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Effects of elevated CO_2 on growth and carbon/nutrient balance in the deciduous woody shrub *Lindera benzoin* (L.) Blume (Lauraceae)

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Abstract. We examined the effects of elevated CO_2 on growth and carbon/nutrient balance in a natural population of the deciduous temperate zone shrub *Lindera benzoin*. Our data concern whole plant, leaf, and stem growth for the first two seasons of a long-term field experiment in which CO_2 levels were manipulated in situ. In addition to growth parameters, we evaluated changes in leaf and stem chemistry, including total nitrogen, nonstructural carbohydrates, and total phenolics. Over the course of this study, *L. benzoin* appeared to respond to elevated CO_2 primarily by physiological and biochemical changes, with only a slight enhancement in aboveground growth (ramet height). Positive effects on aboveground growth were primarily evident in young (nonreproductive) ramets. Our results suggest that nitrogen limitation may have constrained plants to allocate carbohydrates produced in response to elevated CO_2 primarily to storage and belowground growth, and perhaps to increased secondary chemical production, rather than to increased stem and leaf growth. We discuss our results in terms of changes in carbon/nutrient balance induced by elevated CO_2, and provide predictions for future changes in this system based upon constraints imposed by intrinsic and extrinsic factors and their potential effects on the reallocation of stored reserves.

Key words: Carbon dioxide, elevated – Carbon/nutrient balance – Growth - Nitrogen – Carbohydrates

Data concerning recent changes in global atmospheric CO_2 concentration generally concur that levels have risen from pre-industrial concentrations of about 275–285 ppm (Neftel et al. 1985) to present levels of about 340–360 ppm (Overdieck and Forstreuter 1991). Models of future global CO_2 increase are also in general agreement that levels will continue to rise in the near future, with predicted levels reaching 600 to 800 ppm by the year 2100 (cf., Conway et al. 1988). Much interest has been generated as a result of these conclusions, particularly with respect to the potential effects of CO_2 elevation on global warming as well as direct effects on plant growth and vegetation change (cf., Bazzaz et al. 1990; Kienast 1991; Coleman and Bazzaz 1992).

Most studies of the direct effects of increased CO_2 on photosynthesis and plant growth have focused on agricultural crops, or seedlings and juveniles of native species grown in pots or small growth chambers (see review in Eamus and Jarvis 1989). Of these studies, most find that short term increases in photosynthesis and growth are counterbalanced by longer term decreases thought to result primarily from nutrient and space limitation (see review in Drake and Leadley 1991) or from limitation of available carbon sinks (Kramer 1981). Concern has arisen over the validity of extrapolations from short term, small scale experiments, particularly because of the potential interacting effects of factors such as nutrient and space limitation on photosynthetic acclimation via feedback mechanisms. Additionally, there is a paucity of data concerning long-term effects on native woody perennials growing under natural habitat conditions (Eamus and Jarvis 1989; Overdieck and Forstreuter 1991), despite the fact that these plants form a major component of global phytomass. Questions remain concerning the degree to which intrinsic and extrinsic factors regulate woody plant responses to elevated CO_2 under natural conditions. Given that large scale models of regional and global vegetation dynamics require parameterization with data consistent with changes expected in plants under natural conditions, it is imperative that larger scale in situ experiments with woody plants be undertaken (cf., Wullschleger et al. 1992). Here we report effects of elevated CO_2 on growth and carbon/nutrient balance in a natural population of the temperate zone shrub *Lindera benzoin* (L.) Blume (Lauraceae). We report data concerning whole plant and modular (leaf and stem) growth for the first two seasons of a long term field

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experiment in which CO_2 levels were manipulated via the use of open-top chambers.

In association with photosynthetic and growth responses, internal chemical changes such as the accumulation of storage carbohydrates or shifts in carbon/nutrient balance may be an early indication of plant response to increased carbon availability (Curtis et al. 1989; Fajer et al. 1992; Wullschleger et al. 1992). In particular, an extention of the carbon/nutrient balance hypothesis (Coley et al. 1985) suggests that an increased availability of carbon should, under conditions of nitrogen limitation, result in increased production of non-structural carbohydrates, lowered tissue nitrogen levels, and increased allocation to C-based secondary products (cf., Fajer et al. 1992). Thus in addition to effects on growth parameters, we report effects of CO_2 elevation on nitrogen, total non-structural carbohydrates, and total phenolic content of leaf and stem tissues.

Materials and methods

1. *Study species*

Individuals (genets) of the deciduous woody shrub *L. benzoin* bear unisexual flowers (Boyle 1980) and reproduce vegetatively via the initiation of ground stems (ramets) from a central root bole. At our study site, Smithsonian Environmental Research Center (SERC), Edgewater, Maryland, plants are concentrated in the understories of mid- to late-successional riparian and floodplain forests dominated by *Liriodendron tulipifera* L., *Liquidambar styraciflua* L., *Platanus occidentalis* L., *Fagus grandifolia* Ehrh., *Acer rubrum* L., *Quercus* spp., and *Carya* spp. (i.e., the "tulip-poplar association" of Brush et al. 1980 and Parker et al. 1989). Shade tolerance has been considered an important factor influencing distribution and growth in this species, although plants may show substantial morphological and growth responses to increased light (Moore and Willson 1982; Veres and Pickett 1982; Niesenbaum 1992; M. Cipollini and D. Wallace-Senft, unpublished data). Plants flower when leafless in early April, followed by new leaf and stem initiation in early May. Female plants produce single-seeded fleshy fruits that are dispersed by birds in September and October (Stiles 1980; Johnson et al. 1985). Estimates of the within-year biomass and mineral costs of female reproduction are much higher than those for male reproduction, and leaf and stem growth rates may be lower for female plants (Niesenbaum 1992; Cipollini and Whigham 1994). In light of the potential importance of available resource sinks on plant photosynthetic response to CO_2 elevation, it was necessary to consider plant reproductive status (male, female, nonreproductive) as a potential factor influencing growth in *L. benzoin*.

2. *Study site and experimental design*

In early June 1991, just after leaf initiation, plants growing in a midsuccessional lowland forest on SERC property were assigned to experimental treatments for a long term study of the effects of CO_2 elevation on photosynthesis. The site, which was dominated by mature *L. tulipifera* and *L. styraciflua* in the overstory, was occupied almost exclusively by *L. benzoin* in the shrub layer. The soil at this site is listed as Adelphia sandy loam (Kirby and Matthews 1973). Within this site, a 60×30 m area was divided into three 20×30 m blocks arranged in parallel along a slight slope (about 3%). Within each block, plants were grouped into three experimental treatments: control, ambient, and elevated. For elevated and ambient treatments, plants were enclosed within 3.4 m tall $\times$ 3.8 m wide octagonal footprint chambers constructed using PVC tubing supports covered with clear 300 gauge polyester film (Courtauld's Performance Films, Martinsville, VA). Except for a 25 cm centrally-inclined frustrum (angled at 30°), designed to maintain within-chamber air circulation, the chambers were open-topped.

These chambers were similar in construction to those used in a study of effects of CO_2 enrichment on a nearby saltmarsh ecosystem (Drake et al. 1989), except for differences in the ventilation system. In each of the chambers used in this study, a 50 cm diameter polyethylene duct extending from an external blower (Dayton Model 5C092) was connected to a manifold of five 13 cm diameter polyethylene tubes in which pairs of 2 cm holes were punched every 13 cm. The ventilation tubes were arranged in parallel on the ground within each chamber, and provided full air exchanges at a rate of 1.28 per minute.

Carbon dioxide levels were monitored in each of the ambient and elevated chambers using 6 mm $\times$ 3.4 m sampling tubes perforated with pairs of 1.5 mm diameter holes spaced every 0.5 m. Tubes were horizontally suspended at midcanopy (1.8 m high) within each chamber, and were connected to 6 mm diameter tubing leading to valves that allowed regular CO_2 determinations using an infra-red gas analyzer (Binos Model 092). Using this system, air from each chamber was routinely sampled at 3 minute intervals for 30 second sampling periods. Carbon dioxide concentration ranged from about 340 to 400 ppm in the ambient chambers (G. Peresta, unpublished data), and was maintained at about 680 to 740 ppm (+340 ppm) within elevated chambers by injecting pure CO_2 gas into ventilation blower inlets. Carbon dioxide elevation was maintained for the time plants were in leaf (June 1991 through October 1991; May 1992 through October 1992). Methods and equipment used for manipulating and monitoring CO_2, and for monitoring light, temperature and humidity in these chambers were similar to those described in Drake and Leadley (1991). Control plots were identical in area to ambient and elevated chambers, but were not subjected to experimental manipulation.

The primary reason for establishing the ambient treatment was to account for potential effects resulting from plant enclosure within the chambers. As a means of reducing potential chamber effects, chambers were opened on two sides during the periods when CO_2 levels were not being manipulated. Available data suggest only slight effects of enclosure on microclimatic parameters, including a buffering of exterior wind fluctuations (G. Peresta, unpublished data). No differences between chambers and control plots were detected in comparisons of temperature or humidity in 1992 (W. Dugas, unpublished data). Light levels, which were monitored by single photosensors located at the top center of six of the plots (two ambient, two elevated and two control), were low and highly variable, typically ranging from 10 to 100 umol/m^2/min, with occasional sunfleck peaks ranging from 100 to 1000 umol/m^2/min (G. Peresta, unpublished data).

3. *Plant censuses and growth measurements*

In June 1991, we tagged and measured basal diameter (at 10 cm above ground) of individual ramets of most adult and vegetative genets within each of the nine plots. We also counted the number of leaves and branches (sum of all orders) for each of the tagged ramets. In April 1992, a more complete census was undertaken, so that all genets were located and tagged. At this time, newly produced ramets on previously-tagged genets were also marked. As of the second census period, there were 102, 89, and 97 individually-tagged ramets in control, ambient and elevated treatments respectively. We measured basal diameter of each ramet and counted flower clusters on all ramets at this time. In June 1992, we again measured basal diameter, and counted total leaf and branch number on each marked ramet. In August 1992, we counted the total number of mature fruits on each of the female ramets.

In September 1992, we subsampled five of the largest genets within each plot in order to estimate new stem and leaf growth. For each genet, we randomly collected five newly-produced terminal stems with attached leaves. Position within plant for stem samples

was standardized by restricting collection to terminal stems on side branches of the main axial ramet. We separated leaves from the stems, counted them, and determined total leaf area using a LI-COR Model 3100 area meter. We determined leaf and stem mass (g) before and after oven-drying at 60° C. From these data, we estimated dry mass per leaf, area per leaf, leaf density (dry mass/wet mass), dry mass per stem, and stem density (dry mass/wet mass). Leaf and stem samples were ground to pass a #40 mesh screen, and were stored at −20° C in capped polyethylene vials prior to chemical analyses.

In November 1992, we remeasured basal diameter and determined the length of the main axial stem of each ramet. Ramet length increase from November 1991 to November 1992 was also measured at this time, and in doing so, ramet length at the end of the previous year's growing season (November 1991) was determined. We estimated the following growth parameters for each ramet: basal area increase in cm^3 (June 1991 to November 1992), basal area increase in cm^3 (April 1992 to November 1992), leaf number increase (June 1991 to June 1992), and branch number increase (June 1991 to June 1992). Values for each growth parameter were estimated as the difference between the log-transformed final and initial measurements.

4. *Chemical analyses*

For each stem and leaf sample we determined total nitrogen (percent dry mass) using micro-Kjeldahl digestion followed by auto-analysis (Williams 1984). We determined total non-structural carbohydrates using amylase extraction (Smith 1981) followed by the anthrone colorimetric assay (Newfield and Ginsberg 1966), with results expressed as percent dry mass glucose equivalents. We estimated total phenolics using the Prussian Blue colorimetric assay (0.016 M potassium ferricyanide + 0.1 M ferric ammonium sulphate; Budini et al. 1980; Graham 1992) with results expressed as mg/g dry mass catechin equivalents. The Prussian Blue assay, like the analogous Folin-Denis assay (Harborne 1989), is subject to error if phenolic profiles differ strongly among samples, e.g., if there are differences among samples in the relative concentrations of individual compounds. In an attempt to account for this possibility, we characterized the phenolic profiles for bulk samples of male and female tissues in each of the treatment groups using thin-layer chromatography (silica gel TLC; toluene-acetone-formic acid [6:6:1]); Prussian Blue reagent; A. Hagerman, Miami University – Ohio, pers. comm.). Because thin layer chromatograms of both crude and acid-hydrolyzed extracts showed no detectable differences in phenolic profiles of plants from each of the treatment groups, we assumed that the results of the total phenolic assay represented relative quantitative differences (but see Discussion below).

5. *Statistical analyses*

Multivariate analyses of variance and covariance (MANOVA, MANCOVA) formed the primary statistical analyses (PROC GLM: SAS Institute, Inc. 1982). The MANCOVA approach is analogous to multiple analysis of variance using relative growth data in that it accounts for size-dependent growth, but this method avoids statistical problems associated with the analysis of ratios. The covariate used for MANCOVA was initial ramet basal area, which was the variable most strongly correlated with the various growth parameters. In order to normalize distributions and to linearize variate-covariate relationships prior to statistical analysis, size data were log-transformed and percentage data were arcsine-transformed. We verified homogeneity of variances for all main effect groups using Bartlett's test (Sokal and Rohlf 1981).

When significant overall effects were found in multivariate analyses, we employed analogous ANCOVA tests as a means of determining the effects for individual variables. *A postiori* main effect means comparisons were Bonferroni T-tests which controlled the Type 1 error rate at a confidence level of 0.95. Using these methods, potentially correlated data (e.g., height increase and basal area increase) were first analyzed using full models incorporating all main effects (treatment, block, sex, and basal area [covariate]) and their interactions. In these initial analyses, sex and block effects were sometimes significant, but means for control and ambient treatments never differed significantly ($P > 0.10$). Thus, data for control and ambient treatments were combined, and sex and block effects were dropped where possible, so as to enhance resolution of the main effect of CO_2 elevation. Where block effects were retained, we tested for treatment effects using a split-plot design ($F_{treatment} = MS_{treatment}/MS_{treatment \times block}$). In separate homogeneity-of-slopes tests there were no significant effects of treatment, block, or sex on the slopes of the regressions of variates on their covariates (no significant main effect × covariate interactions).

Using this approach, we analyzed data for all individual ramets, as well as for reproductive (flowering) and nonreproductive ramets separately. Imbalanced design, resulting from considerable variation in numbers of ramets within genets and a predominance of genets with only a single ramet, made nested analyses (ramets nested within genets) inappropriate. Yet, because of potential resource translocation among ramets within genets, which makes measurements on individual ramets not truly independent, we also analyzed data for only the largest ramet within each genet. Results from these latter analyses were consistent with those based upon individual ramets, thus we focus on results obtained from the first set of analyses.

We used MANOVA to determine the effects of CO_2 treatment on leaf and stem characteristics, and multiple linear regression analysis to examine relationships among leaf and stem characteristics.

Results

1. *Whole ramet growth*

The only significant effect of CO_2 elevation was on ramet length (height) increase, which was greater for plants in the elevated treatment for all ramets combined and for nonreproductive ramets analyzed separately (Table 1). In response to the elevated CO_2 treatment, ramet length increase was about 29% greater for reproductive ramets and about 38% greater for nonreproductive ramets. Consistent, albeit non-significant, trends included greater basal area growth (for both time periods analyzed), and reduced leaf number for plants in the elevated treatment. Similar patterns and magnitudes of response were found when data for only the largest ramet within each genet were analyzed, except that the treatment effect on leaf number increase was found to be significant, and a trend appeared for a reduction in the rate of new branch growth with CO_2 elevation (results not shown).

Results of MANCOVA analyses suggested that the significant treatment effect for height increase resulted primarily from the enhanced growth of nonreproductive ramets (Table 2). In most analyses, the covariate explained the largest amount of variation, which is an expected result of size-dependent growth. Block effects were significant in analyses involving leaf, branch and basal area increase (June 1991 to November 1992). This result was associated with the effects of a drought during early summer 1991, which resulted in leaf wilt and stem die-back primarily within the one of the three blocks (the

Table 1. Effect of CO_2 elevation on height (cm), basal area (cm^3), leaf number, and stem number increase in *L. benzoin* ramets (1991 to 1992). Data are means and standard errors for all individual ramets measured. Significance levels refer to the main effect of treatment in analyses of covariance

Reproductive ramets:	Control + Ambient		Elevated	
Variable	N	Mean (s.e.)	N	Mean (s.e.)
HTINC: Height increase 11/91–11(92	41	17.510 (4.05)	31	22.629 (3.53)
BAINC1: Basal area increase 11/91–11/92	41	0.387 (0.12)	33	0.456 (0.22)
BAINC2: Basal area increase 4/92–6/92	41	0.085 (0.07)	33	0.186 (0.07)
LEAFINC: Leaf no. increase 6/91–6/92	41	58.683 (78.75)	33	−16.186 (107.9)
BRCHINC: Branch no. increase 6/91–6/92	41	45.370 (20.99)	33	53.818 (36.00)

Non-reproductive ramets:	Control + Ambient		Elevated	
Variable	N	Mean (s.e.)	N	Mean (s.e.)
HTINC: Height increase 11/91–11/92	130	20.269 (2.26)	91	28.022 (2.95)**
BAINC1: Basal area increase 11/91–11/92	68	0.055 (0.05)	33	0.125 (0.03)
BAINC2: Basal area increase 4/92–6/92	112	0.046 (0.04)	64	0.086 (0.02)
LEAFINC: Leaf no. increase 6/91–6/92	67	57.194 (18.93)	33	−12.061 (27.05)
BRCHINC: Branch no. increase 6/91–6/92	67	28.910 (6.38)	33	10.667 (3.46)

** $P \leq 0.01$

one at the lowest point on the slope). The general conclusions reached here concerning overall treatment effects were not substantively affected when this block was excluded from analyses. Reproductive variables (flower number, fruit number) were not found to differ significantly among treatments (data not reported), and thus we will not comment further on these data.

2. *Modular leaf and stem growth*

Leaf and stem characteristics were affected by the elevated CO_2 treatment. Based upon MANOVA analysis, the treatment effect was significant for leaf characteristics (Wilk's Lambda = 0.6515; $F = 2.83$; df = 1/43; $P \leq 0.05$), and for stem characteristics (Wilk's Lambda = 0.7695; $F = 2.46$; df = 1/43; $P \leq 0.05$). Nevertheless, the only modular growth parameter that was significantly affected by the elevated CO_2 treatment was leaf density (dry mass/wet mass), which was significantly greater in the elevated treatment (Table 3). None of the other modular growth parameters (leaf number per stem, leaf mass, leaf area, stem mass) were significantly affected by the treatment.

3. *Chemical parameters*

Tissue chemical makeup was affected by the elevated CO_2 treatment. Leaf and stem nitrogen was significantly reduced for plants held at elevated CO_2, whereas leaf and stem total phenolic content was significantly increased in these plants (Table 3). There were slight trends for increased total nonstructural carbohydrates in leaf and stem tissues for plants in the elevated CO_2 treatment, but these effects were not statistically significant. Results of multiple linear regression indicated strong relationships between leaf nitrogen and leaf phenolics (partial regression coefficient = −51.43, $T = 3.833$, df = 1/38, $P < 0.0001$), between leaf density and leaf phenolics (partial regression coefficient = 5.783, $T = 2.993$, df = 1/38, $P < 0.01$), and between stem mass and stem phenolics (partial regression coefficient = −0.670, $T = 2.444$, df = 1/40, $P < 0.05$).

Discussion

Elevated CO_2 and plant growth responses

This study is unique in reporting growth responses to elevated CO_2 in adults and juveniles of a woody perennial grown in situ in its native habitat for more than one growing season. The primary difficulties in analyzing growth response to elevated CO_2 in this natural population involve the complex lifeform of *L. benzoin* (with potential resource sharing among ramets), potential effects of environmental heterogeneity (e.g., light, moisture), and size dependent effects on growth. The numbers, sizes, locations and sexes of individual plants used in this study were constrained by the logistical and economical considerations of constructing and maintaining large CO_2 chambers in the field. Despite these difficulties, we have been able to demonstrate significant treatment effects on at least one growth parameter (height increase), as well as a trend for increased basal area of ramets over the course of the first two years of the experiment.

Plants in the elevated CO_2 treatment produced increased ramet growth despite reduced leaf number that was not counterbalanced by enhanced area per leaf. This would suggest that during the initial phases of this experiment, leaves of plants in the elevated treatment became relatively more efficient photosynthetically. Because all leaves of *L. benzoin* are produced in a single flush in May

Table 2. Results of MANCOVA for ramet growth data (all ramets measured), with data for control and ambient chambers combined. ANOVA statistics are *F* values and associated significance levels. For variable definitions, refer to Table 1

MANCOVA 1: Reproductive ramets

ANOVA statistics						MANOVA Test Criteria	
Source	df	HTINC	BAINC1	BRCHINC	LEAFINC	Wilks' Lambda	*F*
Treatment	1	0.59	0.10	0.27	0.15	0.97405662	0.41
Block	2	0.14	3.69*	1.05	3.94*	0.71991521	2.77**
Basal area 6/91 (Covariate)	1	11.51**	0.28	43.30****	28.35****	0.59008930	10.77****
Treatment × Block	2	1.45	0.22	0.09	0.68		
Error	65						

MANCOVA 2: Reproductive ramets only

ANOVA statistics				MANOVA Test Criteria	
Source	df	HTINC	BAINC2	Wilks' Lambda	*F*
Treatment	1	0.49	0.99	0.98302210	0.59
Basal area 4/92 (Covariate)	1	9.35**	2.84	0.87162283	5.01**
Error	69				

MANCOVA 3: Nonreproductive ramets only

ANOVA statistics						MANOVA Test Criteria	
Source	df	HTINC	BAINC1	BRCHINC	LEAFINC	Wilks' Lambda	*F*
Treatment	1	0.05	0.59	1.29	0.39	0.91881069	1.99
Block	2	0.86	0.41	3.79*	1.76	0.70074613	4.38****
Basal area 6/91 (Covariate)	1	7.50**	7.55**	6.60*	16.72****	0.76713555	6.83****
Treatment × Block	2	0.89	4.11*	2.81	2.64		
Error	93						

MANCOVA 4: Nonreproductive ramets only

ANOVA statistics				MANOVA Test Criteria	
Source	df	HTINC	BAINC2	Wilks' Lambda	F
Treatment	1	8.21**	0.29	0.95135665	4.35*
Basal area 4/92 (Covariate)	1	40.14****	18.15****	0.73349329	30.88****
Error	171				

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$

Table 3. Comparison of leaf and stem characteristics for *L. benzoin* plants in control, ambient and elevated treatments (N = 15 plants per treatment). Values are means and standard errors, and significance levels refer to the main effect of treatment in ANCOVA

Variable	Control	Ambient	Elevated
Leaves per stem	6.867 (0.358)	6.480 (0.254)	6.360 (0.196)
Dry mass (g) per leaf	0.065 (0.005)	0.070 (0.004)	0.069 (0.003)
Mean leaf area (cm^2)	23.724 (1.773)	26.503 (1.345)	24.621 (1.428)
Leaf density	0.251 (0.005)	0.256 (0.004)	0.272 (0.005)**
Dry mass (g) per stem	0.163 (0.032)	0.152 (0.029)	0.141 (0.020)
Stem density	0.509 (0.023)	0.510 (0.016)	0.494 (0.007)
Leaf nitrogen (%)	2.763 (0.100)	2.749 (0.042)	2.587 (0.069)*
Leaf carbohydrates (%)	8.476 (0.747)	9.115 (0.385)	9.507 (0.335)
Leaf phenolics (mg/g)	1.292 (0.084)	1.322 (0.047)	1.683 (0.067)****
Stem nitrogen (%)	0.793 (0.028)	0.800 (0.011)	0.751 (0.012)*
Stem carbohydrates (%)	4.205 (0.169)	4.377 (0.120)	4.564 (0.154)
Stem phenolics (%)	0.772 (0.065)	0.780 (0.039)	0.949 (0.051)*

* $P \leq 0.05$; ** $P \leq 0.01$; **** $P \leq 0.0001$

and leaf numbers were counted in June, it is improbable that differences in leaf number relate to differences in leaf production phenology. We do not have data for leaf area in 1991; thus, we can only speculate from leaf number data that total leaf area of plants in the elevated treatment was reduced, which is a reasonable assumption given the magnitude of the difference. In response to elevated CO_2, plants in this experiment have shown substantive increases in leaf photosynthetic capacity (C. Grietner and B. Drake, unpublished data), as well as slight decreases in respiration (G. Thompson and B. Drake, unpublished data). Rates of evapotranspiration differed little between plants from elevated and ambient treatments in the summer of 1992 (W. Dugas and B. Drake, unpublished data), which was consistent with data showing little effect of CO_2 elevation on stomatal function during that time (C. Grietner and B. Drake, unpublished data). In short, available physiological data are consistent with enhanced efficiency of carbon uptake under the elevated CO_2 treatment.

We do not have data concerning belowground allocation in these plants. The reduction in leaf and stem nitrogen coupled with only slightly increased leaf and stem carbohydrate content would suggest that increased carbon allocation to belowground storage or growth is possible. For plants growing under conditions of nutrient limitation, enhanced allocation to storage or belowground tissues is a common early result of CO_2 enrichment (Curtis et al. 1989; Eamus and Jarvis 1989; El-Kohen et al. 1992; Norby et al. 1992). Increased allocation to storage is particularly likely for shade-tolerant species like *L. benzoin* that have inherently low growth rates (Chapin et al. 1990). As such, we might expect to find proportionately greater increases in aboveground growth later in the study, as enhanced root/shoot ratios begin to balance belowground nutrient acquisition with aboveground growth. Allocation to belowground growth or storage may play a role in maintaining low aboveground growth and relatively low nonstructural carbohydrate levels in the face of carbon dioxide enrichment.

We did not partition total carbohydrates into mobile (e.g., sucrose) and immobile (e.g., starch) fractions. It is thus possible that our data showing only slight increases in total carbohydrates with CO_2 elevation reflect shifts in relative concentrations, such that starch levels increase while sucrose levels decrease or remain unaltered (cf., Hendrix and Grange 1991). Such shifts in carbohydrate partitioning have been noted for tree seedlings such as *L. tulipifera* L. and *Quercus alba* L. (Wullschelger et al. 1992), and *Betula pendula* (Pettersson and McDonald 1992), for artificial tropical forest communities (Korner and Arnone 1992), and for agricultural crops such as soybeans (*Glycine max* (L.) Merr.; Huber et al. 1984; Finn and Brun 1982). A more detailed examination of patterns of carbohydrate partitioning is currently being undertaken to address this issue for *L. benzoin* at SERC (J. Jacob and B. Drake, unpublished data).

Plants that have the capacity to respond strongly to elevated CO_2, such that enhanced photosynthetic rates and growth are maintained over the long term, tend to be those with sustained availability of active carbon sinks (cf., Kramer 1981; Idso et al. 1991). Growth in the deciduous *L. benzoin* is structured such that new stems are initiated from overwintering buds in a single flush in early May, and these units attain nearly full size within one month. On the other hand, basal ramet initiation and growth occurs continually throughout the growing season. Our data show relatively greater effects on growth of young and newly initiated ramets (nonreproductive ramets), which suggests that the primary effects on aboveground growth are presently concentrated in young ramets. This is not surprising, given that juvenile and adult plants often show distinct physiological differences (cf., Donovan and Ehleringer 1991). If reallocation from storage occurs in the near future, we expect to continue to see relatively greater growth in younger ramets within mature genets.

In this study, flowers and fruits did not apparently provide suitable carbon sinks. Flowers typically represent only a small fraction of total biomass allocation in *L. benzoin* (less than 1%; Cipollini and Whigham 1994). Depending upon fruit set level, fruit production can account for a much greater fraction of total allocation, a cost which is borne only by female plants. As such, we had expected male and female plants to respond differently to elevated CO_2. Whereas initial analyses of growth parameters revealed that nonreproductive ramets often differed from reproductive ramets, means for male and female ramets never differed significantly. In general, fruit production was very low in all three treatments, which perhaps precluded fruits from acting as major resource sinks. The low levels of fruit set recorded (about 1–2% of initial ovule number) were somewhat lower than the 5–20% levels typically seen in *L. benzoin* (Cipollini and Whigham 1994). The relatively low fruit set may be partially attributable to low ambient light levels at the study site (Niesenbaum 1992).

Carbon/nutrient balance

Leaf and stem characteristics were influenced by treatment, with significantly greater leaf density, lower concentrations of leaf and stem nitrogen, and higher concentrations of leaf and stem phenolics for elevated-CO_2 plants. The lowering of tissue nitrogen content is a common result of CO_2 elevation in woody plants, especially under conditions of nutrient limitation (cf., Conroy et al. 1992; El-Kohen et al. 1992). Such shifts are generally associated with relatively greater allocation to storage carbohydrates, fiber or other types of predominantly C-based metabolites (e.g., phenolics) that tend to increase leaf density or leaf specific weight. Our results concerning total phenolics differ somewhat from those of Fajer et al. (1992), who found that CO_2 enrichment and associated increased C/N ratios were not sufficient, in and of themselves, to increase allocation to C-based secondary chemicals in *Plantago lanceolata*. Fajer et al. hypothesized that other factors (hormonal changes, direct nutrient effects) that are typically intimately associated with C/N balance are necessary to produce shifts

in secondary chemical production. In contrast, our results suggest that carbon dioxide enrichment can produce decreased tissue nitrogen levels and increased levels of total phenolics. A strong negative relationship was found between nitrogen content and phenolic content of leaves, and a similar (but non-significant) negative relationship for stems. The negative relationship observed between stem dry mass and phenolics may be explained partially by tradeoffs with new stem growth (cf., Palo et al. 1992). As such, our results are general agreement with carbon/nutrient balance theory.

Because our total phenolic estimates resulted from the use of a simple colorimetric assay, we must be cautious in interpretation of these particular data. Although we found no apparent differences in phenolic profiles among bulked samples from each treatment (as determined by silica gel TLC), differences among individual samples in the numbers and types of phenolics present could affect results of colorimetric tests such as the Prussian Blue or Folin-Denis assays (J. Schultz, pers. comm.). We have also made the assumption that allocation to phenolics correlates strongly with total allocation to C-based secondary compounds; yet, we know that *L. benzoin* also contains other classes of C-based secondary compounds, including mono- and diterpenoids (Gibbs 1974). Although oven-drying may reduce extractability of phenolic compounds (cf., Lindroth and Pajutee 1987), we have assumed that these effects were identical among treatments. At the minimum, our data on total phenolics can be interpreted as showing significant effects of elevated CO_2 on phenolic metabolism, and thus we believe these results should encourage future tests of carbon/nutrient balance theory using the elevated CO_2 approach. Such studies should attempt to more rigorously quantify total allocation to C-based secondary compounds. Additionally, analyses of leaf and shoot chemical characteristics should be examined over the entire course of the seasonal cycle, as a means of accounting for potential effects of CO_2 enrichment on phenological patterns.

Over the early stages of this study, it appears that *L. benzoin* has responded to elevated CO_2 primarily through physiological and biochemical changes, with only small effects on growth. Our results suggest that, as a possible result of nutrient limitation, carbohydrates produced in response to elevated CO_2 may have been allocated primarily to storage and belowground growth, and perhaps to secondary chemicals, rather than to increased stem and leaf growth. Plants subjected to elevated CO_2 in this study seem to be in the process of reallocating resources; leaf and branch production on existing ramets was decreased while growth of young and newly initiated ramets was increased. Alternatively, it is possible that unexamined genetic or environmental factors have constrained growth in these plants, so as to preclude stronger effects of CO_2 elevation. For instance, variation in the light environment can play a large role in growth patterns in *L. benzoin* (Niesenbaum 1992), yet interactions of light and CO_2 elevation remain unaddressed. Because we plan to maintain and monitor the in situ manipulation of CO_2 for several more years, these early results should not be taken to represent final conclusions. Photosynthetic acclimation and feedback effects involving storage and reallocation are expected to take a matter of years to equilibrate.

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