Oecologia (1999) 121:193–200 

D. Alexander Wait · Clive G. Jones · Jules Wynn
F. Ian Woodward

# The fraction of expanding to expanded leaves determines the biomass response of *Populus* to elevated $CO_2$

Received: 28 September 1998 / Accepted: 23 June 1999

**Abstract** We examined whether the effects of elevated $CO_2$ on growth of 1-year old *Populus deltoides* saplings was a function of the assimilation responses of particular leaf developmental stages. Saplings were grown for 100 days at ambient (approximately 350 ppm) and elevated (ambient + 200 ppm) $CO_2$ in forced-air greenhouses. Biomass, biomass distribution, growth rates, and leaf initiation and expansion rates were unaffected by elevated $CO_2$. Leaf nitrogen (N), the leaf C:N ratio, and leaf lignin concentrations were also unaffected. Carbon gain was significantly greater in expanding leaves of saplings grown at elevated compared to ambient $CO_2$. The Rubisco content in expanding leaves was not affected by $CO_2$ concentration. Carbon gain and Rubisco content were significantly lower in fully expanded leaves of saplings grown at elevated compared to ambient $CO_2$, indicating $CO_2$-induced down-regulation in fully expanded leaves. Elevated $CO_2$ likely had no overall effect on biomass accumulation due to the more rapid decline in carbon gain as leaves matured in saplings grown at elevated compared to ambient $CO_2$. This decline in carbon gain has been documented in other species and shown to be related to a balance between sink/source balance and acclimation. Our data suggest that variation in growth responses to elevated $CO_2$ can result from differences in leaf assimilation responses in expanding versus expanded leaves as they develop under elevated $CO_2$.

D.A. Wait (✉)[1] · C.G. Jones
Institute of Ecosystem Studies,
Millbrook, NY 12545, USA

J. Wynn · F.I. Woodward
Department of Animal and Plant Sciences,
University of Sheffield,
Sheffield, S10 2UQ, UK

*Present address*:
[1]Department of Biology,
901 South National Avenue,
Southwest Missouri State University,
Springfield, MO 65804, USA,
e-mail: daw385f@mail.smsu.edu,
Tel.: +1-417-8365802, Fax: +1-417-8364204

**Key words** Elevated $CO_2$ · Leaf development · Biomass accumulation · Gas exchange · Rubisco

## Introduction

Although many $C_3$ plants show enhanced growth and net $CO_2$ assimilation (assimilation) when grown at elevated concentrations of atmospheric $CO_2$, these responses vary substantially within and between species (Kimball 1983; Eamus and Jarvis 1989; Hunt et al. 1991; Bazzaz et al. 1990; Poorter 1993; Ceulemans and Mousseau 1994; Curtis 1996; Curtis and Wang 1998). Curtis and Wang (1998), in their meta-analysis of 508 reports on the effects of elevated $CO_2$ on woody biomass, showed that, on average, biomass under conditions of high resource availability (i.e., high light, water, and nutrients) increased by 31%, while assimilation increased by 54%. However, responses of total biomass to elevated $CO_2$ ranged from approximately −10% (inhibition) to +150% (stimulation) (Curtis and Wang 1998), while relative assimilation effects (elevated/ambient assimilation rate) ranged from approximately −50% to +450% (Curtis 1996). In some species, increases in growth rates reasonably match increased leaf carbon (C) gain (Norby and O'Neill 1989). In others, leaf C gain and growth rates may increase initially, but then decline (Tolly and Strain 1984). In yet other species, growth increases are small or negligible relative to observed increases in C gain (Norby et al. 1992). For example, the biomass of Euramerican poplar cuttings grown in open-top chambers was not significantly greater under elevated $CO_2$ after 200 days compared to ambient $CO_2$, while photosynthetic rates were enhanced by 121% (see Ceulemans and Mousseau 1994). Biomass of *Populus grandidentata* cuttings grown in open-top chambers under elevated $CO_2$ for 47 days was 34% greater than that of ambient-$CO_2$-grown cuttings, while photosynthetic rates were enhanced by 84% (Zak et al. 1993). Biomass of *Salix dasyclados* cuttings grown in a greenhouse under elevated $CO_2$ for 120 days was 46%

greater than ambient-$CO_2$-grown cuttings, while photosynthetic rates were enhanced by 131%. Ceulemans and Mousseau's (1994) literature survey of various coniferous and deciduous broad-leaved tree species provides numerous examples of differences in biomass versus photosynthesis responses within species. While different growth conditions, differences in storage, or differences in the rate of utilization and accumulation of carbohydrates account for some of the variation in plant responses to $CO_2$ enrichment (Stitt 1991; Poorter 1993; Ceulemans and Mousseau 1994; Loehle 1995), the effect of leaf development on the response of plants to $CO_2$ enrichment is less well understood (Reekie 1996; and see Miller et al. 1997).

Although species vary in leaf developmental rates, across development, assimilation changes in more or less the same way in all species: photosynthetic capacity increases as leaves develop, becoming maximal at or around full expansion, and then declines later in maturity (Kozlowski et al. 1991). Young leaves are typically strong sinks for photosynthate, whereas mature leaves are typically strong sources (Vogelmann et al. 1982). Because most studies have been conducted on a limited number of leaf developmental stages, we do not know in general if elevated $CO_2$ differentially affects photosynthetic capacity as leaves develop. If there is no change in photosynthetic capacity at any developmental stage, or if the magnitude of change is uniform at all stages, then the effects of elevated $CO_2$ on leaf lifetime C gain and plant biomass should generally be predictable from assimilation measures made at any developmental stage. However, instantaneous measures of assimilation are unlikely to estimate leaf lifetime C gain and plant biomass accurately if elevated $CO_2$ has different effects at different leaf developmental stages. Here, the net effect on leaf lifetime C gain relative to ambient $CO_2$ would depend on both the degree of change in assimilation at a given developmental stage and the duration of that developmental stage. To our knowledge, assimilation responses at specific leaf developmental ages have not been specifically invoked as an explanation for variation in plant biomass responses to $CO_2$ enrichment.

Recent data suggest that assimilation responses to $CO_2$ enrichment may be related to leaf development in many species (e.g., Porter and Grodzinski 1984; Besford et al. 1990; Kelly et al. 1991; Norby and O'Neill 1991; Pearson and Brooks 1995; Van Oosten and Besford 1995; Hikosaka 1996; Miller et al. 1997). For example, Van Oosten and Besford (1995) found that after an initial increase in assimilation during early expansion, there was an accelerated ontogenetic decline in assimilation of tomato leaves grown at elevated compared to ambient $CO_2$. They concluded that the effects of $CO_2$ enrichment on tomato assimilation rates, assimilation responses to intracellular $CO_2$ concentration, Rubisco activity, various chloroplast proteins, mRNA, and carbohydrate content all changed as leaves developed. Pearson and Brooks (1995) showed that assimilation declined in *Rumex obstusifolius* leaves at a faster rate as leaves matured in elevated than in ambient $CO_2$. In mature leaves, assimilation rates were actually lower under elevated than in ambient $CO_2$. In their study, total and aboveground biomass, leaf expansion rates, the average area of leaves, and duration of leaf retention did not differ between elevated and ambient $CO_2$ treatments. They concluded that the effects of $CO_2$ enrichment on whole-plant C gain depended on the degree of photosynthetic down-regulation of individual leaves. Leadley and Reynolds (1989) demonstrated close correlations between leaf growth dynamics and photosynthesis in soybean. In this case, leaf size did not change with $CO_2$ enrichment, but initial rates of leaf expansion were more rapid and the period of expansion declined with increasing $CO_2$. Miller et al. (1997) found a significant relationship between rapid initial leaf expansion and higher photosynthetic rate with $CO_2$ enrichment in tobacco, similar to the results found with tomato (Besford et al. 1990). Gunderson et al. (1993) observed no effects of $CO_2$ on timing of leaf senescence in *Lirodendron tulipifera* and *Quercus alba* over a period of 3 years of $CO_2$ enrichment, and found that the two species maintained photosynthetic enhancement under $CO_2$ enrichment.

Here we present data from studies on the effects of elevated $CO_2$ on 1-year-old saplings of a fast-growing, indeterminate-flushing species (*P. deltoides*, clone ST-109) grown for 100 days under conditions of unlimited nutrients and water in large pots. Growth and physiology of other *Populus* species have been shown to be sensitive to elevated $CO_2$ via both increased leaf area and assimilation per unit leaf area (Gaudillere and Mousseau 1989; Radoglou and Jarvis 1990; Van Volkenburgh and Taylor 1996; and see Ceulemans and Mousseau 1994). For example, Gaudillere and Mousseau (1989) found that whole-plant $CO_2$ exchange, leaf photosynthesis, and leaf area were significantly increased by $CO_2$ enrichment, but that the photosynthetic activity in mature leaves was inhibited by $CO_2$ enrichment. They concluded that an increase in photosynthetic activity probably occurred when the plant had active sinks. Ceulemans et al. (1997) have shown that $CO_2$ enrichment leads to increases in both leaf size and expansion rate in a number of different poplar hybrids, with no downward acclimation over one growing season. In addition, Ceulemans et al. (1995) found that interactions with leaf age and/or leaf position significantly confound the $CO_2$ treatment effects on stomatal and epidermal cell densities. Finally, growth, leaf development, and assimilation of the *Populus* clone used in this study are known to be sensitive to resource availability and damage (e.g., Jones and Coleman 1988; Wait et al. 1996, 1998), but leaf developmental stage responses to $CO_2$ have not been studied.

Our data indicate that the net effect of $CO_2$ enrichment on *P. deltoides* biomass resulted from differences in leaf photosynthetic capacity between expanding and expanded leaves under elevated compared to ambient $CO_2$. This effect may be an important cause of the apparent variation between growth and photosynthetic

responses to $CO_2$ enrichment in other species. Finally, we present a simple graphical model that shows how gross patterns of total plant biomass can vary depending on how photosynthetic capacity changes with leaf development under $CO_2$ enrichment.

## Materials and methods

### Growth

One-year-old *P. deltoides* Bartr. saplings (clone ST-109, developed in Stoneville, Miss.) were grown for 100 days in three separate forced-air greenhouses at the University of Sheffield, UK. Each greenhouse was divided into two halves, one with ambient (350 ppm) and one with elevated (ambient plus 200 ppm) $CO_2$. Saplings were propagated vegetatively at the Institute of Ecosystem Studies (IES), Millbrook, N.Y., USA, planted in tree tubes, and shipped dormant to Sheffield. Saplings were grown in 6-l pots using John Innes no. 2 soil medium. Roots were not pot bound in these pots after 100 days. Saplings that had between three to five leaves and were 10–12 cm tall were used in the experiment; therefore, saplings of uniform age and mass (see Wait et al. 1998) were selected before being randomly assigned to treatments and chambers. Fifteen saplings were placed randomly into each half of the three greenhouses and rotated within the greenhouse once a week. Saplings were well watered and fertilized once weekly from day 21 to 100 with a N:P:K fertilizer added in the ratio of 20:5:10 at an application rate of 5 g N $m^{-2}$. Temperature and light (PAR) were recorded continuously throughout the experiment, but not controlled. Thus, they varied with ambient conditions. Mean daily PAR ranged between 90–380 μmol $m^{-2}$ $s^{-1}$. Mean daylight temperatures in glasshouses ranged between 14–21°C with a minimum temperature of 13 and maximum temperature of 34°C. Mean nighttime temperatures in glasshouses ranged between 9–18°C with a minimum temperature of 8 and maximum temperature of 25°C. We calculated Pearson product moment correlations between relative height growth rates (RHGRs) and mean daily PAR, mean daily temperature, and mean nightly temperature. We found that both ambient- and elevated-$CO_2$-grown sapling RHGRs were significantly ($P < 0.05$) and positively correlated with daily PAR and temperatures.

Continuous measurements of height growth, leaf initiation rates, and the leaf position at full expansion were measured on nine saplings per $CO_2$ treatment and greenhouse half from day 50 until harvest on day 100 as described in Wait et al. (1998). Measurements were made on 18 different days at intervals varying between 1–5 days. Sapling height was measured from an acrylic paint mark at the stem base to the tip of the primary node (stem apex). Leaf initiation rate and time to full expansion were determined as follows. On day 50, every leaf on a plant was numbered starting at the base. Every leaf subsequently initiated was numbered and the length of that leaf was measured until it ceased expanding. A new leaf was recorded as having been initiated when it was greater than 2 cm in length. At that length, the lamina had unrolled to its midpoint and exponential growth had started (Larson and Isebrands 1971; Wait et al. 1998). The leaf at full expansion (i.e., the leaf closest to the stem apex that had not expanded between two measurement intervals) was marked with flagging tape at each measurement. From these continuous leaf length measurements, leaves were categorized into two leaf developmental stage classes as described by Wait et al. (1998). Expanding leaves were leaves that were one to two leaf positions above the leaf at full expansion. Fully expanded leaves were those that were one to two leaf positions below the leaf at full expansion.

Plants were harvested on day 100. Leaf area by leaf position and leaf developmental stage was measured using a Delta-T leaf area meter (Delta-T Devices, Cambridge, UK). Leaf, stem, petiole, and root fresh weight were recorded for each plant. Dry weights were obtained for all plant parts after drying to constant weight at 80°C.

### Gas exchange

Measurements of the response of photosynthesis (net $CO_2$ assimilation rate, μmol $m^{-2}$ $s^{-1}$) to intercellular $CO_2$ concentration ($A$/Ci response, $A_{max}$) were made between days 55–65 on three saplings per $CO_2$ treatment on both an expanding and a fully expanded leaf. Measurements were made in the laboratory using an LCA-3 portable IRGA (Analytical Development Company, Hoddesdon, UK) attached to a Parkinson leaf chamber. A range of $CO_2$ concentrations from 0 to 1000 ppm was supplied from cylinders, mixed and regulated by a mass flow meter. Actinic light at 1200 μmol $m^{-2}$ $s^{-1}$ was supplied by a Schott KL 1500-T lamp. In addition, the maximum net $CO_2$ assimilation rate of expanding leaves was measured in the greenhouses 30 min after the transfer of four saplings per treatment from ambient to elevated $CO_2$, and from elevated to ambient $CO_2$. Measurements were made using the same apparatus at the same PAR. Monitoring of $CO_2$ in greenhouses showed that the airflow in chambers and limited time spent in chambers was sufficient to keep $CO_2$ within the range of day-to-day variation while working in the greenhouses.

Instantaneous measures of assimilation were determined in greenhouses between 1000 and 1400 hours on days 60–62 on two expanding and two fully expanded leaves on nine saplings per $CO_2$ treatment. Measurements were obtained at ambient temperature, relative humidity, and PAR. Only measurements at a PAR above light saturation (800 μmol $m^{-2}$ $s^{-1}$; Wait 1992) were used for analysis. Measurements were made with a Li-Cor 6200 portable photosynthesis system (Li-Cor, Lincoln, Neb.).

### Leaf chemistry

Leaf nitrogen, carbon, and lignin concentrations were determined on dried (80°C) green leaf tissue harvested on days 56 and 100 from three plants per harvest date per $CO_2$ treatment. All leaves from an individual were pooled for chemical analysis; therefore, leaf developmental-age effects were not examined for these variables. Nitrogen and carbon were determined by combustion in a Carlo Erba NA 1500 N/C analyzer, and lignin was determined by the permanganate method (Van Soest and Wine 1968), by Natural Resource Management Ltd, Berkshire, UK.

### Rubisco content

The ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) content of leaves was determined on day 100 on two expanding leaves and one fully expanded leaf from four saplings per $CO_2$ treatment. Leaf discs were removed at midday and frozen in liquid nitrogen. Extracts were prepared for determination of Rubisco content as described in Quick et al. (1991). The extract was prepared for measurement by adding 150 μl of sample to 50 μl of preheated buffer containing 0.8 g SDS, 15.26 ml glycerol, 2 ml mercaptoethanol, and 80 μl bromophenol blue (5% in ethanol) made up to 20 ml with 1.88 M Tris-HCL (pH 6.8).

Proteins were then separated at 25 mA for 1 h using 10% SDS-PAGE as a running gel and 3% SDS-PAGE as a stacking gel. Samples were loaded on an equal-area basis. Purified oilseed rape Rubisco extract was used as a standard. Gels were stained for 1 h with Coomassie blue (5% in ethanol) and fixed in 45% ethanol. The optical density of the Rubisco band was measured using a video imaging system, and the amount of Rubisco was calculated by comparison with the optical density of the standards.

### Statistical analyses

Biomass data were analyzed by one-way ANOVA. Tissue composition data were analyzed by two-way ANOVA with $CO_2$ and time as main factors. Height growth rate and leaf development data were analyzed using repeated-measures ANOVA. Instantaneous

assimilation rates and leaf Rubisco were analyzed by two-way ANOVA with $CO_2$ concentration and leaf developmental stage as main factors. All ANOVA models initially considered the effect of chamber within $CO_2$ concentration, but chamber was never found to be significant ($P > 0.18$); therefore, data were pooled across chambers.

Data from $A$/Ci curves were analyzed with non-linear regression (SAS-proc nonlin). Data were fit to the following model (Potvin et al. 1990):

$$\mathrm{NAR} = A_{\max}[1 - \mathrm{e}^{-W_c(x-\Gamma)}]$$

where NAR is the net $CO_2$ assimilation rate, $A_{\max}$ is the asymptote or maximum rate of $CO_2$ assimilation, $W_c$ is the slope corresponding to the carboxylation efficiency, and $\Gamma$ is the $x$-intercept or $CO_2$ compensation point. The estimated regression parameters $A_{\max}$, $W_c$, and $\Gamma$, derived from curves for individual leaves, were analyzed for effects of $CO_2$ concentration and leaf developmental stage using ANOVA.

## Results

### Growth and leaf chemistry

Total biomass, root biomass, root:shoot ratio, expanding and expanded leaf area, RHGR, leaf initiation rate and leaf position at full expansion were not significantly different between saplings grown at 350 and 550 ppm $CO_2$ for 100 days (Table 1). In addition, leaf N and lignin concentration and C:N ratio were not significantly different between saplings grown at 350 and 550 ppm $CO_2$ for 100 days (Table 1). Therefore, elevated $CO_2$ did not result in greater growth or a change in gross leaf chemical composition in these 1-year-old *P. deltoides* saplings. Gregg (1998) obtained similar results with 1-year-old saplings of the same clone in a growth chamber study where light and temperature were controlled.

### Gas exchange and Rubisco content

There was no detectable effect of $CO_2$ concentration on photosynthetic capacity in expanding leaves, based on $A$/Ci analysis (Fig. 1, Table 2). Assimilation rates of expanding leaves measured at a common $CO_2$ concentration confirmed these findings (Fig. 2). In contrast, $A$/Ci analysis indicated that there was a reduction in photosynthetic capacity in fully expanded leaves on saplings grown at elevated $CO_2$, compared to fully expanded leaves on saplings grown at ambient $CO_2$ (Fig. 1, Table 2).

Assimilation rates measured in situ were significantly affected by leaf developmental stage ($P < 0.0001$). More importantly, there was a significant interaction between $CO_2$ concentration and leaf developmental stage (Fig. 3), indicating that the effects of $CO_2$ were dependent on leaf developmental stage. Expanding leaves of saplings grown at elevated $CO_2$ had 18% higher assimilation rates than expanding leaves in ambient $CO_2$. However, fully expanded leaves at elevated $CO_2$ had 21% lower assimilation rates than fully expanded leaves in ambient $CO_2$ (Fig. 3). This translated

**Table 1** Biomass, growth rate, leaf development, and leaf chemistry of *Populus deltoides* saplings grown at 350 and 550 ppm $CO_2$. All values are means with SEs in parentheses. The $P$-values of main factors and interactions determined by ANOVA (see text for details) are shown at the bottom of the table (*DW* dry weight, *ND* not determined, *NA* not applicable, *RHGR* relative height growth rate)

| $CO_2$ concentration (ppm) | Time (days) | Total biomass (g DW) | Root biomass (g DW) | Root:shoot ratio | Expanding-leaf area ($cm^2$) | Expanded-leaf area ($cm^2$) | RHGR[a] (cm $cm^{-1}$ $day^{-1}$) | Leaf initiation rate[a] (leaves $day^{-1}$) | Leaf position at full expansion[a] | Leaf nitrogen (% DW) | Leaf C:N ratio | Leaf lignin (% DW) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 350 | 56 | ND | ND | ND | ND | ND | NA | NA | NA | 3.18 (0.08) | 13.9 (0.30) | 5.27 (0.70) |
| 550 | 56 | ND | ND | ND | ND | ND | NA | NA | NA | 3.24 (0.11) | 13.8 (0.40) | 5.78 (0.05) |
| 350 | 100 | 29.6 (3.2) | 9.5 (1.5) | 0.45 (0.04) | 511 (28) | 1165 (129) | 0.024 (0.003) | 0.31 (0.02) | 5.9 (0.23) | 3.63 (0.34) | 13.3 (1.94) | 6.88 (0.40) |
| 550 | 100 | 33.6 (3.6) | 12.0 (1.7) | 0.53 (0.04) | 579 (125) | 1342 (140) | 0.023 (0.003) | 0.30 (0.02) | 5.7 (0.23) | 3.77 (0.07) | 12.2 (0.25) | 6.23 (0.35) |
| $CO_2$ ($P$) | | 0.42 | 0.29 | 0.14 | 0.11 | 0.40 | 0.47 | 0.65 | 0.98 | 0.70 | 0.52 | 0.09 |
| Time ($P$) | | | | | | | 0.08 | 0.62 | 0.46 | 0.02 | 0.31 | 0.06 |
| $CO_2 \times$ time ($P$) | | | | | | | 0.83 | 0.96 | 0.99 | 0.91 | 0.64 | 0.27 |

[a] At days 50–100

into a 51% decline in C gain between expanding and fully expanded leaves for saplings grown at elevated $CO_2$, but only a 21% decline for saplings grown at ambient $CO_2$.

Leaf Rubisco content was affected by $CO_2$ concentration ($P = 0.038$) and leaf developmental stage ($P = 0.031$) (Fig. 4). An indication of an interaction between $CO_2$ and leaf developmental stage ($P = 0.11$) suggested that Rubisco content was dependent on leaf developmental age. There was no significant difference in Rubisco content in expanding leaves on saplings grown at elevated compared to ambient $CO_2$, but there was a 59% lower Rubisco content in fully expanded leaves on saplings grown at elevated compared to ambient $CO_2$ (Fig. 4). This translated into a 58% decline in Rubisco content between expanding and fully expanded leaves for saplings grown at elevated $CO_2$, but only a 6% decline for saplings grown at ambient $CO_2$. These data indicate that the larger decline in photosynthetic capacity and C gain as leaves developed on saplings grown at elevated compared to ambient $CO_2$ was due to the greater reduction in Rubisco concentration.

The gas exchange and Rubisco data can be summarized as follows: carbon gain was significantly greater in expanding leaves on saplings grown at elevated compared to ambient $CO_2$ (Figs. 1, 2, Table 2). Carbon gain and photosynthetic capacity were significantly lower in fully expanded leaves on saplings grown at elevated compared to ambient $CO_2$ (Figs. 1, 3). Carbon gain and photosynthetic capacity declined more rapidly as leaves developed on saplings grown at elevated compared to leaves in ambient $CO_2$ (Fig. 3).

## Discussion

Total biomass and biomass distribution of 1-year-old *P. deltoides* saplings were not affected by a 200-ppm increase in the concentration of atmospheric $CO_2$ over a 100-day period. Because leaf initiation and expansion, leaf area, and leaf N were also unaffected, it is unlikely that the lack of any biomass effect was due to a decline in nutrient status (Brown 1991) or accelerated rates of self-shading (Poorter et al. 1988). Ceulemans et al. (1997) have shown for different poplar hybrids that $CO_2$ enrichment did not result in downward acclimation of

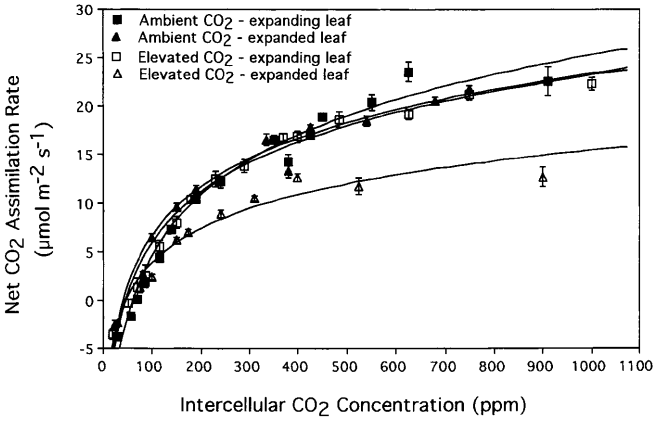


**Fig. 1** Response of photosynthesis to internal $CO_2$ concentration for leaves at two developmental stages grown in 350 and 550 ppm $CO_2$. An expanding leaf is two leaf positions from being fully expanded, a fully expanded leaf is two leaf positions past full expansion. Values are means, error bars are SEs. Curves are best-fit third-order polynomials. Gas exchange characteristics derived from non-linear regression analysis are shown in Table 2

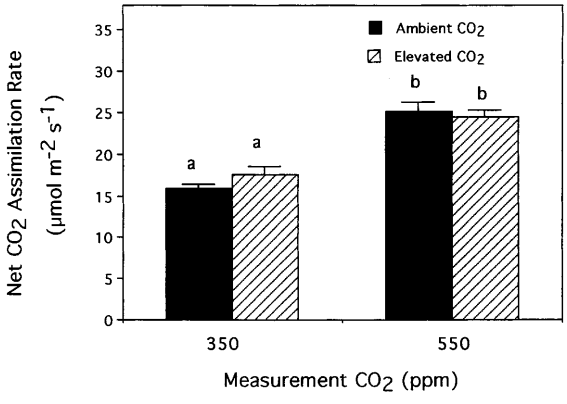


**Fig. 2** Photosynthetic rates of expanding leaves (two leaf positions past full expansion) grown at 350 or 550 ppm $CO_2$ measured at a common $CO_2$ concentration. Values are means, error bars are SEs. Bars with matching letters were found not to be significantly different from each other by Tukey's test ($P > 0.05$). ANOVA indicated that $CO_2$ concentration ($P = 0.595$) and the interaction between $CO_2$ concentration and measurement $CO_2$ ($P = 0.249$) did not, but that measurement $CO_2$ ($P < 0.001$) did significantly affect photosynthetic rates

**Table 2** Gas exchange characteristics [$W_c$ carboxylation efficiency (μmol $m^{-2}$ $s^{-1}$), $A_{max}$ maximum rate of $CO_2$ assimilation (μmol $m^{-2}$ $s^{-1}$), Γ $CO_2$ compensation point (μmol $mol^{-1}$)] for different leaf developmental stages of *P. deltoides* grown at 350 and 550 ppm $CO_2$. All values are means with SEs in parentheses. The *P*-values of main factors determined by ANOVA are shown in the right portion of the table. In a row, numbers in a row with a matching letter are not significantly different from each other (Tukey test, $P > 0.05$)

| | 350 ppm $CO_2$ | | 550 ppm $CO_2$ | | Statistical significance | |
|---|---|---|---|---|---|---|
| | Expanding leaf | Expanded leaf | Expanding leaf | Expanded leaf | $CO_2$ | Leaf stage |
| $W_c$ | $41.2^{a}$ (4.1) | $47.7^{b}$ (4.7) | $45.7^{b}$ (4.7) | $36.3^{a}$ (4.7) | 0.0331 | 0.0162 |
| $A_{max}$ | $24.24^{a}$ (1.10) | $21.15^{a}$ (1.27) | $21.95^{a}$ (1.27) | $12.96^{b}$ (1.27) | 0.0021 | 0.0008 |
| Γ | $67.03^{a}$ (4.29) | $41.79^{b}$ (4.96) | $53.55^{a,b}$ (4.96) | $57.18^{a,b}$ (4.96) | 0.8474 | 0.0508 |

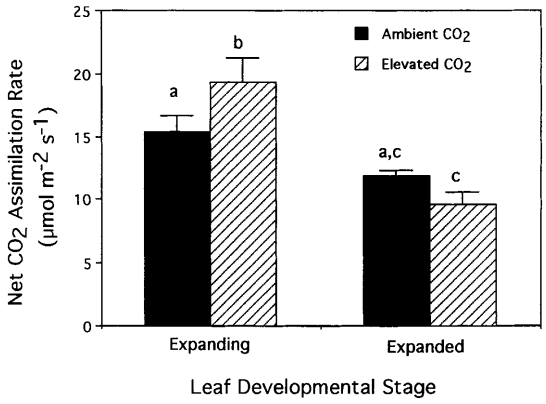


**Fig. 3** Photosynthetic rates of expanding and fully expanded leaves (see legend to Fig. 1 for definition) as a function of $CO_2$ concentration. Values are means, error bars are SEs. Bars with matching letters were found not to be significantly different from each other by Tukey's test ($P > 0.05$). ANOVA indicated that leaf stage ($P < 0.001$) and the interaction between $CO_2$ concentration and leaf stage ($P = 0.028$) did, but that $CO_2$ concentration ($P = 0.550$) did not significantly affect photosynthetic rates

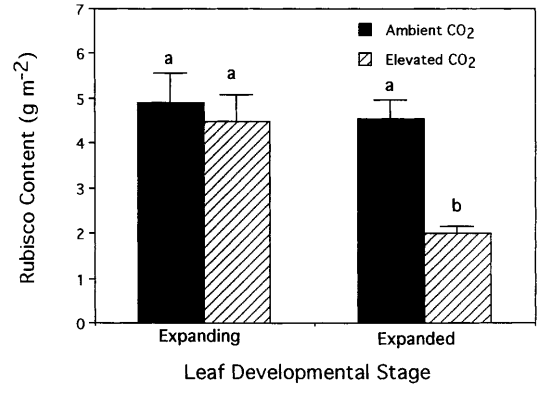


**Fig. 4** Rubisco content of expanding and fully expanded leaves (see legend to Fig. 1 for definition) as a function of $CO_2$ concentration. Values are means, error bars are SEs. Bars with matching letters were found not to be significantly different from each other by Tukey's test ($P > 0.05$). ANOVA indicated that $CO_2$ concentration ($P = 0.038$) and leaf stage ($P = 0.031$) did, but that the interaction between $CO_2$ concentration and leaf stage ($P = 0.110$) did not significantly affect Rubisco content

photosynthesis over one growing season (4 months). However, one of the clones showed downward acclimation over a longer period (17 months) of exposure. Unlike the responses recorded here, however, $CO_2$ enrichment led to increases in both leaf size and leaf expansion rates (Ceulemans et al. 1995). It seems that genetic differences, rather than growth environment differences, would account for these differences in growth between our clone and poplars studied by Ceulemans et al., as the responses they observed were seen in both open-top chambers and glasshouse cabinets.

Our results indicate that leaf developmental-stage-related changes in gas exchange, similar to those reported by Porter and Grodzinski (1984), Kelly et al. (1991), Pearson and Brooks (1995), Van Oosten and Besford (1995), and Miller et al. (1997), were most likely responsible for the lack of an effect of elevated $CO_2$ on biomass. For saplings grown at elevated compared to ambient $CO_2$, increases in C gain in expanding leaves (+18%) were more or less offset by decreases in C gain in expanded leaves (−21%) (Fig. 3). The lower Rubisco concentrations in fully expanded leaves on saplings grown at elevated $CO_2$ compared to ambient $CO_2$ (Fig. 4) indicated leaf-age-dependent $CO_2$-induced down-regulation (see Bowes 1991; Pearson and Brooks 1995), and is consistent with the observed decrease in photosynthetic capacity and C gain measured between days 55–65 (Table 2, Figs. 1, 2, and 3). Thus, compared to ambient $CO_2$, expanding leaves showed increased C gain under elevated $CO_2$, whereas expanded leaves had lower C gain. These changes may have offset each other, so that across the leaf lifetime, C gain and resulting plant biomass were unaffected by elevated $CO_2$.

Our gas exchange and Rubisco data are consistent with those of Miller et al. (1997), who showed that the photosynthetic pattern in elevated-$CO_2$-grown tobacco is shifted temporally to an earlier maximum and subsequent decline, and that leaf developmental age may play a role in the interaction between source/sink balance and acclimation. Therefore, our data support their model that lowered photosynthetic rates observed during acclimation may result from a shift in the timing of the normal photosynthetic stages of leaf ontogeny to an earlier onset of the natural decline in photosynthetic rates.

Reekie (1996) has suggested that differences in developmental patterns have the potential to alter substantially the growth responses of plants to $CO_2$ enrichment, which would have important consequences for predictions on the effects of $CO_2$ enrichment on plant-plant interactions. Our study illustrates that the effects of $CO_2$ enrichment are not necessarily manifested through measurable changes in leaf initiation and expansion, but rather through the effects of $CO_2$ enrichment on leaf developmental age assimilation capacities. Loehle (1995) has suggested that studies with seedlings alone can be misleading with respect to adult growth. Our study suggests that some, but certainly not all, of the variation between seedlings and adults may be related to the proportion of expanding to expanded leaf area and leaf developmental-age-specific responses to $CO_2$ enrichment.

To illustrate the magnitude of C gain that would have occurred in our study without developmental differences in assimilation, measured assimilation rates (Fig. 3) of expanding and expanded leaves (Table 1) were multiplied by their respective leaf area to estimate whole-plant C gain (μmol $s^{-1}$). We estimate that saplings grown at elevated $CO_2$ had a 10.6% greater C gain compared to ambient-$CO_2$-grown saplings (24.24 vs 21.65 mmol $s^{-1}$). We estimate that saplings grown at elevated $CO_2$ would have had a 31% greater C gain compared to ambient $CO_2$ if assimilation of expanding leaves had declined at

the same rate as that in ambient $CO_2$ (28.36 vs 21.65 mmol $s^{-1}$). Since our data suggest that there was a good correspondence between estimated whole-plant C gain (24.24 vs 21.65 mmol $s^{-1}$ in elevated vs ambient $CO_2$) and biomass accumulation (33.6 vs 29.6 g in elevated vs ambient $CO_2$), a 31% greater C gain would presumably have resulted in significantly greater biomass. Our data also suggest that while expanded leaves had over twice the leaf area of expanding leaves (Table 1), and thus potentially have twice the control over total C gain, this was only apparently so in ambient-$CO_2$-grown saplings. We estimate from final harvest data that by the end of the experiment, expanded leaves contributed 45% (13.98 mmol $s^{-1}$) more to total C gain (21.65 mmol $s^{-1}$) than expanding leaves in ambient-$CO_2$-grown saplings, while expanded leaves contributed 17% (13.24 mmol $s^{-1}$) more to total C gain (24.24 mmol $s^{-1}$) than expanding leaves in elevated-$CO_2$-grown saplings.

We conclude that there was no net effect of elevated $CO_2$ on biomass in this study because net increases in C gain in expanding leaves of elevated-$CO_2$-grown saplings were offset by net decreases in C gain in their fully expanded leaves. We further suggest that changes in the effects of elevated $CO_2$ on C gain throughout the development of a leaf, and the distribution of leaf developmental stages during an entire experiment, may determine whether elevated $CO_2$ results in greater, the same, or less biomass than plants grown at ambient $CO_2$. A simple graphical model can be used to illustrate how gross patterns of total plant biomass could vary depending on how photosynthetic capacity changes with leaf development under elevated $CO_2$ (Fig. 5). In the model we assume that the distribution of leaf area and biomass across leaf developmental stages are not affected by elevated $CO_2$, and that carbon gain is proportional to biomass accumulation. If leaf assimilation rates are enhanced by elevated $CO_2$ at all leaf developmental stages, leaf lifetime C gain and biomass would be substantially greater under elevated than ambient $CO_2$ (Fig. 5A). If leaf assimilation rates are enhanced by elevated $CO_2$ at early developmental stages, but then decline to the level found in leaves on plants grown at ambient $CO_2$, leaf lifetime C gain and biomass would still be somewhat greater under elevated than ambient $CO_2$ (Fig. 5A). Suppose, however, that leaf assimilation responses to elevated $CO_2$ are not uniform with respect to leaf development, as seen in our study. For example, if there were no acclimation (sensu Curtis and Wang 1998) early in development, but greater acclimation at later developmental stages, then leaf lifetime C gain and resulting biomass may not differ (Fig. 5B), or may even be less (Fig. 5C) under elevated than ambient $CO_2$. We performed a simple "run" of the model by multiplying end point leaf area data for ambient-$CO_2$-grown saplings (Table 1) and assimilation rates from Fig. 3. We calculated that daily whole-plant C gain would be from 28–50% greater assuming the relationship in Fig. 5A, 7.1% greater assuming the relationship in Fig. 5B, and

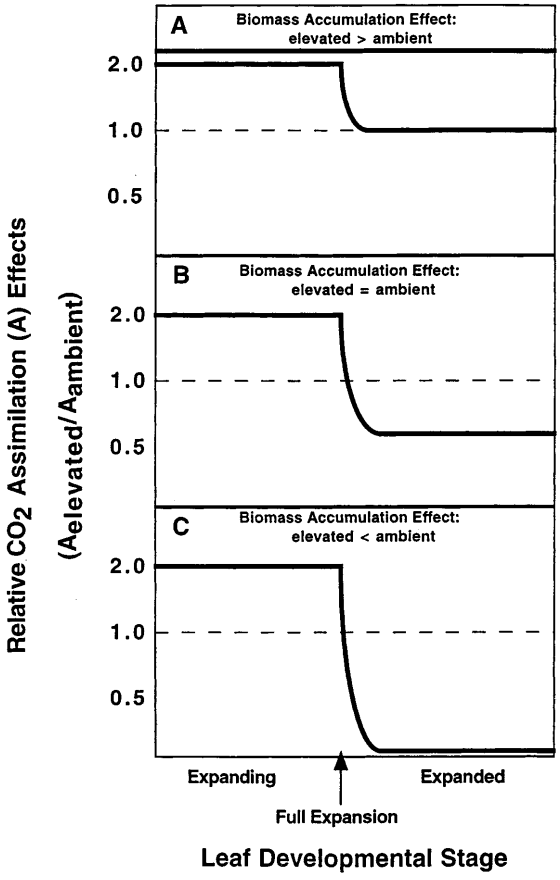


**Fig. 5A–C** A simple graphical model of the relative effects of an elevated concentration of $CO_2$ on net $CO_2$ assimilation rates (elevated/ambient assimilation rate) as a function of leaf developmental stage. Predicted relative biomass responses are given. The model assumes that the distribution of leaf area and mass across leaf developmental stages are not affected by elevated $CO_2$, and that carbon gain is proportional to biomass accumulation. Assimilation rates are enhanced independent of leaf developmental stage, or only when leaves are rapidly expanding (**A**). Assimilation rates are enhanced when leaves are expanding, but decline faster as leaves mature (**B–C**)

23% lower assuming the relationship in Fig. 5C in elevated- compared to ambient-$CO_2$-grown saplings. A more realistic run of the model (i.e., through time) would require leaf area × developmental age × assimilation data through time. Although the model is appealing for its simplicity and potential use (e.g., see Reekie 1996), it is a speculation. We clearly need data on the effects of elevated $CO_2$ on assimilation across leaf developmental stages, leaf lifetime C gain, and resulting plant biomass in other species (Reekie 1996).

**Acknowledgements** We thank G. Cooper, S. Hartley, H. Jones, R. Jones, P. Quick, J. Scholes, and A. Terry for help with data collection, and I. Forseth for comments on the manuscript. Supported by the Core grant to NERC Center for Population Biology, Imperial College, Silwood Park (C.G.J, D.A.W), The John Simon Guggenheim Memorial Foundation (Fellowship to C.G.J.), and the Department of Animal and Plant Sciences, University of Sheffield (J.W.). Contribution to the program of the Institute of Ecosystem Studies.

## References


Bazzaz FA, Coleman JS, Morse SR (1990) Growth responses of seven major co-occurring tree species of the northeastern United States to elevated $CO_2$. Can J For Res 20:1479–1484

Besford RT, Ludwig LJ, Withers AC (1990) The greenhouse effect: acclimation of tomato plants growing in high $CO_2$, photosynthesis and ribulose-1,5-bisphosphate carboxylase protein. J Exp Bot 41:925–931

Bowes G (1991) Growth at elevated $CO_2$: photosynthetic responses mediated through Rubisco. Plant Cell Environ 14:795–806

Brown KR (1991) Carbon dioxide enrichment accelerates the decline in nutrient status and relative growth rate of *Populus tremuloides* Michx. seedlings. Tree Physiol 8:161–173

Ceulemans R, Mousseau M (1994) Effects of elevated atmospheric $CO_2$ on woody plants. New Phytol 127:425–446

Ceulemans R, Jian XN, Shao BY (1995) Growth and physiology of one-year-old poplar (*Populus*) under elevated atmospheric $CO_2$ levels. Ann Bot 75:609–617

Ceulemans R, Taylor G, Bosac C, Wilkins D, Besford RT (1997) Photosynthetic acclimation to elevated $CO_2$ in poplar grown in glasshouse cabinets or in open top chambers depends on duration of exposure. J Exp Bot 48:1684–1689

Curtis PS (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. Plant Cell Environ 19:127–137

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

Eamus D, Jarvis PG (1989) The direct effects of increase in the global atmospheric carbon dioxide concentration on natural and commercial temperate trees and forests. Adv Ecol Res 19:1–55

Gaudillere JP, Mousseau M (1989) Short term effect of $CO_2$ enrichment on leaf development and gas exchange of young poplars (*Populus euramerican* cv I 214). Oecol Plant 10:95–105

Gregg J (1998) The effect of multiple human-accelerated environmental changes on plant growth and physiology in an urban ecosystem. PhD thesis, Cornell University, Ithaca

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 Environ 16:797–807

Hikosaka K (1996) Effects of leaf age, nitrogen nutrition and photon flux density on the organization of the photosynthetic apparatus in leaves of a vine (*Ipomoea tricolora* Cav.) grown horizontally to avoid mutual shading of leaves. Planta 1:144–150

Hunt R, Hand DW, Hannah MA, Neal AM (1991) Response to $CO_2$ enrichment in 27 herbaceous species. Funct Ecol 5:410–421

Jones CG, Coleman JS (1988) Plant stress and insect behavior: cottonwood, ozone, and the feeding and oviposition preference of a beetle. Oecologia 76:51–56

Kelly DW, Hicklenton PR, Reekie EG (1991) Photosynthetic response of *Geranium* to elevated $CO_2$ as affected by leaf age and time of $CO_2$ exposure. Can J Bot 69:2482–2488

Kimball BA (1983) Carbon dioxide and agricultural yield: an assemblage and analysis of 770 prior observations. USDA, ARS Water Conservation Lab, WCL Rep 14

Kozlowski TT, Kramer PJ, Pallardy SG (1991) The physiological ecology of woody plants. Academic Press, New York

Larson PR, Isebrands JG (1971) The plastochron index as applied to developmental studies of cottonwood. Can J For Res 1:1–11

Leadley PW, Reynolds J (1989) Effect of carbon dioxide enrichment on development of the first six mainstem leaves in soybean. Am J Bot 76:1551–1555

Loehle C (1995) Anomalous responses of plants to $CO_2$ enrichment. Oikos 73:181–187

Miller A, Tsai C, Hemphill D, Endres M, Rodermel S, Spalding M (1997) Elevated $CO_2$ effects during leaf ontogeny: a new perspective on acclimation. Plant Physiol 115:1195–1200

Norby RJ, O'Neill EG (1989) Growth dynamics and water use of seedlings of *Quercus alba* L. in $CO_2$ enriched atmospheres. New Phytol 111:491–500

Norby RJ, O'Neill EG (1991) Leaf area compensation and nutrient interactions in $CO_2$-enriched seedlings of yellow-poplar (*Liriodendron tulipifera* L.). New Phytol 117:34–47

Norby RJ, Gunderson CA, Wullschleger SD, O'Neill EG, McCracken MK (1992) Productivity and compensatory responses of yellow-poplar trees in elevated $CO_2$. Nature 357:322–324

Pearson M, Brooks GL (1995) The influence of elevated $CO_2$ on growth and age-related changes in leaf gas exchange. J Exp Bot 46:1651–1659

Poorter H (1993) Interspecific variation in the growth response of plants to an elevated ambient carbon dioxide concentration. Vegetatio 104/105:77–97

Poorter H, Pot S, Lambers H (1988) The effect of an elevated atmospheric $CO_2$ concentration on growth, photosynthesis, and respiration of *Plantago major*. Physiol Plant 73:553–559

Porter MA, Grodzinski B (1984) Acclimation to high $CO_2$ in bean. Plant Physiol 74:413–416

Potvin CP, Lechowicz MJ, Tardif S (1990) The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. Ecology 71:1389–1400

Quick WP, Schurr U, Scheibe R, Schulze E-D, Rodermel SR, Bogorad L, Stitt M (1991) Decreased ribulose-5-phosphate carboxylase-oxygenase in transgenic tobacco transformed with "antisense" rbcS. Planta 183:542–554

Radoglou KM, Jarvis PG (1990) Effects of $CO_2$ enrichment on four poplar clones. I. Growth and anatomy. Ann Bot 65:617–626

Reekie EG (1996) The effect of elevated $CO_2$ on development processes and its implications for plant-plant interactions. In: Körner C, Bazzaz FA (eds) Carbon dioxide, populations, and communities. Academic Press, New York, pp 333–346

Stitt M (1991) Rising $CO_2$ levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environ 14:741–762

Tolly LC, Strain BR (1984) Effects of $CO_2$ enrichment on growth of *Liquidambar stryaciflua* and *Pinus taeda* seedlings under different irradiance levels. Can J For Res 14:343–350

Van Oosten JJ, Besford RT (1995) Some relationships between the gas exchange, biochemistry and molecular biology of photosynthesis during leaf development of tomato plants after transfer to different carbon dioxide concentrations. Plant Cell Environ 18:1253–1266

Van Soest PJ, Wine RH (1968) Determination of lignin and cellulose in acid-detergent fibre with permanganate. J Assoc Off Anal Chem 51:780

Van Volkenburgh E, Taylor G (1996) Leaf growth physiology. In: Stettler RF, Bradshaw HD Jr, Heilman PE, Hinckley TM (eds) Biology of *Populus* and its implications for management and conservation. NRC Research Press, National Research Council of Canada, Ottawa, pp 283–299

Vogelmann TC, Larson PR, Dickson RE (1982) Translocation pathways in the petioles and stem between source and sink leaves of *Populus deltoides* Bart. ex Marsh. Planta 156:345–358

Wait DA (1992) Effect of nutrient addition rates on growth, chemistry, and physiology of *Populus deltoides*, and the feeding preference and consumption of *Chrysomela scripta* and *Lamantria dispar*. MSc thesis. SUNY-ESF, Syracuse, New York

Wait DA, Jones CG, Schaedle M (1996) Controlling growth and chemical composition of saplings by iteratively matching nutrient supply to demand: a bootstrap fertilization technique. Tree Physiol 16:359–365

Wait DA, Jones CG, Coleman JS (1998) Effects of nitrogen fertilization on leaf chemistry and beetle feeding are mediated by leaf development. Oikos 82:502–514

Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL (1993) Elevated atmospheric $CO_2$ and feedback between carbon and nitrogen cycles. Plant Soil 151:105–117