})^{-1}$. Dry masses of leaves collected for physical/chemical analyses were included in our calculations of plant growth and allocation variables.

Foliar chemistry

We quantified levels of phytochemicals that are known to influence interactions of aspen with other organisms [e.g., herbivores

various response variables measured, so the sample cannot be viewed as truly random. Also, the chi-square distribution must be viewed as approximate. Thus, results from analyses comparing the magnitude of variance among clones, across treatments, should be interpreted with caution. Moreover, in these analyses we did not correct the plant growth data for initial mass because we desired to explore variability in the "realized" values. Because variance analyses were conducted separately for each nutrient level, and because nutrient level was the major factor contributing to differential covariate effects, the impact of not correcting for initial mass is minimal.

Results

Growth and allocation

CO₂, nutrient availability, and aspen genotype all influenced the growth of aspen saplings, but the magnitude of effect varied among the different growth variables. Biomass production (total growth) was at most marginally affected by CO₂ concentration (CO₂ × genotype interaction), and strongly affected by nutrient level and genotype (Fig. 1, Table 1). Biomass production averaged 2.7-fold greater for high nutrient plants compared with low nutrient plants. The effects of initial tree size on biomass production were more pronounced under high nutrient conditions than under low nutrient conditions (initial mass covariate × nutrient interaction). The likelihood ratio test revealed that the variance among genotypes tended to be greater at high CO₂ concentrations than at low CO₂ concentrations for low nutrient plants, but not for high nutrient plants. Stem growth of aspen trees differed among nutrient treatments and genotypes (Fig. 1, Table 1). Stem growth responded to enriched CO₂, but differently so in different genotypes (CO₂ × genotype interaction). Moreover, growth of some clones tended to decrease under high CO₂ at low nutrient availability, but increase under high CO₂ at high nutrient availability (CO₂ × nutrient × genotype interaction). The magnitudes of the variance among genotypes did not differ between CO₂ treatments. Consistent with results for stem growth, RGRs of trees responded strongly to nutrient availability and differed among genotypes (Fig. 1, Table 1). CO₂ enrichment accelerated growth in some, but not all, genotypes (CO₂ × genotype interaction). Similarly, nutrient availability had a stronger impact on some clones than on others (nutrient × genotype interaction). As for biomass production, initial tree mass influenced RGRs more strongly at high than at low nutrient levels (covariate × nutrient interaction). Overall, the variance for RGRs among genotypes did not differ between CO₂ treatments. CO₂ enrichment did not significantly alter total leaf mass in any of the aspen genotypes (Fig. 1, Table 1). The variance among genotypes, however, was 2.2-fold greater for CO₂-enriched trees than for ambient trees, in the low nutrient regime. On average, leaf mass increased 3.4-fold in high nutrient, compared with low nutrient, trees, and the effect of initial tree size on final leaf mass was greater under high nutrient conditions. Nutrient availability altered leaf mass in some genotypes more strongly than

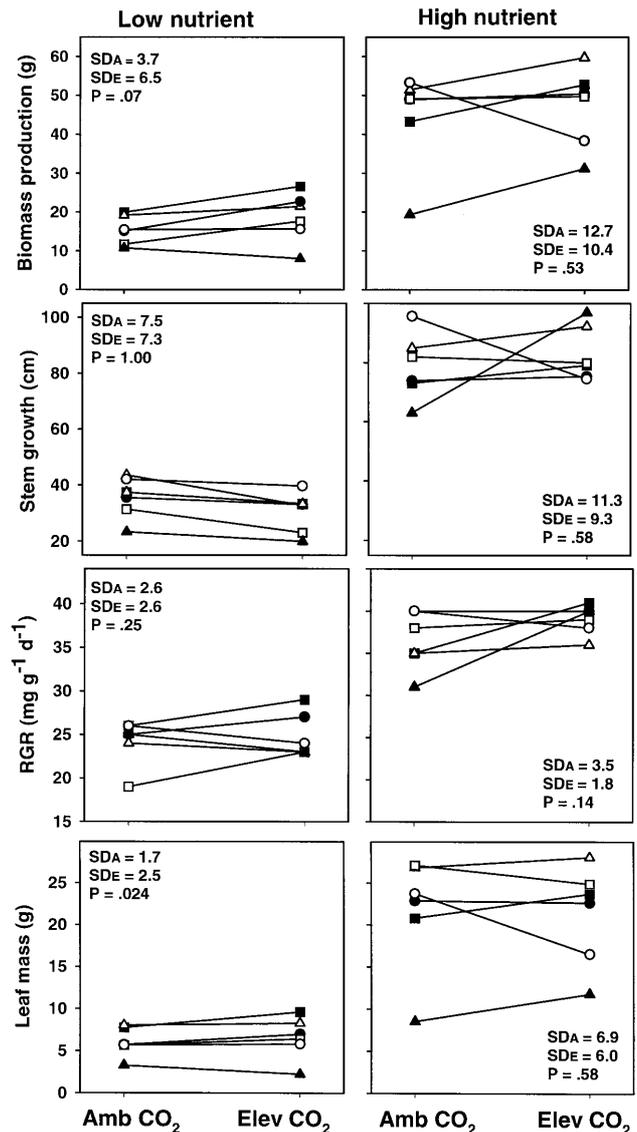


Fig. 1 Norms of reaction for growth of aspen in response to CO₂ and nutrient availability. Each line represents a single clone; each point represents an average of 12 trees for four of the clones (4 rooms, 3 trees/room), and 8 trees for the remaining two clones (4 rooms, 2 trees/room). SD_A and SD_E represent the standard deviation among genotypes at ambient and elevated CO₂, respectively. P values show the level of significance associated with the likelihood ratio test for equality of the among-genotype variances between CO₂ treatments

in others, such that genetic variation was greater under high nutrient conditions than under low nutrient conditions (nutrient × genotype interaction).

CO₂, nutrient availability, and genotype also affected biomass allocation in aspen saplings. Leaf mass per unit area increased an average of 13% in high CO₂, decreased an average of 32% with high nutrient availability, and varied among genotypes (Fig. 2, Table 1). Elevated CO₂ increased the among-genotype variance for trees in the high nutrient treatment. Leaf mass ratios (the proportion of total plant mass consisting of leaves) were unaffected by CO₂ environment (Fig. 2, Table 1). They varied, how-

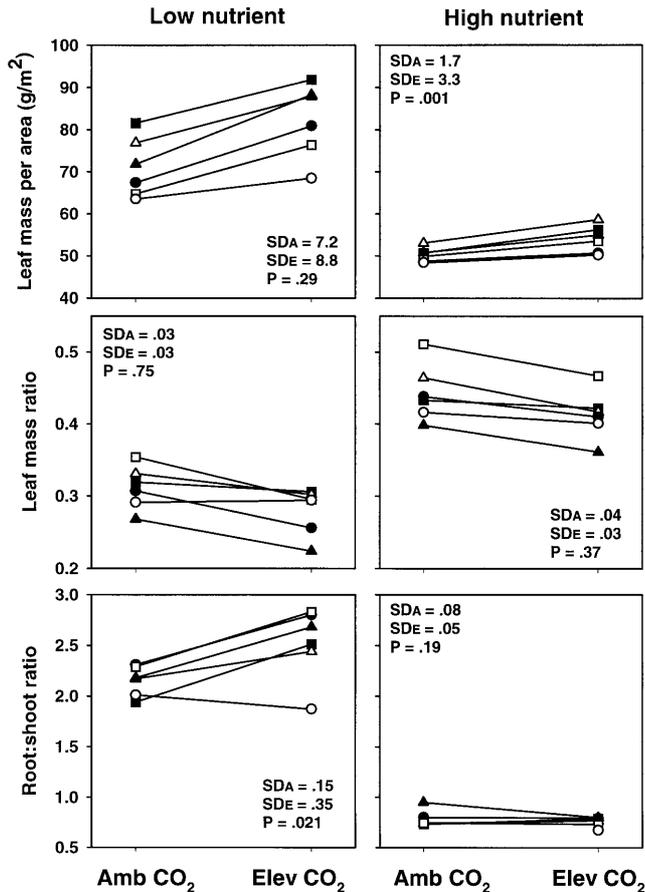


Fig. 2 Norms of reaction for leaf mass per unit area and biomass allocation of aspen genotypes in response to CO₂ and nutrient availability. Format of figure is as described for Fig. 1

ever, among genotypes, especially under high nutrient conditions (nutrient \times genotype interaction). The effect of initial tree size on leaf mass ratios was influenced by both nutrient treatment and genotype (covariate \times nutrient and covariate \times genotype interactions). Root:shoot ratios increased with high CO₂ in low nutrient trees (all but one genotype), but were unaffected by CO₂ in high nutrient trees (CO₂ \times nutrient and CO₂ \times nutrient \times genotype interactions; Fig. 2, Table 1). Enriched CO₂ also increased the variance among genotypes (5.4-fold) in low nutrient trees, but did not affect variance in high nutrient trees. Root:shoot ratios declined markedly with high nutrient availability and differed among genotypes, especially at low nutrient levels (nutrient \times genotype interaction). The effect of initial tree size on root:shoot ratios was influenced by a complex set of interactions with CO₂, nutrient availability and genotype.

Foliar chemistry

CO₂, nutrient availability, and genotype influenced levels of both primary and secondary metabolites in aspen leaves. Water concentrations were not affected by CO₂, but differed by an average of 10.6% (fresh leaf mass) be-

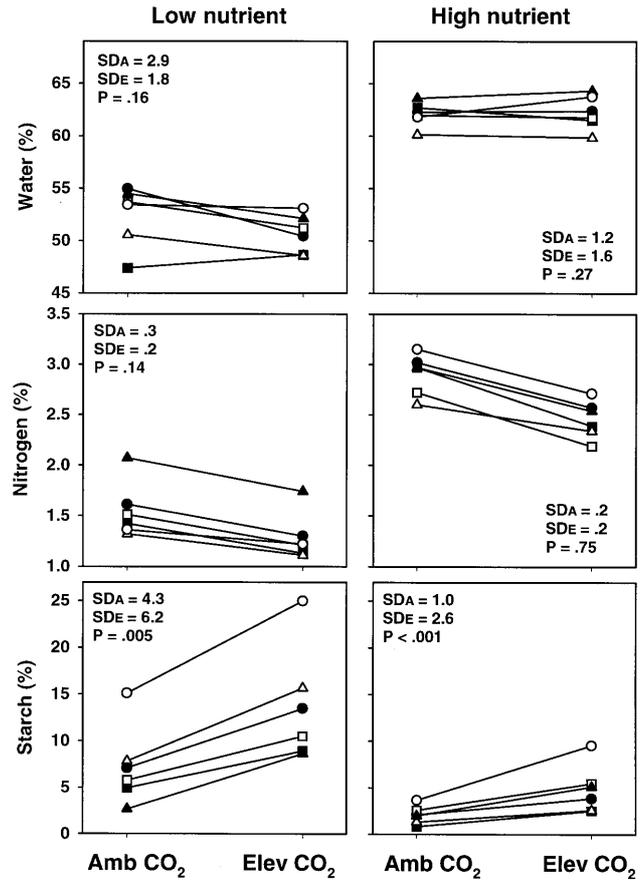


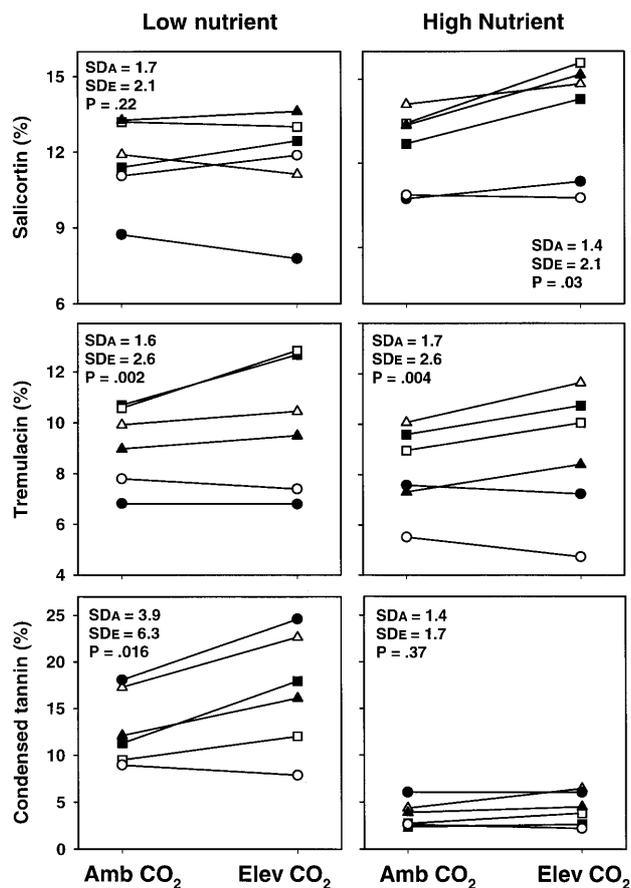
Fig. 3 Norms of reaction for foliar water, nitrogen and starch concentrations of aspen genotypes in response to CO₂ and nutrient availability. Values for water are percent fresh mass; for nitrogen and starch are percent dry mass. Format of figure is as described for Fig. 1

tween nutrient treatments and varied among genotypes (Fig. 3, Table 2). Nitrogen concentrations decreased by 16% in high CO₂, increased by 90% with high nutrient availability, and differed substantially among genotypes (Fig. 3, Table 2). CO₂ treatment did not alter the magnitudes of the variance among aspen genotypes. The aspen genotypes responded differently to altered nutrient availability (nutrient \times genotype interaction). Concentrations of starch were also influenced by all three treatment factors (Fig. 3, Table 2). Levels increased 100%, but declined 67%, with high CO₂ and nutrient availability, respectively. Starch concentrations were more variable among genotypes at elevated CO₂ than at ambient CO₂, for both soil fertility levels.

With regard to the major carbon-based secondary metabolites in aspen, we found that levels of the phenolic glycoside salicortin were not markedly affected by CO₂ (Fig. 4; Table 2). Enriched CO₂ did, however, increase the among-genotype variance in the high nutrient treatment. Salicortin levels also tended to increase with high nutrient availability, although responses differed among genotypes (nutrient \times genotype interaction). In contrast, levels of the phenolic glycoside tremulacin tended to increase under high CO₂ but decreased with

Table 2 Summary of *P* values for the effects of atmospheric CO₂, soil nutrient availability and genotype on foliar chemical composition of aspen

Main effects and interactions	Water	Nitrogen	Starch	Salicortin	Tremulacin	Total phenolic glycosides	Condensed tannins
CO ₂	0.639	0.005	0.040	0.160	0.103	0.102	0.086
Nutrient	<0.001	<0.001	0.004	0.008	0.044	0.590	<0.001
CO ₂ × nutrient	0.224	0.445	0.282	0.195	0.843	0.567	0.097
Genotype	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CO ₂ × genotype	0.649	0.550	0.144	0.211	0.016	0.042	0.029
Nutrient × genotype	0.112	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CO ₂ × nutrient × genotype	0.529	0.844	0.814	0.306	0.674	0.653	0.107

**Fig. 4** Norms of reaction for foliar phenolic glycoside (salicortin and tremulacin) and condensed tannin concentrations (percent dry mass) of aspen genotypes in response to CO₂ and nutrient availability. Format of figure is as described for Fig. 1

high nutrient availability (Fig. 4; Table 2). Responses to both treatments varied among aspen genotypes (CO₂ × genotype and nutrient × genotype interactions). Variability among genotypes was greater under high CO₂ than under ambient CO₂. When data for salicortin and tremulacin were combined for statistical analysis, we found that both CO₂ and nutrient availability altered total phenolic glycoside levels, but differently so among genotypes (CO₂ × genotype and nutrient × genotype interactions; Table 2). Moreover, the variance among genotypes was greater under enriched CO₂ conditions. Fi-

nally, levels of condensed tannins tended to increase under enriched CO₂, but more strongly in some genotypes than in others (CO₂ × genotype interaction; Fig. 4, Table 2). High CO₂ increased the among-genotype variance by 2.6-fold in low nutrient trees, but did not affect variance in high nutrient trees. Tannin levels declined with increased nutrient availability, and the magnitude of change varied among genotypes (nutrient × genotype interaction).

Discussion

Assessment of genetic variation in the effects of CO₂ enrichment on plant characteristics influencing fitness is an important step toward elucidating potential evolutionary consequences of global environmental change (Geber and Dawson 1993). Results from this study illustrate that enriched atmospheric CO₂ will differentially affect some growth, allocation and phytochemical characteristics of aspen genotypes. Moreover, the magnitude of such effects will be modulated by other environmental factors, such as soil nutrient availability.

Several caveats are appropriate here in relation to the short-term nature of this study. First, our results may have been influenced by pre-treatment effects, as all trees were grown under ambient CO₂ prior to experimental use. Trees, however, were 3- to 5-fold (low nutrient) or 8- to 15-fold (high nutrient) larger at the end of the study than at the beginning. This substantial growth likely occluded significant carryover of effects from the pre-treatment CO₂ environment. Moreover, foliar chemical responses observed in this study were similar to those observed in work with trees subjected to enriched CO₂ for several seasons (Roth et al. 1998). Second, allocation data may reflect CO₂- or nutrient-induced differences in developmental stage, characterized by unique allometric relationships, rather than direct effects of the treatments themselves (Gebauer et al. 1996). Third, we recognize that differential responses of aspen genotypes to CO₂ enrichment may change over longer time spans. Given that such responses appeared over a period as short as even several months, however, should be considered as a conservative test of the hypothesis of differential genotypic response.

Growth and allocation

We examined seven different traits for plant growth and biomass allocation. Considering general responses averaged across nutrient treatments and genotypes, only two traits (leaf mass per unit area and root:shoot ratio) were significantly affected by CO₂ treatment (i.e., significant CO₂ main effect). Conversely, all plant growth/allocation variables were strongly affected by soil nutrient availability. Similarly, all such variables varied markedly among aspen genotypes.

Of particular interest in this study, however, are not CO₂ main effects but CO₂ × genotype interactions, as those highlight the potential for differential evolutionary responses to enriched CO₂ in natural aspen populations. Aspen clones responded differently to CO₂ with respect to total biomass production (marginally significant), stem growth, RGR, and root:shoot ratio. For example, shifts in RGRs under high CO₂ (and high nutrient availability) varied from -5 to +29% among clones. In addition, most of these responses were themselves influenced by soil nutrient levels, illustrating that evolutionary responses of plants to enriched CO₂ will likely be influenced by a complex of interacting factors.

Also of interest were cases in which the magnitudes of among-genotype variance differed in response to CO₂ treatment. Contrary to our initial prediction, genetic variability in growth differed relatively little between CO₂ treatments. Likelihood ratio tests indicated that the variance among genotypes increased under enriched CO₂, but only in low nutrient treatments and only for biomass production and total leaf mass.

Comparison of results between this and similar studies with aspen reveals that a complex of genetic and environmental factors determines the growth response of aspen to enriched CO₂. Lindroth et al. (1993) documented that for aspen from a full-sib seed source, elevated CO₂ increased biomass production, RGR and root:shoot ratio. Later, Kinney and Lindroth (1997) showed that growth and allocation responded to both CO₂ and soil nitrate availability, although the effects were largely independent (noninteractive). Recently, Zak et al. (2000) reported that enriched CO₂ stimulated growth of aspen, especially in high fertility soil, but that all genotypes evaluated responded similarly. Moreover, enhanced growth did not substantially alter above- and below-ground biomass allocation. Such diversity of responses among studies is not surprising, given that the studies were conducted with a limited number of genotypes, under different experimental conditions, for different periods of time.

Results from our work are consistent with those of several other studies that have examined genetic variation in the growth responses of trees to enriched CO₂. Most of such studies have been conducted with members of the plant genus *Populus*. For example, elevated atmospheric CO₂ accelerated the growth of hybrid poplar, but differentially so among genotypes (Radoglou and Jarvis 1990; Ceulemans et al. 1996; Dickson et al. 1998). Differences in responses among genotypes was minimal,

however, in similar research with willow (*Salix myrsinifolia*; Julkunen-Tiitto et al. 1993) and jack pine (*Pinus banksiana*; Cantin et al. 1997). Interspecific variation in the magnitude of genotypic responses to CO₂ indicates, not surprisingly, that plant species will vary with respect to evolutionary responses to environmental change.

Foliar chemistry

We also investigated six foliar chemical constituents known to, or likely to, mediate interactions between aspen and other organisms (Lindroth and Hwang 1996a). Again considering average responses across nutrient treatments and genotypes, only nitrogen and starch concentrations were affected by CO₂ (significant main effects), exhibiting typical decreases and increases, respectively (Lincoln et al. 1993; Lindroth 1996). Levels of all chemical constituents, however, were influenced by soil nutrient availability, and differed among genotypes. With high soil fertility, nitrogen levels increased whereas starch and condensed tannin levels decreased. Such responses are consistent with the predictions of carbon-nutrient balance theory (Bryant et al. 1983) and parallel patterns observed in earlier studies in which CO₂ and/or nutrient availability were altered (Lindroth et al. 1993; Kinney et al. 1997; Hemming and Lindroth 1999). Surprisingly, levels of salicortin tended to increase with high soil fertility, whereas those of tremulacin tended to decrease. Overall, differences in phenolic glycoside concentrations were substantial among genotypes and minimal among resource treatments. This pattern is consistent with those observed in our other work with aspen (Hemming and Lindroth 1999; Osier and Lindroth, unpublished data).

Of greater interest than CO₂ main effects on phytochemistry were CO₂ × genotype interactions. Interactions did not occur for levels of primary metabolites (water, nitrogen, starch), but were evident with respect to levels of tremulacin and condensed tannins. For example, under low nutrient conditions, enriched CO₂ elicited relative changes in concentrations of tremulacin from -5 to +21%, and of tannins from -12 to +59%, among genotypes.

In accordance with carbon-nutrient balance theory (Bryant et al. 1983), we initially predicted that the magnitude of among-genotype variance would be greatest under enriched CO₂ and low nutrient availability, as these conditions contribute to accumulation of photosynthate in excess of that required for growth. Our results partially support that prediction. In every case in which the variances differed between CO₂ environments, it was larger with enriched CO₂. The relative increase in the magnitude of the variance, however, was greater in low nutrient, compared to high nutrient, treatments, only for concentrations of tremulacin and condensed tannins. These results suggest that enriched CO₂ atmospheres may magnify genetic differences in the carbon-based chemistry of aspen clones.

Only a few other studies have evaluated the effects of high CO₂ levels on intraspecific variation in phytochemistry. In research similar to ours, Mansfield et al. (1999) reported that enriched CO₂ increased levels of condensed tannins in aspen, but differently so for different genotypes. Julkunen-Tiitto et al. (1993) reported no significant CO₂ × genotype interactions for levels of nitrogen and sugar, but strong interactions for levels of phenolic glycosides and other phenolics in *Salix myrsinifolia*. Fajer et al. (1992) found no significant CO₂ × genotype interactions for concentrations of iridoid glycosides in *Plantago lanceolata*. Most recently, Goverde et al. (1999) reported that for *Lotus corniculatus*, levels of condensed tannins, but not of cyanoglycosides, were influenced by CO₂ × genotype interactions.

Implications of CO₂ × genotype effects

Differential effects of elevated atmospheric CO₂ on aspen growth and chemistry are likely to affect both population and community structure via interactions such as competition and herbivory. Shifts in growth rates and biomass allocation will likely alter competitive interactions among aspen clones, as well as between aspen and herbaceous (Landhauser and Lieffers 1998) and woody (Peterson and Squiers 1995) vegetation. In eastern North America, aspen is part of a successional sere transitional to other hardwood or coniferous forests (Sakai et al. 1985; Peterson and Squiers 1995). Asymmetric effects of enriched CO₂ on the fitness and genetic structure of populations of aspen and competing plant species may alter rates of ecological succession.

Aspen plays an important role in early successional forests as food for a plethora of vertebrate and invertebrate herbivores, and as host for a variety of pathogens. Central to this role is chemical composition. Phenolic glycosides, for example, determine the susceptibility of aspen to insects, mammals and pathogens (Lindroth and Hwang 1996a). Thus, differential effects of enriched CO₂ on the chemical composition of aspen genotypes will likely shift susceptibility to herbivory and/or disease within aspen populations. Moreover, future CO₂ environments could potentially alter the expression of tradeoffs between growth and defense in aspen. Strong, negative genetic correlations exist between growth and defense in aspen growing in carbon- and nutrient-poor environments (Osier and Lindroth, unpublished data). The negative correlations disappear, however, in resource-rich environments. Thus, CO₂ enrichment may enhance the potential for aspen to maintain (largely) genetically-determined levels of defense compounds at a reduced cost to growth, although the cost decrement may differ among genotypes.

In conclusion, this research demonstrates that elevated atmospheric CO₂ can alter characteristics important to the fitness of aspen, and do so differentially among genotypes. Moreover, enriched CO₂ tends to increase the magnitude of the variance among aspen genotypes, particularly at low nutrient levels. Thus CO₂ alone, and in

combination with other biotic and abiotic factors, is likely to change the evolutionary trajectories of aspen populations. Clearly, assessment of genetic variation in response to global environmental change should be accorded a higher priority by global change scientists. Equally clearly, evaluation of multiple responses of numerous genotypes of many species in diverse environmental contexts is a formidable, if not impossible, task. As a first step, we recommend focus on species that play especially important ecological roles, have broad geographical distributions, and display high degrees of genetic variation.

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References

- Bazzaz FA, Jasienski M, Thomas SC, Wayne P (1995) Microevolutionary responses in experimental populations of plants to CO₂-enriched environments: parallel results from two model systems. *Proc Natl Acad Sci* 92:8161–8165
- Bryant JP, Chapin FS III, Klein DR (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40:357–368
- Cantin D, Tremblay MF, Lechowicz MJ, Potvin C (1997) Effects of CO₂ enrichment, elevated temperature, and nitrogen availability on the growth and gas exchange of different families of jack pine seedlings. *Can J For Res* 27:510–520
- Ceulemans R, Mousseau M (1994) Tansley review no. 71: effects of elevated atmospheric CO₂ on woody plants. *New Phytol* 127:425–446
- Ceulemans R, Shao BY, Jiang XN, Kalina J (1996) First- and second-year aboveground growth and productivity of two *Populus* hybrids grown at ambient and elevated CO₂. *Tree Physiol* 16:61–68
- Curtis PS, Snow AA, Miller AS (1994) Genotype-specific effects of elevated CO₂ on fecundity in wild radish (*Raphanus raphanistrum*). *Oecologia* 97:100–105
- Dickson RE, Coleman MD, Riemenschneider DE, Isebrands JG, Hogan GD, Kamosky DF (1998) Growth of five hybrid poplar genotypes exposed to interacting elevated CO₂ and O₃. *Can J For Res* 28:1706–1716
- Fajer ED, Bowers MD, Bazzaz FA (1992) The effect of nutrients and enriched CO₂ environments on production of carbon-based allelochemicals in *Plantago*: a test of the carbon/nutrient balance hypothesis. *Am Nat* 140:707–723
- Gebauer RLE, Reynolds JF, Strain, BR (1996) Allometric relations and growth in *Pinus taeda*: the effect of elevated CO₂ and changing N availability. *New Phytol* 134: 85–93
- Geber MA, Dawson TE (1993) Evolutionary responses of plants to global change. In: Kareiva PM, Kingsolver JG, Huey RB (eds) *Biotic interactions and global change*. Sinauer, Sunderland, Mass., pp 179–197
- Goverde M, Bazin A, Shykoff JA, Erhardt A (1999) Influence of leaf chemistry of *Lotus corniculatus* (Fabaceae) on larval development of *Polyommatus icarus* (Lepidoptera, Lycaenidae) effects of elevated CO₂ and plant genotype. *Funct Ecol* 13:801–810
- Hagerman AE, Butler LG (1980) Condensed tannin purification and characterization of tannin-associated proteins. *J Agric Food Chem* 28:947–952

- Hemming JDC., Lindroth RL (1999) Effects of light and nutrient availability on aspen: growth, phytochemistry and insect performance. *J Chem Ecol* 25:1687–1714
- Hwang S-Y, Lindroth RL (1997) Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. *Oecologia* 111:99–108
- Julkunen-Tiitto R, Tahvanainen J, Silvola J (1993) Increased CO₂ and nutrient status changes affect phytomass and the production of plant defensive secondary chemicals in *Salix myrsinifolia* (Salisb.). *Oecologia* 95:495–498
- Kimball BA (1983) Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agron J* 75:779–788
- Kinney KK, Lindroth RL (1997) Responses of three deciduous tree species to atmospheric CO₂ and soil NO₃⁻ availability. *Can J For Res* 27:1–10
- Kinney KK, Lindroth RL, Jung SM, Nordheim EV (1997) Effects of CO₂ and NO₃⁻ availability on deciduous trees: phytochemistry and insect performance. *Ecology* 78:215–230
- Kubiske ME, Pregitzer KS, Zak DR, Mikán CJ (1998) Growth and C allocation of *Populus tremuloides* genotypes in response to atmospheric CO₂ and soil N availability. *New Phytol* 140:251–260
- Landhausser SM, Lieffers VJ (1998) Growth of *Populus tremuloides* in association with *Calamagrostis canadensis*. *Can J For Res* 28:396–401
- Lang CA (1958) Simple microdetermination of Kjeldahl nitrogen in biological materials. *Anal Chem* 30:1692–1694
- Lincoln DE, Fajer ED, Johnson RH (1993) Plant-insect herbivore interactions in elevated CO₂ environments. *Trends Ecol Evol* 8:64–68
- Lindroth RL (1996) CO₂-mediated changes in tree chemistry and tree-Lepidoptera interactions. In: Koch GW, Mooney HA (eds) Carbon dioxide and terrestrial ecosystems. *Physiological ecology series*. Academic Press, San Diego, pp 105–120
- Lindroth RL, Hwang S-Y (1996a) Diversity, redundancy and multiplicity in chemical defense systems of aspen. In: Romeo J (ed) Recent advances in phytochemistry, vol 33. Plenum Press, New York, pp 25–56
- Lindroth RL, Hwang S-Y (1996b) Clonal variation in foliar chemistry of quaking aspen (*Populus tremuloides* Michx.). *Biochem Syst Ecol* 24:357–364
- Lindroth RL, Kinney KK, Platz CL (1993) Responses of deciduous trees to elevated atmospheric CO₂: productivity, phytochemistry and insect performance. *Ecology* 74:763–777
- Littel RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute, Cary, N.C., USA
- Mansfield JL, Curtis PS, Zak DR, Pregitzer KS (1999) Genotypic variation for condensed tannin production in trembling aspen (*Populus tremuloides*, Salicaceae) under elevated CO₂ and in high- and low-fertility soil. *Am J Bot* 86:1154–1159
- Mitton JB, Grant MC (1996) Genetic variation and the natural history of quaking aspen. *Bioscience* 46:25–31
- Owensby CE, Coyne PL, Ham JM, Auen LM, Knapp AK (1993) Biomass production in a tallgrass prairie ecosystem exposed to ambient and elevated CO₂. *Ecol Appl* 3:644–653
- Palo RT (1984) Distribution of birch (*Betula* spp.), willow (*Salix* spp.), and poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. *J Chem Ecol* 10:499–520
- Parkinson JA, Allen SE (1975) A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. *Commun Soil Sci Plant Anal* 6:1–11
- Peñuelas J, Estiarte M (1998) Can elevated CO₂ affect secondary metabolism and ecosystem function? *Trends Ecol Evol* 13:20–24
- Peterson CJ, Squiers ER (1995) Competition and succession in an aspen – white-pine forest. *J Ecol* 83:449–457
- Porter LJ, Hrstich LN, Chan BG (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* 25:223–230
- Potvin C, Vasseur L (1997) Long-term CO₂ enrichment of a pasture community: species richness, dominance, and succession. *Ecology* 78:666–677
- Radoglou KM, Jarvis PG (1990) Effects of CO₂ enrichment on four poplar clones. I. Growth and leaf anatomy. *Ann Bot* 65:617–626
- Roth SK, Lindroth RL (1995) Elevated atmospheric CO₂: effects on phytochemistry, insect performance and insect parasitoid interactions. *Global Change Biol* 1:173–182
- Roth S, Lindroth RL, Volin JC, Kruger EL (1998) Enriched atmospheric CO₂ and defoliation: effects on tree chemistry and insect performance. *Global Change Biol* 4:419–430
- Sakai AK, Roberts MR, Jolls CL (1985) Successional changes in a mature aspen forest in northern lower Michigan: 1974–1981. *Am Midl Nat* 113:271–282
- SAS (1989) User's guide: statistics. SAS Institute, Cary, NC
- Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO₂ atmosphere. *New Phytol* 139:395–436
- Ward JK, Strain BR (1999) Elevated CO₂ studies: past, present and future. *Tree Physiol* 19:211–220
- Zak DR, Pregitzer KS, Curtis PS, Vogel CS, Holmes WE, Lussenhop J (2000) Atmospheric CO₂, soil-N availability, and allocation of biomass and nitrogen by *Populus tremuloides*. *Ecol Appl* 10:34–46