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Consequences of CO_2 and light interactions for leaf phenology, growth, and senescence in *Quercus rubra*

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Abstract

We investigated how light and CO_2 levels interact to influence growth, phenology, and the physiological processes involved in leaf senescence in red oak *(Quercus rubra)* seedlings. We grew plants in high and low light and in elevated and ambient CO_2. At the end of three years of growth, shade plants showed greater biomass enhancement under elevated CO_2 than sun plants. We attribute this difference to an increase in leaf area ratio (LAR) in shade plants relative to sun plants, as well as to an ontogenetic effect: as plants increased in size, the LAR declined concomitant with a decline in biomass enhancement under elevated CO_2

Elevated CO_2 prolonged the carbon gain capacity of shade-grown plants during autumnal senescence, thus increasing their functional leaf lifespan. The prolongation of carbon assimilation, however, did not account for the increased growth enhancement in shade plants under elevated CO_2. Elevated CO_2 did not significantly alter leaf phenology. Nitrogen concentrations in both green and senesced leaves were lower under elevated CO_2 and declined more rapidly in sun leaves than in shade leaves. Similar to nitrogen concentration, the initial slope of A/C_i curves indicated that Rubisco activity declined more rapidly in sun plants than in shade plants, particularly under elevated CO_2. Absolute levels of chlorophyll were affected by the interaction of CO_2 and light, and chlorophyll content declined to a minimal level in sun plants sooner than in shade plants. These declines in N concentration, in the initial slope of A/C_i curves, and in chlorophyll content were consistent with declining photosynthesis, such that elevated CO_2 accelerated senescence in sun plants and prolonged leaf function in shade plants. These results have implications for the carbon economy of seedlings and the regeneration of red oak under global change conditions.

Keywords: CO_2 and light interactions, functional leaf lifespan, nitrogen, phenology, *Quercus rubra*, senescence

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Introduction

A realistic understanding of the effects of rising concentrations of CO_2 on plants in heterogeneous environments requires knowledge of the interactive effects of CO_2 with other resources. In this study, we investigated how CO_2 interacts with light to influence growth, phenology, and the physiological processes involved in senescence in red oak, *Quercus rubra*. Red oak is a dominant forest species in many communities in the North-eastern United States and is an important species for carbon sequestration and forest function in this region (Kleiner *et al*. 1992; Kubiske & Abrams 1992; Wofsy *et al*. 1993; Bassow 1995; Goulden *et al*. 1996).

Although many CO_2 experiments on plants are carried out under natural or high light conditions, those experiments that have incorporated contrasting light regimes have often found higher CO_2 enhancement ratios (ratio of biomass at elevated CO_2 to biomass at ambient CO_2; E/A) under low light than under high light in both woody (Miao *et al*. 1992; Wayne & Bazzaz 1997) and herbaceous species (Ford & Thorne 1967; Gifford 1977; Wheeler *et al*. 1991; Rufty *et al*. 1994). A meta-analysis on the effects of elevated CO_2 on woody plants

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showed that growth increased under elevated CO_2 relative to ambient CO_2 by an average of 52% under conditions of low light compared to only 31% at higher light levels (Curtis & Wang 1998). In the present study, we sought to determine whether this phenomenon was true for red oak, and if so, whether it could be accounted for by changes in phenology and senescence. We also investigated the physiological changes accompanying senescence in order to gain insights into the mechanisms involved. Specifically, we tested the following hypotheses:

1 Changes in phenology and leaf lifespan of plants contribute significantly to differential biomass enhancement of sun-and shade-grown plants under elevated CO_2.

2 In contrasting light regimes, CO_2 differentially affects N dynamics [including N translocation, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity, and chlorophyll degradation] during senescence, which can explain changes in carbon gain capacity.

Influences of light and CO_2 on phenology and senescence

Previous studies have shown that leaf phenology and leaf lifespan change with light availability (e.g. Williams *et al*. 1989; Ackerly & Bazzaz 1995). Differences in leaf emergence and senescence between the canopy and the understorey have been considered important in the regeneration dynamics of understorey seedlings (Uemura 1994; Kurosawa 1995; Gill *et al*. 1998); seedlings often put out leaves in the understorey prior to budbreak in the canopy. Leaf senescence in the canopy also may precede leaf senescence of understorey seedlings (Uemura 1994). Thus, seedlings may experience a longer growing season than overstorey trees.

Elevated CO_2 has also been shown to change leaf phenology in both herbaceous (Cook *et al*. 1998) and woody species (Jach & Ceulemans 1999). In herbaceous species, elevated CO_2 is thought to accelerate phenological processes such as bud break, flowering, and senescence (Garbutt *et al*. 1990; Reekie & Bazzaz 1991; Navas *et al*. 1997). In woody species, however, the effects are less clear. Among evergreen coniferous species, CO_2 accelerated leaf emergence in *Pinus sylvestris* (Jach & Ceulemans 1999), although not in *Picea abies* (Roberntz 1999), and senescence was not affected in *Pseudotsuga menziesii* (Olszyk *et al*. 1998). CO_2 had no effect on the timing of leaf senescence in several deciduous species, including *Quercus alba*, *Liriodendron tulipfera* (Gunderson *et al*. 1993) and *Populus tremuloides* (Curtis 1999). *Populus grandidentata*, however, showed delayed senescence under elevated CO_2 (Curtis 1992), and *Castanea sativa* seedlings showed delayed bud break and slightly earlier senescence under elevated CO_2 (Mousseau *et al*. 1996). McConnaughay *et al*. (1996) found lower end-of-season photosynthetic rates in five species of deciduous tree seedlings under elevated-CO_2, although there were no clear patterns in rates of leaf loss.

Effects of light and CO_2 on photosynthesis and the physiological processes during senescence

Leaf senescence is a highly coordinated process in which the photosynthetic apparatus within the chloroplast is dismantled, and the chloroplast contents are remobilized (Crafts-Brandner *et al*. 1990). Degradation of chlorophyll is known to be a primary process in leaf senescence (e.g. Dean *et al*. 1993; Embry & Nothnagel 1994; Masoni *et al*. 1994), and is regulated by phytochrome, which responds to changes in light quality and quantity (Okada *et al*. 1992). Decreasing photoperiod and high light both increase chlorophyll degradation (Hidema *et al*. 1992; Okada *et al*. 1992). Chlorophyll loss during senescence is accompanied by degradation of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and other leaf proteins, leaf N resorption, as well as declining photosynthetic capacity (Adams *et al*. 1990; Tobias *et al*. 1995). Photosynthesis is thought to cease when the chlorophyll and other components of the photosynthetic apparatus decline below a minimum level (Tobias *et al*. 1995; Aerts 1996). Little is known about how elevated CO_2 interacts with light to influence these senescence processes.

Materials and methods

Experimental design

Plants were grown from acorns in glasshouse facilities at Harvard University in two light and two CO_2 regimes for three years. Germinants were transplanted into individual pots within 1–2 weeks after emergence. Soil was taken from a red oak stand at the Harvard Forest (Petersham, MA) to ensure that seedlings would be exposed to typical soil conditions with respect to soil microflora and other factors. The high light treatment was achieved by natural light filtering through the glass of the greenhouse (~70% of full sun), and the low light treatment was achieved with neutral density shade cloth (~10% of full sun). CO_2 treatments were ambient (~380 $\mu L\,L^{-1}$) and elevated CO_2 (700 $\mu L\,L^{-1}$), and each treatment was replicated in three blocks. There were 30 plants in each of four treatments (10 plants per block) for a total of 120 plants. Care was taken to make sure that plants were not pot limited and were sequentially transplanted each growing season in larger pots starting in 10 L, then 40 L, and finally 120 L. New field soil was provided when seedlings were transplanted to larger

pots making addition of nutrients unnecessary. Relatively high leaf N levels (Fig. 4b) were indicative of ample nutrient supply.

Simulated autumnal senescence. We imposed an autumnal senescence treatment on seedlings starting in October at the end of the first and second year of growth. Measurements were made only after the second year of growth. Temperature and photoperiod were decreased for seven weeks. The plants were initially removed from glasshouse conditions at 25 °C day/19 °C night and moved to six growth chambers at 22 °C day/15 °C night. The temperature was dropped by 3 °C each week until a steady temperature of 9 °C day/3 °C night was reached beginning in week five. The photoperiod in the chambers began at 11 h, similar to the natural photoperiod that the plants had been experiencing in the glasshouse. Each week the photoperiod was decreased by 16 minutes for five weeks until the photoperiod reached 9 h and 40 min, similar to the natural reduction in photoperiod during fall in New England. Within each chamber, shade cloth was hung dividing it diagonally into two compartments, one with high light (700 μmol m^{-2} s^{-1}) and one with low light (80 μmol m^{-2} s^{-1}). These light levels are comparable to forest edges and understorey conditions, respectively, in which red oak would naturally regenerate (George 1996). Five plants were put into each growth chamber compartment so that there were 10 plants per compartment and 60 plants in total. Seedlings were not allowed to touch each other or the chamber walls. Each week, seedlings were rearranged randomly within their treatments to minimize possible effects of spatial heterogeneity.

Growth

Sixty plants were harvested at the end of their second year of growth to determine total dry biomass, root:shoot ratio, total leaf area, leaf mass per area (LMA), stem height, and leaf-area ratio (leaf area: total biomass; LAR). At the end of the third year, the remaining 60 plants were harvested for the same measurements. Total leaf area and stem height were measured at the end of the first growing season, but no plants were harvested. Leaf area was measured on intact leaves for the first year using a portable leaf-area meter (Li-Cor Li-3000, Lincoln, NB, USA) and on detached leaves for years 2 and 3 using an Li-3100.

Leaf-level measurements

Leaf phenology. Leaf emergence was recorded for each plant by labelling leaf flushes with the dates when the leaves emerged. The rate of leaf senescence, as indicated by leaf browning, was determined first by measuring total leaf area of intact leaves for each plant (as above) and then by estimating the percentage of pigmented leaf area (leaf area that was not brown) every week for all leaves of each plant during the senescent period.

Photosynthesis and carbon gain. During the senescent period, photosynthetic rates and transpiration rates were measured once weekly from one leaf of each flush for all of the experimental seedlings using portable gas exchange systems (Li-Cor 6400; Li-Cor, Lincoln, Nebraska). Light level, CO_2 concentration and temperature within the leaf cuvette were matched to the environmental conditions of the growth chambers. Whole-plant photosynthetic rate was calculated by multiplying the photosynthetic rate, measured on one leaf per flush, by the estimated functional leaf area of each flush. By making the assumption that leaves within a flush had the same photosynthetic rate, these data allowed calculation of whole plant photosynthetic rate on a flush-by-flush basis each week. We tested this assumption prior to and during the senescent period and found it to hold across treatments and flushes (data not shown).

A/C_i curves. The response of assimilation to decreasing internal CO_2 partial pressures (C_i) was measured on three plants per treatment (one from each block) for a total of 12 plants. Measurements were made before the start of senescence in August of the second year, and at three times during senescence on the same individuals, using the Li-Cor 6400 under saturating light (1700 μmol m^{-2} s^{-1}) at 25 °C. Consistent environmental conditions were desired so that slopes could be compared at different times throughout senescence. Leaves were acclimated to each CO_2 concentration for a minimum of 10 min. Nine points were measured for each curve, with a maximum C_i of ~60 Pa. Initial slopes of the relationship determined from the linear part of the curve were used as an indication of Rubisco activity (von Caemmerer & Farquhar 1981; Wullschleger 1993).

Chlorophyll content. Relative chlorophyll content on an area basis was measured on intact leaves with a Minolta SPAD-502 meter (Spectrum Technologies, Inc., Plainfield, IL, USA). Measurements were made on one leaf of each flush, taking the average of six measurements per leaf. These values were converted to actual concentrations by a regression between SPAD values and measured concentrations. Thirty 1-cm^2 leaf samples from plants at different stages of senescence throughout the experiment were extracted in N,N-dimethylformamide for chlorophyll. SPAD values were measured prior to removing leaf tissue. Absorbance of chlorophyll was measured with a dual beam spectrophotometer (Shimadzu, Tokyo,

Japan) at wavelengths of 664.5 and 647 nm. We calculated chlorophyll concentrations according to previously described procedures (Moran & Porath 1980; Inskeep & Bloom 1985). The following calibration curve was used to calculate chlorophyll concentration from SPAD values: $y = 0.0147x + 0.227$ ($R^2 = 0.89$), where y = chlorophyll concentration ($\mu g\,cm^{-2}$) and x = SPAD absorbance (relative units).

Nitrogen concentration. At three different time periods, mid-August, week three of the senescent period, and two weeks after the final physiological measurements were taken, 3-cm^2 leaf pieces were cut from the tip next to the midvein from one leaf in the top flush of each plant. The leaf tissue (excluding 1° or 2° veins) was ground finely in liquid N using a mortar and pestle. Approximately 200 µg of this homogenate was used to determine percentage leaf N using a Europa Elemental Analyser (model ANCA-sl) attached to a Europa model 20–20 Ssotope Analyser via a capillary interface (Europa Instruments, England).

Statistical analysis

Bootstrapping (resampling with replacement, 1000 randomizations) was used to generate 95% confidence intervals to determine whether CO_2 enhancement ratios of sun-grown and shade-grown plants at the end of two and three years of growth were significantly different from 1.0 using the software package Resampling Statistics (Simon 1995). A permutation test in which CO_2 labels were randomly re-assigned to data without replacement (1000 randomizations) within light treatments, was then used to generate a null distribution of differences between sun- and shade-CO_2 enhancement ratios. The 95% confidence limits generated from this were used to determine whether the observed difference between CO_2 enhancement ratios in sun and shade plants was significantly different from 0. CO_2 enhancement ratios are usually calculated by taking the ratio of the means of total plant biomass in elevated CO_2 to those in ambient CO_2, producing a derived statistic for which we have no *a priori* knowledge of the probability distribution. By using bootstrapping and assuming our data are a random sample of the total population, we were able to empirically derive the distribution of our statistic under the null hypotheses and determine confidence limits for it.

Two-way repeated-measures anova was used to determine the main fixed effects of CO_2, light, block, and their interactions over time on photosynthesis, canopy photosynthesis, timing of leaf senescence, initial slopes of A/C_i curves, chlorophyll content, and N content. Analyses were performed using Statistica (StatSoft 1995). All main effects, including time, were treated as fixed factors with their mean squares tested over the mean square of the error, except time and interactions with time, which were tested over the mean square of the within-subject error. This error term is referred to as the repeated measures error in Table 2. Prior to anova, percentage data (for timing of leaf senescence and leaf N content) were transformed using the arcsine of the square root of the value (Sokal & Rohlf 1995, pp. 419–421).

Two-way anova was used to determine the main effects of CO_2, light, block, and their interactions on leaf emergence date. Blocks were nested in CO_2, all main effects were treated as fixed factors, and their mean squares were tested over the residual means square.

Results

Growth

The results of the bootstrapping analysis show that at the end of three years shade-grown plants had an enhancement ratio (E/A) of 1.8, which was significantly greater than the CO_2 enhancement ratio of 1.5 for sun-grown plants as determined by 95% confidence limits. Second-year enhancement ratios also reflected this trend but the differences were not significant (Table 1). In all cases, enhancement ratios were significantly different from 1. Greater enhancement under elevated CO_2 in shade plants was paralleled by higher LAR in shade plants relative to sun plants at the end of the third growing season (Table 1). This is reflected in the steeper slope of the relationship between leaf area and total biomass in shade plants relative to sun plants (Fig. 1a). These data show that for a given biomass, shade plants had higher leaf area than sun plants, or alternatively, that shade plants produced less biomass per unit leaf area.

LAR decreased with increasing biomass (Fig. 1b). This decline in LAR was paralleled by a decline in CO_2 enhancement ratio (E/A) with size (Fig. 1c). This suggests an important ontogenetic effect, such that as plants get larger their LAR and enhancement under elevated CO_2 decline, regardless of light availability. Root-to-shoot ratios showed no significant differences after the second year of growth. After the third year under elevated CO_2, however, sun plants had significantly higher root biomass relative to shoot biomass than shade plants (Table 1). LMA was significantly higher in sun plants than in shade plants for both years, while there was no significant difference between CO_2 treatments. LMA was consistent between years and did not change with plant size (Table 1).

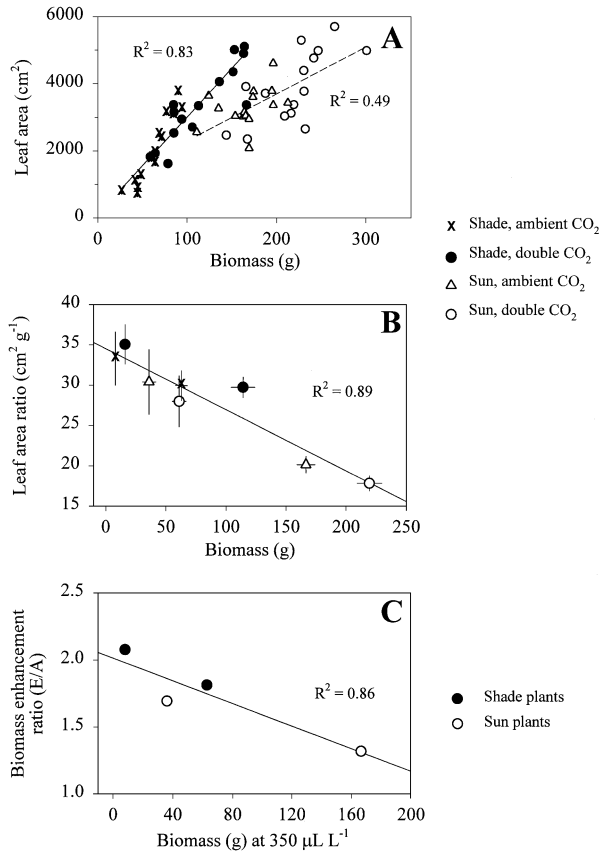

Fig. 1 (a) The relationship between leaf area and biomass for all plants at the end of the third year of growth. There were significant differences in slopes between contrasting light treatments, but not between slopes of contrasting CO_2 treatments. Therefore, regression slopes are shown only for sun and shade treatments with the CO_2 treatments pooled. Shade plants had significantly higher leaf area per unit biomass than sun plants. (b) Treatment means of leaf-area ratio at the end of the second and third years of growth showing a decline in LAR with size. In both (a) and (b), X, shade plants in ambient CO_2; ●, shade plants in elevated CO_2; △, sun plants in ambient CO_2; ○, sun plants in elevated CO_2. Error bars in (b) are ± 1 SE. (c) The biomass enhancement ratio (E/A; ratio of biomass under elevated CO_2 to biomass under ambient CO_2) plotted against biomass under ambient CO_2 for years two and three. This shows that E/A declines as plants get bigger, indicating a strong ontogenetic effect. Open circles depict sun plants, and closed circles depict shade plants.

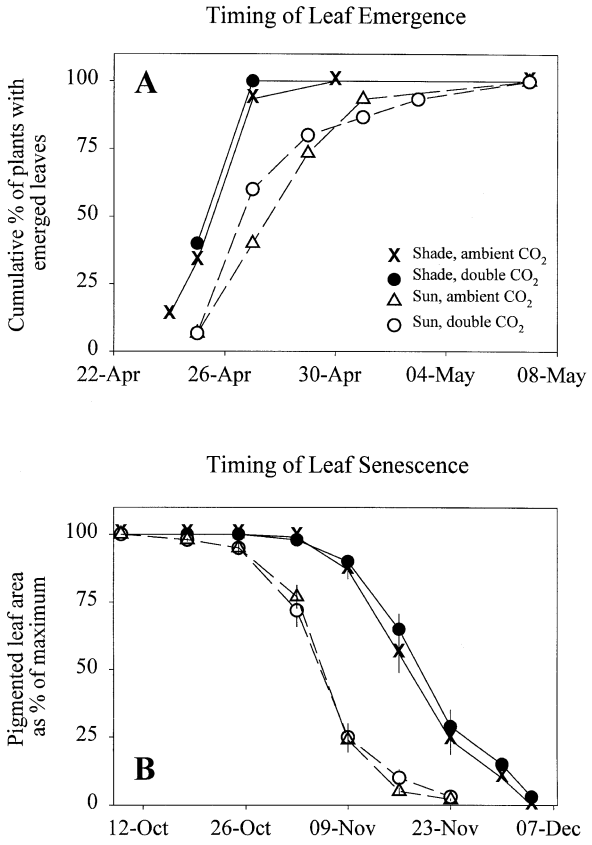

Fig. 2 (a) Timing of spring leaf emergence as shown by the cumulative percentage of plants with emerged leaves, and (b) timing of leaf senescence measured by the pigmented leaf area as a percentage of maximum ($N = 60$). Dashed and solid lines represent sun and shade plants, respectively. Symbols are the same as in Fig 1a and b. Error bars in (b) are ± 1 SE.

Phenology

The timing of leaf emergence was not significantly affected by light or CO_2 (Fig. 2a), although a trend toward delayed emergence is apparent in sun plants. High light significantly accelerated leaf senescence (leaf browning), while elevated CO_2 had no significant effect (Fig. 2b, Table 2).

Physiological processes during senescence

Leaf-level and whole plant photosynthesis. Elevated CO_2 and high light led to increased initial photosynthetic rates and whole plant carbon gain. However, under high light, leaf-level photosynthesis and canopy photosynthesis declined more rapidly in both elevated and ambient CO_2 during senescence (Fig. 3a,b). Among the shade plants, elevated CO_2 prolonged leaf-level and whole plant photosynthetic rates, leading to a sharp increase during the last two weeks in the ratio of canopy photosynthesis under elevated CO_2 to that under ambient CO_2 (inset Fig. 3b). The influence of CO_2 in prolonging photosynthesis in shade plants contrasts with the phenological results showing only a significant influence of light on loss of pigmented leaf area (Table 2).

During the last few weeks of the senescence period, shade plants under elevated CO_2 had higher photosyn-

Table 1. Growth and allometric data for red oak seedlings under contrasting light and CO_2 treatments. Biomass enhancement ratios under elevated CO_2 are shown for shade and sun plants after the second and third years of growth. Means are shown for total biomass, total leaf area, stem height, root : shoot, leaf mass per area (LMA), and leaf area ratio (LAR), for all treatments after years 1, 2, and 3 or for years 2 and 3 only. SE are given after the mean, and means are shown as different (within years) if $P < 0.05$. See text for details.

	Shade						Sun					
Biomass enhancement ratio			2.06		a				1.69		a	
Year 2			1.81		a				1.32		b	
Year 3	Ambient CO_2			Elevated CO_2			Ambient CO_2			Elevated CO_2		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE	
Biomass (g)												
Year 2	8.06	1.30	a	16.14	2.34	a	36.07	5.61	b	61.08	5.62	c
Year 3	63.01	4.99	a	114.17	9.98	c	166.67	7.68	b	219.42	10.43	d
Total Leaf Area (cm^2)												
Year 1	153.76	11.64	a	213.04	15.16	ab	204.84	14.84	ab	300.19	23.63	b
Year 2	230.21	32.99	a	535.68	69.65	a	1051.58	165.26	b	1561.89	146.11	c
Year 3	1982.37	254.16	a	3355.52	296.91	b	3296.74	151.56	b	3917.02	279.24	c
Stem Height (cm)												
Year 1	11.62	1.21	a	14.70	1.14	a	17.85	1.66	a	19.26	2.04	a
Year 2	18.35	1.06	a	30.94	2.69	a	37.74	3.80	b	46.08	3.69	b
Year 3	59.40	7.37	a	90.23	10.54	b	65.60	4.97	ac	73.50	5.20	c
Root : Shoot ($g\ g^{-1}$)												
Year 2	2.14	0.27	a	1.79	0.28	a	1.67	0.28	a	1.93	0.27	a
Year 3	2.21	0.35	ab	1.53	0.18	a	2.47	0.12	ab	2.75	0.17	b
LMA ($mg\ cm^{-2}$)												
Year 2	4.89	0.10	a	5.22	0.17	a	7.18	0.13	b	6.99	0.17	b
Year 3	5.42	0.16	a	4.91	0.22	a	7.50	0.44	b	7.40	0.13	b
LAR ($cm^2\ g^{-1}$)												
Year 2	33.29	3.31	a	35.05	2.49	a	30.38	3.99	a	27.97	3.18	a
Year 3	29.94	1.88	a	29.72	1.31	a	20.13	1.04	b	17.83	0.92	b

Table 2. Analysis of variance for photosynthesis (μmol CO_2 $m^{-2}s^{-1}$), carbon gain (μmol CO_2 $plant^{-1}$ s^{-1}), chlorophyll content (mg cm^{-2}), leaf nitrogen content (g $100g^{-1}$ (arcsin transformed)), rubisco activity (initial slope of ACi curve), leaf emergence (date of bud-break, Julian days), leaf senescence (pigmented leaf area, arcsin transformed % of max.). Photosynthesis, carbon gain, chlorophyll, nitrogen content, rubisco, and leaf senescence were tested as repeated measures ANOVAs with CO_2, light level and block treated as fixed factors; block was nested in CO_2 treatment; time was the repeat factor. Mean squares of all main effects and their interactions were tested over residual a, except for time or interactions with time, which were tested over residual b. (Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$.)

	df	MS	F	P
Photosynthesis (in situ)				
Block	4	17.62	1.95	0.117
CO_2	1	308.04	34.10	0.000***
Light	1	198.33	21.95	0.000***
Time	6	432.79	97.52	0.000***
Block*Light	4	14.25	1.58	0.195
CO_2*Light	1	35.56	3.94	0.053
Block*Time	24	2.84	0.64	0.905
CO_2*Time	6	6.29	1.42	0.208
Light*Time	6	55.22	12.44	0.000***
Block*Light*Time	24	3.15	0.71	0.841
CO_2*Light*Time	6	17.14	3.86	0.001***
Residual a	48	9.03		
Residual b	288	4.44		
Carbon gain				
Block	4	25433976	2.04	0.104
CO_2	1	549108736	44.11	0.000***
Light	1	636188160	51.10	0.000***
Time	6	447525664	161.08	0.000***
Block*Light	4	9132025	0.73	0.574
CO_2*Light	1	8140109	0.65	0.423
Block*Time	24	4945152	1.78	0.015**
CO_2*Time	6	46455668	16.72	0.000***
Light*Time	6	193863744	69.78	0.000***
Block*Light*Time	24	2580131	0.93	0.563
CO_{22}*Light*Time	6	13862179	4.99	0.000***
Residual a	47	12449957		
Residual b	282	2778325		
Timing of leaf emergence				
Block	4	13.4	0.562	0.691
CO_2	1	9	0.377	0.542
Light	1	28.3	1.183	0.282
Block*Light	4	33.9	1.419	0.242
CO_2*Light	1	30.1	1.261	0.267
Residual	48	23.9		
Timing of leaf senescence				
Block	4	337.8	1.48	0.225
CO_2	1	75.5	0.33	0.568
Light	1	40685.0	177.83	0.000**
Time	6	47661.0	716.79	0.000***
Block*Light	4	557.3	2.44	0.061
CO_2*Light	1	45.2	0.20	0.659
Block*Time	24	89.7	1.35	0.132
CO_2*Time	6	107.1	1.61	0.144
Light*Time	6	4242.6	63.81	0.000***
Block*Light*Time	24	81.3	1.22	0.221
CO_2*Light*Time	6	15.0	0.23	0.968
Residual a	46	228.8		
Residual b	276	66.5		
Chlorophyll content				
Block	4	290	2.15	0.089
CO_2	1	4	0.03	0.857
Light	1	7887	58.48	0.000***
Time	6	2941	256.56	0.000***
Block*Light	4	42	0.32	0.866
Light*CO_2	1	1088	8.06	0.007**
Block*Time	24	11	0.95	0.528
Light*Time	6	59	5.16	0.000***
CO_2*Time	6	18	1.54	0.166
Block*Light*Time	24	8	0.71	0.839
Light*CO_2*Time	6	25	2.15	0.048*
Residual a	47	135		
Residual b	282	11		
Initial slope of A/C_i curves				
CO_2	1	0.00014	7.73	0.024*
Light	1	0.000066	3.65	0.092
Time	3	0.001968	38.78	0.000***
Light*CO_2	1	0.000149	8.23	0.021*
CO_2*Time	3	0.00012	2.37	0.096
Light*Time	3	0.000189	3.72	0.025*
Light*CO_2*Time	3	0.000031	0.60	0.620
Residual a	8	0.000018		
Residual b	24	0.000051		
Nitrogen content				
Block	4	5.49	4.05	0.007**
CO_2	1	34.92	25.77	0.000***
Light	1	71.30	52.61	0.000***
Time	2	464.26	1232.24	0.000***
Block*Light	4	1.38	1.02	0.408
CO_2*Light	1	8.40	6.20	0.017*
Block*Time	8	0.67	1.78	0.094
CO_2*Time	2	0.41	1.10	0.338
Light*Time	2	12.41	32.95	0.000***
Block*Light*Time	8	1.30	3.46	0.002**
CO_2*Light*Time	2	1.64	4.34	0.016*
Residual a	41	1.36		
Residual b	82	0.38		

thetic rates, and therefore, presumably higher carbon gain than sun plants. Rough calculations of the magnitude of carbon gain, however, indicate that the extra amount gained by shade plants under elevated CO_2 during the senescent period was insignificant relative to the total carbon acquired by plants throughout the growing season. This small amount of additional late-season carbon in shade plants under elevated CO_2 could not account for the overall difference in biomass enhancement between sun and shade plants. Nevertheless, it could be important for new leaf produc-

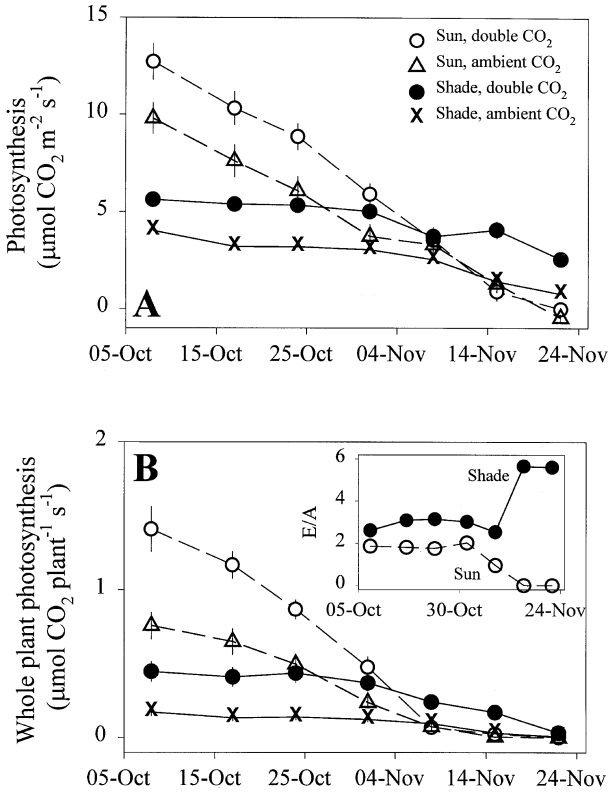

Fig. 3 (a) Weekly photosynthetic rates, and (b) whole plant photosynthesis throughout the 7-week period of simulated autumnal senescence ($N = 60$). Dashed and solid lines represent sun and shade plants, respectively. Error bars are ± 1 SE. The ratio of whole plant photosynthesis under elevated CO_2 to that under ambient CO_2 (E/A) is shown for the same time period in the inset to (b). Closed circles depict shade plants, and open circles depict sun plants.

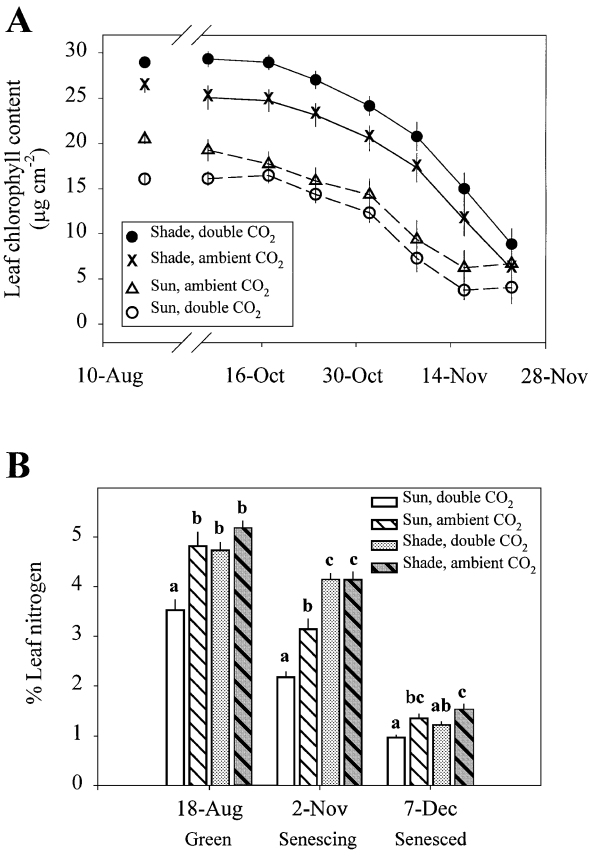

Fig. 4 (a) Chlorophyll content throughout the course of autumnal senescence ($N = 12$); symbols are the same as in Figs 2 and 3. Measurements in August prior to the onset of senescence are shown before the break in the x-axis. (b) Nitrogen concentration of leaves (g N [100 g leaf]$^{-1}$), measured in August before the onset of senescence, during senescence, and after all leaves had turned brown. Leaves had completely senesced by December. Treatments are shown as different if $P < 0.05$ (Scheffé's posthoc multiple comparisons). Error bars are ± 1 SE.

tion the following spring, depending on how end-of-season photosynthate is allocated. For example, there was a sharp, and significantly greater, relative increase in leaf production during the third year of growth in shade plants under elevated CO_2 compared with the other treatments (Table 1).

Chlorophyll, A/C_i curves, and N concentration. By inspection, all of the chlorophyll degradation curves had approximately the same shape (Fig. 4). This indicates that there was no difference in the degradation rate between the different treatments. Repeated measures anova indicates a significant change in the rate of chlorophyll degradation over time due to light but not to CO_2 (Table 2). This light-by-time interaction appears to result from the fact that sun plants reached their minimum chlorophyll content in week 6 and then plateaued.

Light significantly affected the absolute chlorophyll content. Seedlings grown in the shade had significantly higher chlorophyll content than those grown in the sun ($P < 0.0001$) prior to the onset of senescence (data not shown), and throughout the senescent period (Fig. 4a). CO_2 interacted with light to affect chlorophyll content such that elevated CO_2 decreased chlorophyll content in high light and increased chlorophyll content in the shade.

At the onset (18 August), there was no significant difference in the initial slopes of the A/C_i curves suggesting similar Rubisco activity (Fig. 5a–c). The rate at which these initial slopes declined, however, differed significantly between treatments. The initial slopes of A/C_i curves in sun plants – particularly under elevated CO_2 – declined markedly by mid-November, in contrast to shade plants. This decline in sun plants was significantly greater than for shade plants as indicated

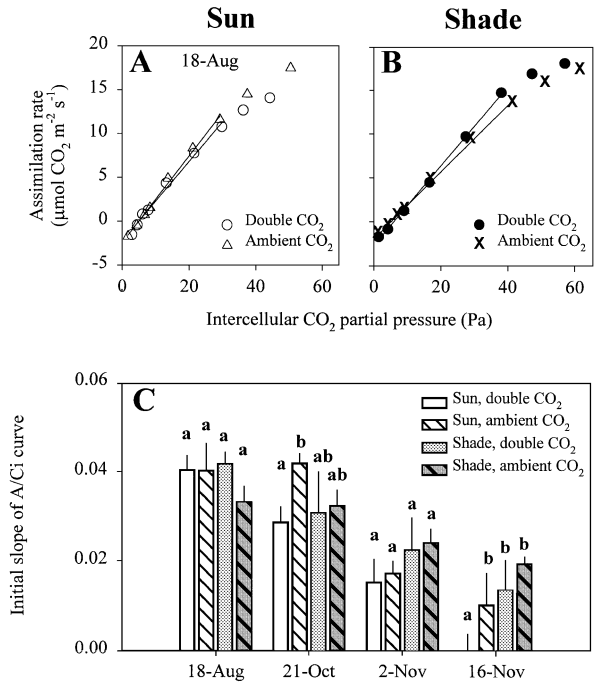

Fig. 5 Representative A/C_i curves for all treatments, measured in August prior to senescence for (a) sun plants and (b) shade plants. Initial slopes were used to indicate Rubisco activity. There were no significant differences between slopes prior to senescence. (c) Means of initial slopes of A/C_i curves, measured in August and at three time-points throughout the course of senescence. Treatments are shown as different if $P < 0.05$ (Scheffé's posthoc multiple comparisons). Error bars are ± 1 SE.

by the light-by-time interaction (Table 2, Fig. 5). CO_2 also significantly affected the rate of decline in initial slopes, such that within the sun plants elevated CO_2 led to a more rapid decline, but within the shade plants there was no significant difference between the CO_2 treatments (Table 2). Consistent with chlorophyll content and initial slopes of the A/C_i curves, N concentration declined significantly earlier in sun plants than in shade plants (Fig. 4). The same pattern results if N content is calculated on a leaf-area basis from measurements of LMA.

Discussion

We demonstrate in this study that growth enhancement in response to elevated CO_2 is significantly increased in shade relative to sun in *Quercus rubra* after three years of growth. This finding is consistent with other studies that show that elevated CO_2, on average, has a greater effect on growth in low light than in high light (reviewed in Curtis & Wang 1998). The mechanism for this interaction of CO_2 and light on growth has not yet been determined. We tested whether changes in leaf phenology or leaf lifespan could account for the differential effect that CO_2 has in the sun relative to the shade. In the shade we found that elevated CO_2 prolonged the carbon gain capacity of leaves significantly, thereby extending functional leaf lifespan. At the same time, in the sun, photosynthesis declined more quickly under elevated CO_2 than under ambient CO_2. Although these trends are consistent with the increased CO_2 biomass enhancement in the shade, the extension of functional leaf lifespan did not contribute significantly to this effect. Therefore, we can reject our initial hypothesis that changes in phenology and senescence are the cause of greater CO_2 biomass enhancement in the shade.

Analysis of the growth data, however, revealed an important ontogenetic effect. The ratio of biomass under elevated CO_2 to biomass under ambient CO_2 (E/A) declined with size for both sun- and shade-grown plants (Fig. 1c). This decline can be explained, in large part, by the decrease in leaf area ratio with size (Fig. 1b). As plants increased in size, the allocation to photosynthetic tissue decreased relative to allocation to woody tissue, thereby diminishing the effect of photosynthetic enhancement on overall biomass. Note, however, that even when controlling for size, shade plants still had greater leaf area than sun plants by the end of the third growing season (Fig. 1a). Indeed, increased LAR is a common response to shading (Phares 1971; Peace & Grubb 1982). Thus, higher LAR in shade plants, independent of size, also likely contributed to greater growth enhancement under elevated CO_2 in shade plants.

Osborne *et al*. (1997) showed that CO_2 had a greater relative effect on light-limited photosynthesis than on light-saturated photosynthesis in Indiana strawberry. This effect, if present in red oak, could also have contributed to the higher CO_2 biomass enhancement under low light in red oak, although we did not test this in the present study.

During simulated autumnal senescence, sun plants experienced earlier and more rapid decline in photosynthesis and in degradation of the chloroplast machinery, as indicated by changes in N dynamics. High light is known to accelerate leaf senescence via a phytochrome-mediated process (Hidema *et al*. 1992; Okada *et al*. 1992). The onset of chlorophyll degradation occurred sooner in sun plants, particularly under elevated CO_2, and this was accompanied by an accelerated decline in the initial slopes of A/C_i curves and in photosynthetic rates of sun plants, particularly under elevated CO_2. Of all the treatments, sun plants under elevated CO_2 also had the most rapid N loss in leaf tissue.

Prior to the onset of senescence, however, there was no significant difference in the initial slopes of the A/C_i curves, suggesting similar Rubisco activity among treat-

ments (Fig. 5a–c). This indicates that there was no decline of Rubisco activity in red oak under elevated CO_2 after two years. Rubisco activity and photosynthetic rates have been shown to decline in some species under elevated CO_2, and mechanisms for this have been proposed (Bowes 1991; Heineke *et al*. 1999; Stitt & Krapp 1999); although Drake *et al*. (1997) showed that Rubisco activity could decline without an accompanying decline in photosynthetic rate. Other evidence indicates that neither Rubisco activity nor photosynthetic rates decline under elevated CO_2 (Gunderson *et al*. 1993), and that photosynthetic response is not necessarily linked to biomass enhancement (Ceulemans *et al*. 1999; Norby *et al*. 1999).

Shade plants under elevated CO_2 appear to have allocated more N to chlorophyll, and higher chlorophyll content was maintained by shade plants under elevated CO_2 throughout the senescent period. There was no significant difference in the inferred Rubisco activity within the shade treatments at the end of the senescent period, and final N content was actually lower under elevated CO_2. Therefore, higher chlorophyll content may be responsible for the prolonged leaf functioning in elevated CO_2 plants in the shade.

The interactive effects of contrasting CO_2 and light regimes on red oak seedlings have implications for seedling survival under global change conditions. It is possible that even small increases in end-of-season carbon gain could enhance leaf production in the spring, depending on how that carbon is allocated. Moreover, different species may show different interactive effects of elevated CO_2 and light availability on phenology, which could influence species' interactions. Finally, under global change conditions, the regeneration niche of red oak may expand if seedlings and saplings are able to survive in more shaded areas of the forest.

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References

Ackerly DD, Bazzaz FA (1995) Leaf dynamics, self-shading and carbon gain in seedlings of a tropical pioneer tree. *Oecologia*, **101,** 289–298.

Adams WW, Winter K, Schreiber U, Schramel P (1990) Photosynthesis and chlorophyll fluorescence characteristics in relationship to changes in pigment and element composition of leaves of *Platanus occidentalis L.* during autumnal leaf senescence. *Plant Physiology*, **93,** 1184–1190.

Aerts R (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? *Journal of Ecology*, **84,** 597–608.

Bassow SL (1995) *Canopy photosynthesis and carbon cycling in a deciduous forest: implications of species composition and rising concentrations of CO_2*. Thesis, Harvard University, Cambridge, MA.

Bowes G (1991) Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. *Plant, Cell and Environment*, **14,** 795–806.

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, **153,** 376–387.

Ceulemans R, Janssens IA, Jach ME (1999) Effects of CO_2 enrichment on trees and forests: lessons to be learned in view of future ecosystem studies. *Annals of Botany*, **84,** 577–590.

Cook AC, Tissue DT, Roberts SW, Oechel WC (1998) Effects of long-term elevated [CO_2] from natural CO_2 springs on Nardus stricta: Photosynthesis, biochemistry, growth and phenology. *Plant Cell and Environment*, **21,** 417–425.

Crafts-Brandner S, Salvucci M, Egli D (1990) Changes in ribulosebisphosphate carboxylase/oxygenase and ribulose 5-phosphate kinase abundances and photosynthetic capacity during leaf senescence. *Photosynthesis Research*, **23,** 223–230.

Curtis PS (1992) Seasonal responses of leaf gas exchange to elevated carbon dioxide in *Populus grandidentata*. *Canadian Journal of Forest Research*, **22,** 1320–1325.

Curtis PS (1999) Elevated CO_2 effects on leaf senescence and patterns of litterfall. In: *Abstracts of the Ecological Society of America Annual Meeting, Spokane, WA.*

Curtis P, Wang X (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia*, **113,** 299–313.

Dean MA, Letner CA, Eley JH (1993) Effect of autumn foliar senescence on chlorophyll a: b ratio and respiratory enzymes of *Populus tremuloides*. *Bulletin of the Torrey Botanical Club*, **120,** 269–274.

Drake BG, Gonzalez Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology*, **48,** 609–639.

Embry JL, Nothnagel EA (1994) Photosynthetic light-harvesting during leaf senescence in *Panicum miliaceum*. *Plant Science*, **95,** 141–152.

Ford MA, Thorne GN (1967) Effect of CO_2 concentration on growth of sugar beet, barley, kale, and maize. *Annals of Botany*, **31,** 629–644.

Garbutt K, Williams WE, Bazzaz FA (1990) Analysis of the differential response of five annuals to elevated CO_2 during growth. *Ecology*, **71,** 1185–1194.

George LO (1996) *The Understory as an Ecological Filter.* Harvard University, Cambridge, MA.

Gifford RM (1977) Growth pattern, carbon dioxide exchange and dry weight distribution in wheat growing under different photosynthetic environments. *Australian Journal of Plant Physiology*, **4,** 99–100.

Gill DS, Amthor JS, Bormann FH (1998) Leaf phenology, photosynthesis, and the persistence of saplings and shrubs in a mature northern hardwood forest. *Tree Physiology*, **18,** 281–289.

Goulden ML, Munger JW, Fan S-M, Daube BC, Wofsy SC (1996) Exchange of carbon dioxide by a deciduous forest: response to interannual climate variability. *Science*, **271,** 1576–1578.

Gunderson CA, Norby RJ, Wullschleger SD (1993) Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated CO_2: no loss of photosynthetic enhancement. *Plant, Cell and Environment*, **16,** 797–807.

Heineke D, Kauder F, Frommer W *et al.* (1999) Application of transgenic plants in understanding responses to atmospheric change. *Plant Cell and Environment*, **22,** 623–628.

Hidema J, Makino A, Kurita Y, Mae T, Ojima K (1992) Changes in the levels of chlorophyll and light-harvesting chlorophyll a/b protein of PSII in rice leaves aged under different irradiances from full expansion through senescence. *Plant Cell Physiology*, **33,** 1209–1214.

Inskeep WP, Bloom PR (1985) Extinction coefficients of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. *Plant Physiology*, **77,** 483–485.

Jach ME, Ceulemans R (1999) Effects of elevated atmospheric CO_2 on phenology, growth and crown structure of Scots pine (*Pinus sylvestris*) seedlings after two years of exposure in the field. *Tree Physiology*, **19,** 289–300.

Kleiner KW, Abrams MD, Schultz JC (1992) The impact of water and nutrient deficiencies on the growth, gas exchange and water relations of red oak and chestnut oak. *Tree Physiology*, **11,** 271–287.

Kubiske M, Abrams MD (1992) Photosynthesis, water relations and leaf morphology of xeric versus mesic *Quercus rubra* ecotypes in central Pennsylvania in relation to moisture stress. *Canadian Journal of Forest Research*, **22,** 1402–1407.

Kurosawa T (1995) Ecological differentiation of *Euphorbia lasiocaula* and *E. sinanensis* (Euphobiaceae) plant height, phenology and allocation to stems and leaves. *Journal of Plant Research*, **108,** 277–281.

Masoni A, Ercoli L, Mariotti M, Barberi P (1994) Changes in spectral properties of aging and senescing maize and sunflower leaves. *Physiologia Plantarum*, **91,** 334–338.

McConnaughay KDM, Bassow SL, Bernston GM, Bazzaz FA (1996) Leaf senescence and decline of end-of-season gas exchange in five temperate deciduous tree species grown in elevated CO2 concentrations. *Global Change Biology*, **2,** 25–33.

Miao SL, Wayne PM, Bazzaz FA (1992) Elevated CO_2 differentially alters the responses of coocurring birch and maple seedlings to a moisture gradient. *Oecologia*, **90,** 300–304.

Moran R, Porath D (1980) Chlorophyll determination in intact tissues using N,N-dimethylformamide. *Plant Physiology*, **65,** 478–479.

Mousseau M, Dufrene E, Kohen AE *et al.* (1996) Growth strategy and tree response to elevated CO_2: A comparison of beach (*Fagus sylvatica*) and sweet chestnut (*Castanea sativa*). In: *Carbon Dioxide and Terrestrial Ecosystems* (eds Koch GW, Mooney HA). Academic Press, Boston, MA.

Navas M-L, Sonie L, Richarte J, Roy J (1997) The influence of elevated CO_2 on species phenology, growth and reproduction in a Mediterranean old-field community. *Global Change Biology*, **3,** 523–530.

Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW, Ceulemans R (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. *Plant, Cell and Environment*, **22,** 683–714.

Okada K, Inoue Y, Satoh K, Katoh S (1992) Effects of light on degradation of chlorophyll and proteins during senescence of detached rice leaves. *Plant Cell Physiology*, **33,** 1183–1191.

Olszyk D, Wise C, VanEss E, Apple M, Tingey D (1998) Phenology and growth of shoots, needles, and buds of Douglas-fir seedlings with elevated CO_2 and (or) temperature. *Canadian Journal of Botany-Revue Canadienne de Botanique*, **76,** 1991–2001.

Osborne CP, Drake BG, LaRoche J, Long SP (1997) Does long-term elevation in CO_2 concentration increase photosynthesis in forest floor vegetation? *Plant Physiology*, **114,** 337–344.

Peace WJ, Grubb PJ (1982) Interactions of light and mineral nutrient supply in the growth of. *Impatiens Parviflora. New Phytologist*, **90,** 127–150.

Phares RE (1971) Growth of red oak (*Quercus rubra* L.) seedlings in relation to light and nutrients. *Ecology*, **52,** 669–672.

Reekie EG, Bazzaz FA (1991) Phenology and growth in four annual species grown in ambient and elevated carbon dioxide. *Canadian Journal of Botany*, **1991,** 2475–2481.

Roberntz P (1999) Effects of long-term CO_2 enrichment and nutrient availability in Norway spruce. I. Phenology and morphology of branches. *Trees-Structure and Function*, **13,** 188–198.

Rufty TW, Thomas RB, Cure JD, Cure WW (1994) Growth response of cotton to CO_2 enrichment in differing light environments. *Physiologia Plantarum*, **91,** 503–509.

Simon JL (1995) *Resampling Statistics.* Resampling Stats, Inc., Arlington, VA.

Sokal RR, Rohlf FJ (1995) *Biometry*. W.H. Freeman, New York.

StatSoft (1995) *Statistica 6.0*. Microsoft Corporation.

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell and Environment*, **22,** 583–621.

Tobias DJ, Ikemoto A, Nishimura T (1995) Leaf senescence patterns and photosynthesis in four leaf flushes of two deciduous oak (*Quercus*) species. *Photosynthetica*, **31,** 231–239.

Uemura S (1994) Patterns of leaf phenology in forest understory. *Canadian Journal of Botany*, **72,** 409–414.

Wayne PM, Bazzaz FA (1997) Light acquisition and growth by competing individuals in CO_2-enriched atmospheres: Consequences for size structure in regenerating birch stands. *Journal of Ecology*, **85,** 29–42.

Wheeler RM, Tibbits TW, Fitzpatrick AH (1991) Carbon dioxide effects on potato growth under different photoperiods and irradiance. *Crop Science*, **31,** 1209–1213.

Williams K, Field CB, Mooney HA (1989) Relationships among leaf construction cost, leaf longevity, and light environment in rain-forest plants of the genus Piper. *American Naturalist*, **133,** 198–211.

Wofsy SC, Goulden ML, Munger JW *et al.* (1993) Net exchange of CO_2 in a mid-latitude forest. *Science*, **260,** 1314–1317.

Wullschleger (1993) Biochemical limitations to carbon assimilation in C3 plants—A retrospective analysis of the A/Ci Curves from 109 species. *Journal of Experimental Botany*, **44,** 907–920.