Interaction of nutrient limitation and elevated CO2 concentration on carbon assimilation of a tropical tree seedling (Cedrela odorata)

F. E. CARSWELL,1,2,3 J. GRACE,1,3 M. E. LUCAS1 and P. G. JARVIS1

1 Institute of Ecology and Resource Management, University of Edinburgh, King’s Buildings, Mayfield Road, Edinburgh EH9 3JU, U.K.
2 Present address: Landcare Research, P.O. Box 69, Lincoln 8152, New Zealand
3 Royal Botanic Garden of Edinburgh, Inverleith Row, Edinburgh EH3 5LR, U.K.

Summary  Carbon assimilation by Cedrela odorata L. (Meliaceae) seedlings was investigated in ambient and elevated CO2 concentrations ([CO2]) for 119 days, using small fumigation chambers. A solution containing macro- and micronutrients was supplied at two rates. The 5% rate (high rate) was designed to avoid nutrient limitation and allow a maximum rate of growth. The 1% rate (low rate) allowed examination of the effect of the nutrient limitation–elevated CO2 interaction on carbon assimilation. Root growth was stimulated by 23% in elevated [CO2] at a high rate of nutrient supply, but this did not lead to a change in the root:shoot ratio. Total biomass did not change at either rate of nutrient supply, despite an increase in relative growth rate at the low nutrient supply rate. Net assimilation rates and relative growth rates were stimulated by the high rate of nutrient addition, irrespective of [CO2]. We used a biochemical model of photosynthesis to investigate assimilation at the leaf level. Maximum rate of electron transport (Jmax) and maximum velocity of carboxylation (Vcmax) did not differ significantly with CO2 treatment, but showed a substantial reduction at the low rate of nutrient supply. Across both CO2 treatments, mean Jmax for seedlings grown at a high rate of nutrient supply was 75 µmol m⁻² s⁻¹ and mean Vcmax was 27 µmol m⁻² s⁻¹. The corresponding mean values for seedlings grown at a low rate of nutrient supply were 36 µmol m⁻² s⁻¹ and 15 µmol m⁻² s⁻¹, respectively. Concentrations of leaf nitrogen, on a mass basis, were significantly decreased by the low nutrient supply rate, in proportion to the observed decrease in photosynthetic parameters. Chlorophyll and carbohydrate concentrations of leaves were unaffected by growth [CO2]. Because there was no net increase in growth in response to elevated [CO2], despite increased assimilation of carbon at the leaf level, we hypothesize that the rate of respiration of non-photosynthetic organs was increased.

Keywords: plant growth, nutrient supply, photosynthetic acclimation, respiration.

Introduction  The expected increase in atmospheric carbon dioxide concentra-

Received September 2, 1999

Data from studies of tropical tree species are conflicting. Some workers have found little or no increase in photosynthesis in response to elevated [CO2] (Oberbauer et al. 1985, Reekie and Bazzaz 1989), whereas Ziska et al. (1991) found sizeable increases in both photosynthesis and growth of four tropical tree species. In general, the mean increase in photosynthesis of tree seedlings is about 50% in response to a doubling of [CO2] (reviewed in Ceulemans and Mousseau 1994), but many authors have reported a decline or disappearance of photosynthetic stimulation after extended periods of exposure (Mousseau and Saugier 1992, Grulke et al. 1993). Investigation of acclimation responses, such as down-regulation of photosynthesis with extended exposure to elevated [CO2], based on the use of the relationship between assimilation rate, A, and intercellular [CO2], Ci, is well established for temperate species (e.g., Sage 1994), but has rarely been made on tropical species.
Much of the early work on tropical tree responses to elevated \([\text{CO}_2]\) (Oberbauer et al. 1985, Reekie and Bazzaz 1989) may be of limited value because of the use of small pots that may have reduced sink capacity (Thomas and Strain 1991) and the ability of the tree to “mine” for nutrients (Johnson et al. 1996). However, provided that pot volume is adequate for normal rooting behavior, effects of variable sink strength may be overcome by ensuring stable plant nitrogen concentration (Pettersson and McDonald 1994). By using the Ingestad technique (Ingestad and McDonald 1992), it is possible to control the rate of nutrient addition such that plants are in a steady state of nutrient supply even though the rate may be below that required for maximum growth (Ingestad and Ågren 1992).

The decrease in photorespiration with an increase in intercellular \([\text{CO}_2]\) is arguably the most important effect of an increased concentration of atmospheric \(\text{CO}_2\) (Drake et al. 1997). However, gas exchange \textit{per se} is only partly responsible for the observed growth rate. Other processes may be equally or even more important, such as the allocation of dry matter between leaf, stem and root tissues (Körner 1991). Therefore, the effects of an elevated \([\text{CO}_2]\) should be studied at the whole-plant level, with processes at a finer scale used to explain net responses.

We report net assimilation responses of a tropical seedling, \textit{Cedrela odorata} L. (Meliaceae) to both elevated \([\text{CO}_2]\) and a limiting rate of nutrient supply. Responses at the whole-plant level are explained in terms of the component processes. Specifically, photosynthesis and the allocation of carbon within seedlings were quantified. Estimates of \(J_{\text{max}}\) and \(V_{\text{cmax}}\) (maximum rates of electron transport and carboxylation) were used to assess photosynthetic acclimation to elevated \([\text{CO}_2]\), and they may also be useful in larger-scale models predicting tropical tree responses to this global change (e.g., Lloyd et al. 1995). Nutrient addition rate was controlled to allow elucidation of the response of \textit{C. odorata} to the interaction of elevated atmospheric \([\text{CO}_2]\) with nutrient limitation. We hypothesized that the growth response of \textit{C. odorata} would be reduced when nutrient supply was limiting as a result of a reduction in photosynthesis and a possible increase in nonstructural carbohydrate concentration in the leaves (Norby et al. 1996). Although Wullschleger (1993) back-calculated \(J_{\text{max}}\) and \(V_{\text{cmax}}\) from \(A/C_i\) curves of tropical species, we believe that this is the first published study designed to quantify photosynthetic response to elevated \([\text{CO}_2]\) at limiting and non-limiting rates of nutrient supply for a tropical species.

**Material and methods**

**Plant material and growth conditions**

Seed of \textit{C. odorata} provenances San Antonio and Teupasente from the Oxford Forestry Institute in Costa Rica was supplied by the Institute of Terrestrial Ecology (ITE) and sown in sand. Seedlings were transferred to a Perlite growing medium in 1-dm\(^3\) containers when the seedlings had a mean height of 21 mm and were approximately 4 weeks of age. Eighty-eight seedlings of mixed provenance were then transferred to eight fumigation chambers, 11 per chamber, inside a greenhouse at the Royal Botanic Garden of Edinburgh (55\(^{°}\)57’ N, 3\(^{°}\)12’ W, elevation 40 m). The seedlings were carefully transferred, minimizing root disturbance, to larger pots (4 dm\(^3\)) after six weeks, when their mean height was 70 mm. Each pot stood in its own saucer in which water and nutrient feed could accumulate. All seedlings were irrigated daily with tap water. Seedlings were rotated within a chamber every 2 weeks to minimize the effects of within-chamber variation in temperature, light and \([\text{CO}_2]\).

**Fumigation chambers**

Eight small fumigation chambers, modified from open-top chambers of 2.0 m\(^3\), were used. Four fumigation chambers were maintained at ambient \([\text{CO}_2]\) and four at elevated \([\text{CO}_2]\). Greenhouse temperatures fluctuated from nighttime values of approximately 18 ± 3 °C to daytime values of 19–32 °C, with a mean daytime temperature of 23 °C. A tropical humidity of 65–85% was maintained. Air was blown into the chambers from inside the greenhouse. Air was extracted from the chambers with a centrifugal fan that pulled air from all chambers through an overhead duct at a rate of 0.27 m\(^3\) s\(^{-1}\), giving a flow of 2.0 m\(^3\) min\(^{-1}\) per chamber, or one air change per chamber per minute. Air temperature, photosynthetic photon flux density (PPFD), and relative humidity (RH) were recorded on a data logger (DL2, Delta-T Devices, Cambridge, U.K.).

For the \textit{CO}_2-enriched chambers, incoming air was supplemented with pure \textit{CO}\(_2\) from cylinders (Distillers Plc, Glasgow, U.K.). No ethylene contamination was detected in \textit{CO}_2 from this source (Radoglou and Jarvis 1990). The \([\text{CO}_2]\) in each chamber was continually measured with an infrared gas analyzer (WMA1, PP Systems, Hitchin, U.K.) that was calibrated regularly against a known concentration of \textit{CO}_2. Sampling and adjustment of \textit{CO}_2 supply was fully automated with the use of a computer and mass flow controllers (Barton 1997). The \([\text{CO}_2]\) of air in the ambient chambers ranged between 325 and 450 \textit{µmol mol}\(^{-1}\) for 66% of the time. In the elevated-\([\text{CO}_2]\) chambers, \([\text{CO}_2]\) varied between 600 and 800 \textit{µmol mol}\(^{-1}\) for 75% of the time.

**Nutrient regime**

Half of all seedlings were assigned to a high rate of nutrient supply in a fully randomized design. These seedlings were then distributed evenly between the two \textit{CO}_2 treatments. The remainder of the seedlings were given a low rate of nutrient supply and similarly distributed between the \textit{CO}_2 treatments. Seedlings of different provenances were distributed at random throughout the chambers. One leaf sample from each of four healthy \textit{C. odorata} seedlings growing in a greenhouse in tropical conditions was taken and its nutrient concentration determined. A nutrient solution originally devised for birch by Ingestad and Lund (1986) was modified to match the nutrient concentrations determined in \textit{C. odorata} leaves. This solution contained nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) at concentrations of 100, 7, 59 and 40 g dm\(^{-3}\), re-
respectively. Allowances were made for nutrient concentrations in the tap water supply.

Nutrient solution was applied to the growing medium three times per week. Conductivity and pH of the nutrient solution were monitored at each application. The same volume of diluted stock solution was fed to each seedling each time, and run-off was contained within each plant saucer. For the rate of nutrient supply to match that of growth, dilution of stock solution before addition to seedlings was decreased exponentially as the experiment progressed, on the assumption that seedling growth was exponential (Ingestad and Ågren 1992). We tested a high and a low rate of supply of nutrient solution. Previous work had suggested a maximum growth rate of 3.24% per day for C. odorata growing under greenhouse conditions (Ramos and Grace 1990). Therefore, we supplied seedlings in the high nutrient supply treatment with a rate that would allow growth of 5% per day in the event of increased growth in response to elevated [CO₂]. Seedlings in the low nutrient supply treatment were supplied at a rate of 1% per day.

At the start of the experiment, there were 22 seedlings in each of the four treatments: elevated [CO₂] and high rate of nutrient application (EH), elevated [CO₂] and low rate of nutrient application (EL), ambient [CO₂] and high rate of nutrient application (AH), and ambient [CO₂] and low rate of nutrient application (AL).

**Growth measurements**

Staggered harvests enabled semi-continuous measurements of leaf, root and shoot dry mass, and subsequent calculation of relative growth rate (RGR). For dry mass determination, root samples were carefully washed by hand to minimize loss of fine roots. Root and shoot samples were oven-dried at 80 °C to constant mass.

Nine seedlings were harvested before the start of the experiment to determine initial mass. Two further harvests, each of 16 randomly selected seedlings (one seedling from each nutrient treatment per chamber), were made at 41 and 80 days, respectively. The final harvest of the 56 remaining seedlings (14 per treatment) was made 119 days from the start of the experiment when seedlings were about 21 weeks old. Nutrient analyses were performed on leaf samples at the second harvest to check the nutrient regime.

At the final harvest, leaf areas were measured (LI-3100 area meter, Li-Cor, Inc., Lincoln, NE) and the dry mass for all seedlings determined as above. Shoot samples were separated into stem and leaf components. Mean % increases in dry mass of each component as a result of exposure to elevated [CO₂] were calculated as:

\[ \Delta = \frac{(m_2 - m_1)}{m_1} \times 100 \]  

where \( m_1 \) = mean mass of tissue grown at ambient [CO₂] , and \( m_2 \) = mean mass of tissue grown at elevated [CO₂].

In addition to mean relative growth rate (RGR, \( R \)) for the entire period, calculations were made of mean net assimilation rate (NAR, \( E \)), final leaf area ratio (LAR, \( L \)), and final specific leaf area (SLA, \( S \)) as follows:

\[ R = \frac{(\ln m_2 - \ln m_1)}{t_2 - t_1} \times 100 \]  

\[ E = \frac{m_2 - m_1}{A_{t_2} - A_{t_1}} \frac{\ln A_{t_2} - \ln A_{t_1}}{t_2 - t_1} \]  

\[ L = \frac{A_{t_2}}{m_2} \]  

\[ S = \frac{A_{t_2}}{m_L} \]

where \( t_1 \) = time 1, \( t_2 \) = time 2, \( m_1 \) = tree dry mass at \( t_1 \), \( m_2 \) = tree dry mass at \( t_2 \), \( m_L \) = mass of leaves at \( t_2 \), \( A_{t_1} \) = leaf area at \( t_1 \), and \( A_{t_2} \) = leaf area at \( t_2 \).

It was assumed that mass increased without discontinuity between \( t_1 \) and \( t_2 \) in the calculation of RGR. Values for \( m_1 \) were obtained from the initial harvest, and values for \( A_{t_1} \) were obtained from the allometric relationship between seedling mass and leaf area that was established based on all simultaneous measurements of seedling mass and leaf area. Means of these were used as estimates of initial mass and leaf area of seedlings that were harvested at \( t_2 \). Values of RGR, LAR, NAR and SLA were calculated for each individual seedling, and treatment means were used for statistical comparisons between treatments.

**A/Cᵢ curves**

The rate of net photosynthesis (\( A \)) was measured over a range of intercellular CO₂ concentrations (\( C_i \)) and photosynthetic parameters were derived from the resulting A/Cᵢ curves (Harley et al. 1992). The A/Cᵢ curves, which were obtained for all four treatments, were derived for individual leaves during weeks 9–10 of the experiment when seedlings were approximately 14 weeks old. The terminal leaflet of the uppermost fully expanded, but non-senescent leaf was measured on each of four seedlings selected at random from each of the four nutrient + CO₂ treatments. The \( C_i \) was adjusted in the range 18 to 1300 µmol mol⁻¹ and the resultant changes in \( A \) were measured with a portable gas exchange system (LCA-3, Analytical Development Company, Hoddesdon, U.K.). In all cases, an artificial light source (quartz-halogen lamp with a PPFD of about 1100 µmol m⁻² s⁻¹) was used to ensure light saturation, which occurs at about 800 µmol m⁻² s⁻¹ for this species (Ramos and Grace 1990). Air was taken from within a fumigation chamber and then bubbled through water to ensure a relative humidity of about 75% in the leaf cuvette. No attempt was made to control leaf temperature, which ranged between 20 and 28 °C.

To determine maximum rate of electron transport (\( J_{\text{max}} \)), maximum carboxylation velocity (\( V_{\text{max}} \)) and apparent dark respiration during the day (\( R_d \), the A/Cᵢ curves were fitted with the Farquhar et al. model (1980), as defined by de Pury
and Farquhar (1997). Fitting the model involved an optimization procedure in which the parameters were adjusted to minimize the sums of squares of the residuals between observed and modeled A over the range of C. All curves were scaled to a leaf temperature of 25 °C. Derived photosynthetic parameters were then compared between treatments.

In addition, stomatal conductances (g s) were recorded during A/C i curve measurement when the ambient [CO 2] was equal to that of the growth concentration of the seedlings. These conductances were used to estimate photosynthesis at the operational concentration of CO 2.

### Tissue nutrient concentration

At the final harvest, one sample of each of stem, leaf and root tissue of all 56 seedlings was analyzed for nutrient (N, P, K, Ca, Mg) concentration. Nitrogen and P were assayed colorimetrically following acid digestion (Grimshaw et al. 1989). Calcium, Mg and K were analyzed by atomic absorption spectrophotometry (Unicam 919, Unicam Ltd., Cambridge, U.K.).

### Monosaccharide, oligosaccharide and starch concentrations

Monosaccharide, oligosaccharide and starch were determined for a subsample of leaves at final harvest. Mono- and oligosaccharides were extracted by a modification of the method of Grimshaw et al. (1989) and analyzed by high performance liquid chromatography (Dionex DX500, Dionex (U.K.) Ltd., Camberley, U.K.) on a carbopac (PA1) column. For analysis of starch, the subsample was subjected to acid digestion followed by an iodine colorimetric analysis (adapted from Grimshaw et al. 1989).

### Chlorophyll concentration

Three leaf discs were taken from each seedling at final harvest and incubated in N,N-dimethylformamide (DMF) for 3 days to extract the chlorophyll. The absorption of the extract was measured at 647 and 664 nm with a spectrophotometer (CE 303 Grating, Cecil Instruments Ltd., Cambridge, U.K.). Measurements were also made at 750 nm to zero the baseline. Chlorophyll concentration was then determined based on the extinction coefficients published by Porra et al. (1989). Chlorophyll concentration was expressed per unit leaf area.

### Statistical analysis

The goodness of fit of the A/C i curves derived for individual leaves was tested by regressing predicted versus measured values of A and testing the product moment correlation coefficient, r, for statistical significance (Fowler and Cohen 1990). The range of the coefficient of determination, r 2, is given for the fitted curves. Parameters obtained from the A/C i curves were tested for significant differences by a two-way analysis of variance (ANOVA) (Fowler and Cohen 1990).

Treatment differences in biomass were analyzed by a hierarchical two way ANOVA in SAS for Windows Version 6.12 (SAS Institute Inc., Cary, NC). This test avoids pseudo-replication (Hurlbert 1984) by nesting the CO 2 treatment within chambers and testing the CO 2 effect by using the error associated with variations between chambers, rather than between individuals in the same CO 2 regime (Potvin 1993). We note that the standard errors presented are merely an indication of the variation within each group, because it was not feasible to show all errors according to each level of nesting within a treatment. Assumptions of normality and equal variance were tested with normal plots and Hartley’s test for homogeneity of variance (Milliken and Johnson 1992). Data were transformed by either log or square root transformations where appropriate. Where a treatment difference was indicated, paired comparisons were investigated with Tukey’s honestly significant difference test (Fowler and Cohen 1990).

### Results

Differences between chambers and provenances (San Antonio and Teupasente) were not statistically significant (P > 0.05 in both cases). Therefore, in all subsequent tests the data sets for the two provenances were pooled.

### Whole-plant C assimilation

The low rate of nutrient supply severely reduced RGR (P > 0.05, Figure 1). The RGR calculated over the entire growing season was about 50% of the RGR of seedlings with the high rate of nutrient supply treatment, which is about twice as high as was expected for a nutrient addition rate of 1% per day. Seedlings grown with a high rate of nutrient supply achieved the 5% per day growth rate expected for a 5% per day addition rate of nutrient. The increase in RGR in response to elevated [CO 2] was significant only at the low rate of nutrient supply (P < 0.05). Despite the elevated [CO 2]-induced increase in RGR, seedlings in the elevated [CO 2] + low rate of nutrient supply treatment were not significantly larger than seedlings in the ambient [CO 2] + low rate of nutrient supply at the end of the experiment (P > 0.05, Figure 2), because of the large variation in seedling biomass in the two treatments. The RGR was significantly higher in seedlings in the elevated [CO 2] + high rate of nutrient supply treatment than in seedlings in the ambient [CO 2] + low rate of nutrient supply treatment (P < 0.05, Figure 1). There was no difference in RGR between seedlings in the elevated [CO 2] + high rate of nutrient supply treatment and seedlings in the ambient [CO 2] + high rate of nutrient supply treatment (P > 0.05).

The high rate of nutrient supply significantly increased NAR (P < 0.05, Figure 1), but there was no significant increase in NAR in response to elevated [CO 2]. Neither treatment had a significant effect on SLA (P > 0.05) or LAR (P > 0.05), although LAR showed a tendency to decrease in response to elevated [CO 2] (Figure 1).

The biomass of seedlings grown at the high rate of nutrient supply was 21 times larger than that of seedlings grown at the low rate of nutrient supply (P < 0.05, Figure 2). Only root mass increased in response to elevated [CO 2] and the high rate of nutrient supply (Figure 2). The mean increase in root biomass (Δ) in the elevated [CO 2] + high rate of nutrient supply treatment was 23% (P < 0.05). The elevated [CO 2] + high rate
of nutrient supply treatment had no significant effect on stem dry mass and total dry mass and did not significantly change the root:shoot (R:S) ratios (P > 0.05 in all cases). The R:S ratios were 0.45 ± 0.02 (± SE) and 0.4 ± 0.02 for seedlings grown with the high rate of nutrient supply at elevated and ambient [CO2], respectively. However, there was a large increase in R:S ratio of seedlings grown at the low rate of nutrient supply compared with seedlings grown at the high rate of nutrient supply (P < 0.05). Values of R:S were 0.97 ± 0.05 and 0.88 ± 0.05 for seedlings with the low rate of nutrient supply grown at elevated and ambient [CO2], respectively. Differences in R:S ratio between CO2 treatments at the low rate of nutrient supply were not significant (P > 0.05).

Leaf-scale processes
The A/Ci curves fitted to data from individual leaves showed a good fit. The range of r² values for comparisons of predicted versus measured values of A was from 0.78 to 0.98 with 90% of the relationships having an r² greater than 0.80. There were statistically significant differences in A/Ci curves between nutrient treatments but not between CO2 treatments (P > 0.05, Figure 3). Stomatal conductances (gs) appeared to be reduced at elevated [CO2]. When A at operational

Figure 1. Box plots of (a) relative growth rate (day⁻¹), (b) net assimilation rate (g m⁻² day⁻¹), (c) specific leaf area (m² g⁻¹) and (d) leaf area ratio (m² g⁻¹) for C. odorata grown at either ambient or elevated [CO2] with a high or low rate of nutrient supply. The mean of each data set is shown as the central bar of the box with the delimiters of the box representing the 25th and 75th percentiles. Capped bars represent the 10th and 90th percentiles with the highest value lying outside of these percentiles shown by circles. Each graph shows the four treatments: EH (elevated [CO2] + high rate of nutrient application), EL (elevated [CO2] + low rate of nutrient application), AH (ambient [CO2] + high rate of nutrient application) and AL (ambient [CO2] + low rate of nutrient application) (n = 14 for all treatments).

Figure 2. Mean values (± SE) of dry mass at final harvest of C. odorata for each root, stem and leaf component in each treatment. Treatments were: EH (elevated [CO2] + high rate of nutrient application), EL (elevated [CO2] + low rate of nutrient application), AH (ambient [CO2] + high rate of nutrient application) and AL (ambient [CO2] + low rate of nutrient application) (n = 14 for all treatments and components).
C, was estimated from the A/C curve, it appeared to be more than doubled by elevated [CO₂] (Figure 3). A high rate of nutrient supply also doubled the rate of photosynthesis at operational Cᵋ. Estimates of Vcmax and Jmax differed significantly between seedlings grown at low and high rates of nutrient application (P < 0.05, Figure 4).

Elevated [CO₂] had no significant effect on concentrations of total mono- and oligosaccharides (P > 0.05), or on concentrations of chlorophyll and starch (P > 0.05 in both cases). Starch and chlorophyll concentration were both higher in leaves from seedlings grown with the high rate of nutrient supply than in seedlings grown with the low rate of nutrient supply (P < 0.05, Table 1). Final nutrient concentrations of leaves, stems and roots differed only with nutrient treatment (P < 0.05, Table 2). In all organs, the concentrations of most elements were increased by the high nutrient supply treatment.

Discussion

Whole-plant C assimilation

Elevated [CO₂] did not stimulate RGR or NAR in C. odorata seedlings growing at a high rate of nutrient supply. However, in seedlings growing at a low rate of nutrient supply, elevated [CO₂] stimulated RGR, but had no significant effect on NAR. Consequently the total biomass of the seedlings was not increased in response to elevated [CO₂]. Root biomass increased at elevated [CO₂], but this increase occurred only when the rate of nutrient supply was non-limiting. Both RGR and NAR were stimulated by a high rate of nutrient supply, irrespective of CO₂ treatment, hence seedlings receiving the high rate of nutrient addition were much larger than seedlings receiving the low rate of nutrient addition. The hypothesis of a reduced stimulation of growth in response to elevated [CO₂] when the rate of nutrient supply was limiting was rejected. Although elevated [CO₂] increased RGR at low rates of nutrient supply,

![Figure 3. Assimilation rate versus intercellular [CO₂] (A/C) curves for leaves of C. odorata. Curves were derived from estimates of photosynthesis model parameters Jmax, Vcmax and Rd (de Pury and Farquhar 1997). The estimated average curve per treatment is shown. Treatments were: EH (elevated [CO₂] + high rate of nutrient application), EL (elevated [CO₂] + low rate of nutrient application), AH (ambient [CO₂] + high rate of nutrient application) and AL (ambient [CO₂] + low rate of nutrient application). n = 5 per high nutrient treatment and 4 per low nutrient treatment. The raw data are shown for both high (H) and low (L) nutrient addition rates. The CO₂ treatments had no significant effect on the derived parameters (P > 0.05). Lines are shown relating atmospheric [CO₂] to Cᵋ, the slope of which represents stomatal conductance, gs. Dotted lines represent gs of seedlings in the high rate of nutrient supply treatments. Lines representing gs in elevated and ambient [CO₂] start at a Cᵋ of 700 and 350 µmol m⁻² s⁻¹, respectively.](image)

![Figure 4. Mean estimates (± SE) of parameters of C. odorata seedlings derived from A/C curves based on the Farquhar et al. (1980) model. The fitted parameters for each CO₂ and nutrient treatment are Jmax (maximum rate of electron transport), Vcmax (maximum carboxylation velocity) and Rd (rate of dark respiration in the day). Treatments were: EH (elevated [CO₂] + high rate of nutrient application), EL (elevated [CO₂] + low rate of nutrient application), AH (ambient [CO₂] + high rate of nutrient application), and AL (ambient [CO₂] + low rate of nutrient application) (n = 5 for each high nutrient treatment and n = 4 for each low nutrient treatment).](image)
Chlorophyll

Table 1. Leaf concentrations of chlorophyll and carbohydrate components in *C. odorata* seedlings at final harvest. All concentrations are means (± SE) of all samples analyzed chemically. Treatments were: EH (elevated [CO2] + high rate of nutrient application), EL (elevated [CO2] + low rate of nutrient application), AH (ambient [CO2] + high rate of nutrient application) and AL (ambient [CO2] + low rate of nutrient application). Means followed by different letters within a row are significantly different (*P* < 0.05).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>EH (μg g⁻¹)</th>
<th>EL (μg g⁻¹)</th>
<th>AH (μg g⁻¹)</th>
<th>AL (μg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a (g m⁻²)</td>
<td>0.28 ± 0.01 a, <em>n</em> = 14</td>
<td>0.14 ± 0.01 b, <em>n</em> = 14</td>
<td>0.29 ± 0.01 a, <em>n</em> = 14</td>
<td>0.13 ± 0.01 b, <em>n</em> = 14</td>
</tr>
<tr>
<td>Chlorophyll b (g m⁻²)</td>
<td>0.12 ± 0.01 a, <em>n</em> = 14</td>
<td>0.06 ± 0.005 b, <em>n</em> = 14</td>
<td>0.13 ± 0.005 a, <em>n</em> = 14</td>
<td>0.05 ± 0.004 b, <em>n</em> = 14</td>
</tr>
<tr>
<td>Starch (mg g⁻¹)</td>
<td>15.2 ± 6.1 a, <em>n</em> = 14</td>
<td>30.2 ± 6.9 b, <em>n</em> = 12</td>
<td>7.7 ± 1.1 a, <em>n</em> = 14</td>
<td>25.2 ± 7.5 b, <em>n</em> = 14</td>
</tr>
<tr>
<td>Total mono- + oligosaccharide (mg g⁻¹)</td>
<td>7.95 ± 1.06 a, <em>n</em> = 13</td>
<td>5.43 ± 0.54 bc, <em>n</em> = 12</td>
<td>6.52 ± 0.60 b, <em>n</em> = 13</td>
<td>4.75 ± 0.38 c, <em>n</em> = 14</td>
</tr>
<tr>
<td>Sucrose (mg g⁻¹)</td>
<td>1.58 ± 0.37 a, <em>n</em> = 13</td>
<td>1.36 ± 0.36 a, <em>n</em> = 9</td>
<td>1.02 ± 0.23 a, <em>n</em> = 13</td>
<td>1.15 ± 0.27 a, <em>n</em> = 9</td>
</tr>
</tbody>
</table>

Table 2. Summary of root, stem and leaf nutrient concentrations (mg g⁻¹) of *C. odorata* seedlings at the end of the experiment. Values are means (± SE) with *n* = 14 for each treatment combination: EH (elevated [CO2] + high rate of nutrient application), EL (elevated [CO2] + low rate of nutrient application), AH (ambient [CO2] + high rate of nutrient application) and AL (ambient [CO2] + low rate of nutrient application). Means followed by different letters within a row are significantly different (*P* < 0.05).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>N (mg g⁻¹)</th>
<th>P (mg g⁻¹)</th>
<th>K (mg g⁻¹)</th>
<th>Ca (mg g⁻¹)</th>
<th>Mg (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Root</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EH</td>
<td>41.7 ± 1.9 a</td>
<td>2.4 ± 0.2 a</td>
<td>25.6 ± 1.8 a</td>
<td>5.0 ± 0.3 a</td>
<td>3.0 ± 0.2 a</td>
</tr>
<tr>
<td>EL</td>
<td>15.3 ± 1.1 b</td>
<td>1.2 ± 0.1 b</td>
<td>15.5 ± 0.6 b</td>
<td>4.1 ± 0.2 b</td>
<td>5.5 ± 0.1 b</td>
</tr>
<tr>
<td>AH</td>
<td>37.9 ± 4.2 a</td>
<td>2.3 ± 0.2 a</td>
<td>24.7 ± 1.9 a</td>
<td>5.4 ± 0.5 a</td>
<td>3.3 ± 0.4 a</td>
</tr>
<tr>
<td>AL</td>
<td>15.7 ± 0.9 b</td>
<td>1.3 ± 0.1 b</td>
<td>18.6 ± 0.9 b</td>
<td>4.1 ± 0.3 b</td>
<td>5.9 ± 0.3 b</td>
</tr>
<tr>
<td><strong>Stem</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EH</td>
<td>28.0 ± 1.1 a</td>
<td>2.6 ± 0.2 a</td>
<td>0.4 ± 0.04 a</td>
<td>0.1 ± 0.01 a</td>
<td>0.2 ± 0.01 a</td>
</tr>
<tr>
<td>EL</td>
<td>18.3 ± 4.3 b</td>
<td>2.4 ± 0.2 a</td>
<td>0.4 ± 0.03 a</td>
<td>0.2 ± 0.01 b</td>
<td>0.2 ± 0.02 a</td>
</tr>
<tr>
<td>AH</td>
<td>29.1 ± 2.1 a</td>
<td>3.0 ± 0.2 a</td>
<td>0.5 ± 0.06 a</td>
<td>0.1 ± 0.01 a</td>
<td>0.2 ± 0.01 a</td>
</tr>
<tr>
<td>AL</td>
<td>11.8 ± 2.5 b</td>
<td>2.3 ± 0.2 a</td>
<td>0.4 ± 0.01 a</td>
<td>0.2 ± 0.01 b</td>
<td>0.2 ± 0.01 a</td>
</tr>
<tr>
<td><strong>Leaf</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EH</td>
<td>14.4 ± 0.5 a</td>
<td>2.2 ± 0.1 a</td>
<td>15.9 ± 1.0 a</td>
<td>2.8 ± 1.0 a</td>
<td>3.6 ± 0.1 a</td>
</tr>
<tr>
<td>EL</td>
<td>5.2 ± 0.5 b</td>
<td>1.2 ± 0.1 b</td>
<td>6.0 ± 0.9 b</td>
<td>6.6 ± 1.0 b</td>
<td>5.4 ± 0.6 b</td>
</tr>
<tr>
<td>AH</td>
<td>15.8 ± 0.6 a</td>
<td>2.3 ± 0.1 a</td>
<td>14.5 ± 1.1 a</td>
<td>3.1 ± 0.8 a</td>
<td>4.6 ± 0.3 b</td>
</tr>
<tr>
<td>AL</td>
<td>5.5 ± 0.7 b</td>
<td>1.4 ± 0.2 b</td>
<td>8.3 ± 1.5 b</td>
<td>6.2 ± 1.0 b</td>
<td>5.7 ± 0.6 b</td>
</tr>
</tbody>
</table>

this effect was not translated into increased biomass.

Although we observed a trend toward stimulation of growth by elevated [CO2], the effect was not statistically significant (*P* > 0.05). The lack of an effect of elevated [CO2] on RGR in seedlings receiving a high rate of nutrient supply can be explained, in part, by compensatory responses of NAR and LAR. Mean NAR tended to increase with elevated [CO2], whereas mean LAR tended to decrease with elevated [CO2] (Figure 1). Although the decrease in LAR was not statistically significant (*P* > 0.05), the data indicate that while NAR increased, the fraction of biomass apportioned to leaves (LAR) decreased, contributing to an overall nonsignificant increase in RGR with elevated [CO2] (*P* > 0.05). This is consistent with the results of Norby et al. (1992) who reported an increase in NAR with a concurrent decrease in LAR and consequent lack of increase in either RGR or seedling biomass in yellow poplar. This species has an indeterminate growth pattern and can quickly readjust its carbon allocation pattern. *Cedrela odorata* is also indeterminate in growth pattern and a shift toward increased allocation of C to storage organs was indicated by the increase

TROPICAL TREES IN ELEVATED CO2 CONCENTRATION

983

TREE PHYSIOLOGY ON-LINE at http://www.heronpublishing.com
in root biomass. The elevated [CO2]-induced increase in R:S ratio was not significant. A similar result was also found in the meta-analysis of Curtis and Wang (1998).

Within-treatment variation is proposed as a large contributor to the nonsignificant effect of elevated [CO2] on growth. The main source of variation is thought to be genetic variation within each provenance, because there was little evidence for within- and between-chamber variability ($P > 0.05$). An increase in RGR of 44% would not have been statistically significant because of the variation present within a treatment. Furthermore, the highly significant nutrient effect had a tendency to swamp the effect of elevated [CO2] in the statistical tests.

In addition to a severe reduction in seedling growth when nutrient supply was limiting, there was a shift in allocation of carbon as indicated by a doubling of the R:S ratio. This is consistent with the idea of a “functional balance,” where the combined rates of use and uptake of nitrogen must equal those of carbon (Brouwer 1962). If shoot mass was not changing but nutrient availability was decreasing, the mass of nutrient uptake tissue, i.e., roots, must also have increased to preserve the balance. The concentrations of all macronutrients in root tissue declined under conditions of low supply (Table 1), probably indicating a dilution by carbohydrates.

Leaf-scale processes

Photosynthetic responses cannot explain the lack of growth response to elevated [CO2]. The $A/C_i$ curves showed an absence of down-regulation, and, based on estimates of photosynthetic rates from these curves at operational $C_i$, it appears that photosynthetic rate at least doubled in response to elevated [CO2], regardless of nutrient treatment. Parameters derived from the $A/C_i$ curves did not significantly differ between CO2 treatments ($P < 0.05$, Figure 4), although $J_{max}$ tended to increase with elevated [CO2] for seedlings growing at a low rate of nutrient addition ($P < 0.1$, Figure 3). An absence of down-regulation of photosynthesis in seedlings grown in elevated [CO2] is commonly observed (Curtis and Wang 1998, Norby et al. 1999). However, another meta-analysis based on European forest tree species showed that $J_{max}$ of trees in elevated [CO2] was, on average, 0.88 of that in trees in ambient [CO2] (Medlyn et al. 1999). Similarly, $V_{max}$ in elevated [CO2] was reported to be 0.91 of that at ambient [CO2].

Because the elevated [CO2] treatment did not result in a significant increase in NAR despite the stimulation of photosynthesis, we suggest that any extra carbon assimilated was lost as respiration. There was too much variation in our data to test whether $R_d$ was reduced, although the majority of studies suggest this to be true (Curtis 1996). Our results provide circumstantial evidence that $g_s$ was reduced in the presence of elevated [CO2] (Figure 3). Therefore it seems likely that an increase in respiration of non-photosynthetic organs occurred in response to elevated [CO2].

Values of $J_{max}$ and $V_{max}$ differed between nutrient treatments, suggesting that a low rate of nutrient supply resulted in down-regulation of photosynthesis, with nutrient-limited seedlings showing a reduced photosynthetic capacity. This reduction in photosynthetic capacity did not, however, increase the likelihood of acclimation to elevated [CO2]. On the contrary, exposure to elevated [CO2] appears to have ameliorated the effect of low nutrient supply, because $J_{max}$ of seedlings in elevated [CO2] tended to increase under conditions of low nutrient availability (Figure 4). Published data on low nutrient availability triggering acclimation are inconsistent (reviewed by Pettersson and McDonald 1994). Most studies have used the growth [CO2] to test for acclimation instead of comparing Rubisco activity at both ambient and elevated [CO2] in seedlings grown at high and low nutrient availability (Gunderson and Wullschleger 1994). Furthermore, we observed no evidence of an interaction between growth [CO2] and nutrient supply rate on the acclimation response.

Investment in photosynthetic light capture, as indicated by chlorophyll concentration, decreased in response to a low rate of nutrient supply but was unaffected by [CO2] (Table 2). Similarly, foliar starch increased in seedlings grown at a low rate of nutrient supply but was not affected by growth [CO2]. The concentrations of leaf N ($N_{leaf}$) and P, reported on a mass basis, both decreased significantly in response to a low rate of nutrient supply, but were unaffected by [CO2] (Table 1). This is consistent with the idea of a conserved $N_{leaf}$/photosynthesis relationship (Field and Mooney 1986), because $J_{max}$ and $V_{max}$ values are equivalent at a given $N_{leaf}$. Values of $N_{leaf}$ in the present study were comparable with those found naturally in an Amazonian rain forest in Venezuela (Reich et al. 1994). In addition, our values of $J_{max}$ and $V_{max}$ were well within the range reported for rain forest species in situ (Wullschleger 1993, Carswell et al. 2000). However, our estimates of $J_{max}$ and $V_{max}$ relative to $N_{leaf}$, on an area basis, were higher than in situ measurements suggest (Meir 1996), largely because of the high SLA of our seedlings.

Conclusions

Despite a sustained increase in photosynthesis in response to elevated [CO2], there was no increase in NAR. We postulate that this is a result of the combined effect of an increase in respiration of non-photosynthetic organs in elevated [CO2] and a high amount of within-treatment variation that reduced the power of the statistical tests. Consequently, there was no stimulation of whole-plant biomass by elevated [CO2] although root growth was stimulated by elevated [CO2] at a high rate of nutrient supply. The increase in root growth did not lead to a change in R:S ratio. Leaf concentrations of chlorophyll, starch and nitrogen did not change in response to elevated [CO2]. Rate of nutrient supply did not interact with [CO2], except for root growth where there was no stimulation by elevated [CO2] at a low rate of nutrient supply.

In both CO2 treatments, RGR, NAR and consequent biomass increment were all increased by a high rate of nutrient supply. This is thought to be a consequence of down-regulation of photosynthesis at low nutrient supply, as indicated by significantly lower values of $J_{max}$ and $V_{max}$ in the $A/C_i$ curves.
of seedlings grown at low rates of nutrient supply than at high rates of nutrient supply. Values of $N_{\text{leaf}}$ also decreased, indicating a conservation of the $N_{\text{leaf}}$/photosynthesis relationship as predicted by studies of rain forest leaves in situ. The values of $J_{\text{max}}$ and $V_{\text{cmax}}$ presented in this paper could therefore be used in models that scale up individual-tree responses to whole-forest responses.

Acknowledgments

Financial support was provided by the Commonwealth Scholarship Commission (U.K.). We acknowledge the grounds staff of the Royal Botanic Garden in Edinburgh, who provided much support and technical expertise, specifically Paul Smith and David Mitchell. In addition, we thank Craig Barton, James Irvine, Andy Gray, Diarmuid O’Neill, Alex Harrower, Dave Mackenzie, Bob Astles and Bill Adams from the University of Edinburgh for all of their help with this research.

References


