

Growth, cell walls, and UDP-Glc dehydrogenase activity of *Arabidopsis thaliana* grown in elevated carbon dioxide¹

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Summary

The impact of elevated CO₂ (1000 μmol/mol) was assessed on the common weed, *Arabidopsis thaliana* (Landsberg erecta), which is used as a model plant system. Elevated CO₂ stimulated relative growth rate (RGR) and leaf area gain of *Arabidopsis* beginning from the cotyledon stage and continuing through the juvenile stage. This early advantage in growth enabled the plants grown in elevated CO₂ to gain more DW despite similar RGRs throughout the latter stages of development. The greater accumulation of DW in leaves grown in elevated CO₂ resulted in a lower specific leaf area (SLA). However, the amount of cell wall investment per unit of leaf area, specific "wall" area (SWA), was similar indicating that elevated CO₂ did not affect the distribution of cell carbon to the cell wall of leaves beyond that needed for cell and leaf expansion. Furthermore, cell wall composition changed with time due to developmental changes and was not affected by elevated CO₂. Associated with the increase in RGR by elevated CO₂ was a concomitant increase in the activity of UDP-Glc dehydrogenase (E.C. 1.1.1.22), a key enzyme in the nucleotide–sugar interconversion pathway necessary for biosynthesis of many cell-wall polysaccharides.

Key words: *Arabidopsis thaliana* – cell wall – elevated carbon dioxide – growth – UDP-Glc dehydrogenase

Introduction

The cell wall is a major sink for carbohydrate comprising 20 to 50% of the dry weight of a plant and 70 to 80% of the carbohydrate (Penning de Vries et al. 1974). Much of the research

into the structure and biosynthesis of cell walls is focused on the primary wall, because the changes that occur in the primary wall are crucial in plant development (Carpita and Gibeaut 1993).

There are a number of enzymes involved in the biosynthesis of the cell wall. Cellulose synthase produces cellulose and callose, which are the only polysaccharides known to be made at the plasma membrane (Delmer and Amor 1995). Interlocking glycans and pectins are synthesized and modified in the Golgi apparatus by various synthases. These polysaccharides are packaged in secretory vesicles and transported to the cell wall (Driouch et al. 1993, Gibeaut and Car-

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pita 1994). Further modifications occur in the wall such as de-esterification of pectins, phenolic cross-linking, hydrolysis and turnover of specific polysaccharides (McCann et al. 1995).

UDP-Glc dehydrogenase (E.C. 1.1.1.22) acts upon the largest proportion of polysaccharides in the plant cell wall which pass through the Golgi apparatus. UDP-Glc dehydrogenase catalyzes the conversion of UDP-Glc to UDP-GlcUA, which can then be interconverted to other nucleotide sugars. These sugars are the precursor molecules for the synthesis of the majority of glycosyl units of structural carbohydrates in the cell wall. UDP-Glc dehydrogenase is a good candidate for a control point in the metabolic pathway of cell wall synthesis because it is in low concentration relative to other enzymes in the pathway, operates far from equilibrium, and because so much of the cell wall carbohydrate is acted upon by this enzyme (Amino et al. 1985 a, 1985 b, Dalessandro and Northcote 1977 a, 1977 b, Robertson et al. 1995, Tenhaken and Thulke 1996).

Cells expand because the cell wall is made more extensible by enzymatic activities that are located both in the wall and in the biosynthetic pathway (N. C. Carpita and D. M. Gibeaut 1993, Cosgrove 1997, D. A. Gibeaut and N. C. Carpita 1994, Taiz 1984). Chemical modifications in the wall mediated by expansins, xyloglucan endotransglycoylase (XET), and polysaccharide endo- and exohydrolases, must occur to allow for polymer creep. Concomitantly, the synthesis and secretion of molecules to the cell wall must occur to sustain growth (Brummell and Hall 1985, Edelman et al. 1989, Ray 1987).

Elevated CO₂ increases cell wall extensibility and stimulates cell expansion rates of roots (Ferris and Taylor 1994) and leaves (Ranasinghe and Taylor 1996), possibly by the increased activity of XET (Taylor et al. 1994). However, the activity of XET alone does not promote loosening or extension of isolated cell walls (Macqueen-Mason et al. 1993). The effect of elevated CO₂ on the activities of other cell wall loosening enzymes and cell wall synthesis have not yet been published.

In this study we characterize the growth response of *Arabidopsis* to elevated CO₂ and correlate this response to changes in cell wall composition and activity of one rate-limiting enzyme in cell wall biosynthesis, UDP-Glc dehydrogenase. We present data showing that the activity of UDP-Glc dehydrogenase is increased by elevated CO₂ and is correlated with the RGR of *Arabidopsis*.

Materials and Methods

A. thaliana (Landsberg erecta) was grown in hydroponic culture under a 10-h photoperiod (400 μmol quanta PAR m⁻² s⁻¹), 75% RH, and day/night temperatures of 21/18 °C. We used hydroponic culture for *Arabidopsis* as described (Gibeaut et al. 1997) because hydroponic culture can produce uniform root and shoot material without the complications of water and nutrient stress or root restriction.

Hydroponic culture

Low-density polyethylene tubs, 32-L volume, were covered with acrylic tops that held 35 individual plants in removable, plastic plugs. Cylinders (3 × 1.5 cm) of rockwool (GrodanHP, Agro Dynamics Inc., East Brunswick, NJ) were cored from a slab and placed in the plugs to a depth that allowed contact with a hydroponic solution. Dry seeds were sprinkled on the rockwool then wetted with a wash bottle. Plants were thinned to one plant per plug.

The hydroponic solution comprised the following macronutrients: 1.25 mmol/L KNO₃, 1.50 mmol/L Ca(NO₃)₂, 0.75 mmol/L MgSO₄, 0.50 mmol/L KH₂PO₄; and the following micronutrients, 50 μmol/L KCl, 50 μmol/L H₃BO₃, 10 μmol/L MnSO₄, 2.0 μmol/L ZnSO₄, 1.5 μmol/L CuSO₄, 0.075 μmol/L (NH₄)₆Mo₇O₂₄, 72 μmol/L Fe-diethylenetriamine pentaacetate (Sequestrene 330, Ciba-Geigy, Greensboro, NC). The solution was also supplemented with 0.1 mmol/L Na₂O₃Si (Epstein 1994, Rafi et al. 1997). The final solution pH was 6.0, and the electroconductivity was 0.66 dS m⁻¹. Air from an oil-free compressed air source (ambient CO₂) was bubbled through a 30-cm long air stone for 5 min every 30 min.

Controlled environment chambers

Two large and two smaller (PGW36, and E15, Conviron Products Co., Winnipeg, Manitoba) controlled environment chambers were maintained at desired atmospheric concentrations of CO₂ using a system comprising an infrared gas analyzer (LI 6252, LICOR Inc., Lincoln, NE), remote data logger (CR10T, Campbell Sci. Inc., Logan, UT), real time monitoring on computer (RTMS, Campbell Sci., Inc., Logan, UT), sampling pumps and switching manifold, CO₂ injectors, and a high pressure CO₂ scrubber (PCDA 21200, Puregas, Westminster, CO) for delivery of CO₂-free air.

Experimental protocol

Four experiments (replicated in time) in the smaller chambers were conducted by planting a single tub of 35 plants in each of the chambers with CO₂ concentrations maintained at 360 or 1000 μmol/mol. At each harvest time, five plants from each chamber were randomly selected for growth analysis. Two experiments (replicated in time and alternating chambers) in the larger chambers were conducted for both growth and cell wall analyses, by planting three tubs of 35 plants in each of the chambers with CO₂ concentrations maintained at 360 or 1000 μmol/mol. At each harvest time, three plants were randomly selected for growth analysis, and an appropriate number of plants were selected for cell wall analysis. Harvests were taken between the 8th and 9th h of the photoperiod.

Growth analysis

An average of the initial DW of plants was estimated by weighing approximately 300 seeds. During the first 14 days after planting, an average of whole plant DW was estimated by harvesting 35 plants, drying and weighing them together. The areas of cotyledons and the first few leaves were estimated by measuring their length and width

using a calibrated reticle in a dissecting microscope. Areas were calculated as the area of an ellipse and average values were determined from five plants. Fresh weights were not determined for these young plants.

Beginning on day 17, the plants were large enough to measure the leaf areas, and the FW and DW of individual plants. Plants were removed from the hydroponic tubs one at a time, dissected into root, leaf, and when bolting commenced on day 32 and 37 for plants grown at 1000 and 360 $\mu\text{mol/mol}$ CO₂, respectively, the inflorescence and stem of the plant was dissected using a 1-cm diameter cork bore. FW of the shoot tissues were determined immediately; roots were first rinsed and blotted dry. Leaf area was measured using a portable area meter (LI-3000A, LiCOR Inc., Lincoln, NE). DW of the material was determined after drying for 2 d in an oven at 60 °C.

Plant growth data from individuals of each experiment were pooled for statistical analysis because no statistically significant differences between replicates (either in time or between chambers) for similar experimental treatments were observed. Data were analyzed for RGR by use of the Richards function (Venus and Causton 1979).

Purification and fractionation of cell wall material

The following procedures were done using 50-mL conical tubes (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ). Each washing step was done in 15 mL of solution stirred vigorously with magnetic stir bars. Pelleting was done at 2500 *g* for 15 to 30 min in a desk top centrifuge with a swinging bucket rotor (GPR, Beckman, Palo Alto, CA). All supernatants and extracts were filtered through glass fiber filters (G8, Fisher Scientific, Pittsburgh, PA) to ensure good recovery of the wall materials.

About 3 g fresh leaves were homogenized in 9 mL ice-cold 50 mmol/L Tris-KOH, 50 mmol/L NaCl, 30 mmol/L ascorbic acid, pH 5.5, using a Polytron with a 1-cm probe (PT 10/35, PTA 10S, Brinkman Inc., Westbury, NY). After homogenization, the mixture was brought to 1.5% Triton X-100 to assist membrane disruption. The cell walls and debris were washed sequentially at RT with 0.1 mol/L NaCl (supplemented with 1% Triton X-100) (2 \times), 0.1 mol/L NaCl (3 \times), water (2 \times), methanol (3 \times), and water (2 \times). To remove starch, 10 mL of DMSO were added to the pellets and the suspensions were stirred vigorously overnight (Carpita and Kanabus 1987). The remaining purified cell wall materials were washed with water (2 \times), resuspended in water, and lyophilized.

Pectin was extracted from the purified cell wall materials by stirring in 10 mL of 50 mmol/L ammonium oxalate (AO) for 30 min in a boiling water bath (2 \times). The AO supernatants were combined, dialyzed (Spectra/Por 4, Spectrum Medical Industries, Houston, TX), and lyophilized. The pellets ("pectin-free" cell wall) were washed in water (3 \times) and lyophilized. Crosslinking polysaccharides were extracted from the "pectin-free" cell walls in 10 mL of 4.0 mol/L KOH (supplemented with 3 mg/mL NaBH₄) with vigorous stirring for 1 h. A second KOH extraction was done overnight. The KOH supernatants were neutralized with glacial acetic acid, dialyzed, and lyophilized. The pellets (α -cellulose) were washed in water (3 \times) and lyophilized. Materials tightly bound to cellulose were removed with a single acetic/nitric acid treatment in boiling water for 90 min (Updegraff 1969).

UDP-Glc dehydrogenase assay

UDP-Glc dehydrogenase (E.C. 1.1.1.22) is specific for UDP-Glc, irreversibly converts UDP-Glc to UDP-GlcUA, and has a pH optimum of

8.5 to 8.7 (S.-I. Amino et al. 1985 a, G. Dalessandro and D. H. Northcote 1977 a). The enzyme is inhibited by UDP-Xyl (Abdul-Baki and Ray 1971) which is formed by the activity of UDP-GlcUA decarboxylase. Assays were performed using about 3 g FW of freshly processed material. The entire shoot for small plants (up to day 25), or leaf tissue separated from the shoot apex using a 1-cm cork bore for larger plants, were harvested and homogenized in a glass-glass homogenizer (Duell, Kontes Glass Co., Vineland, NJ) with 3 mL ice-cold buffer, 50 mmol/L Tris-base, 10 mmol/L Na₂-EDTA, 20% glycerol (v/v), pH 8.7. The homogenate was centrifuged for 20 min at 20,000 *g*. The supernatant was collected then 2.5 mL was applied to a Sephadex G-25 desalting column (PD-10, Pharmacia Biotech Inc., Piscataway, NJ) equilibrated in the same buffer. The extract was eluted with 3.5 mL buffer, and the eluant was kept on ice until the assay. The desalting column removed UDP-Xyl and substrates for competing dehydrogenases such as pyruvate for lactate dehydrogenase. The assay was performed by first mixing 0.8 mL of the eluant with 0.1 mL 100 mmol/L NAD⁺ then incubating for 3 min in a 30 °C water bath. The reaction was initiated with 0.1 mL 100 mmol/L UDP-Glc and the rate was monitored for 4 min as the change in A₃₄₀ due to the reduction of NAD⁺. The reaction was linear for this time period (data not shown).

Results

Elevated CO₂ significantly increased dry matter accumulation in the whole plant (Fig. 1). The greatest effect was in the early stages of growth (Fig. 1) and was reflected by the calculation of RGR (Fig. 2). Similar effects on RGR were seen for the root and whole plant (data not shown). In the first three weeks of growth the RGR was about 20% greater for plants in elevated CO₂ than those at ambient CO₂. After about 21 days the RGR

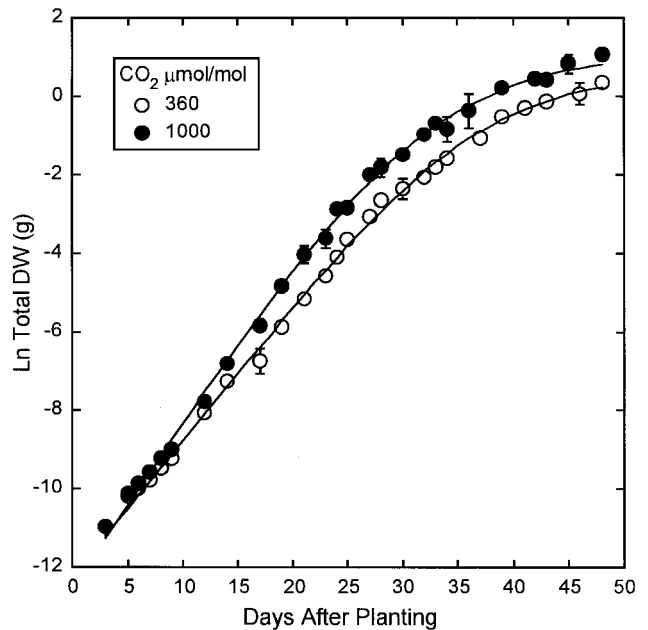


Figure 1. Plants grown at elevated CO₂ accumulated DW faster and to a greater extent than those grown at low CO₂. The lines were fitted using the Richards function. Error bars are 95% confidence values.

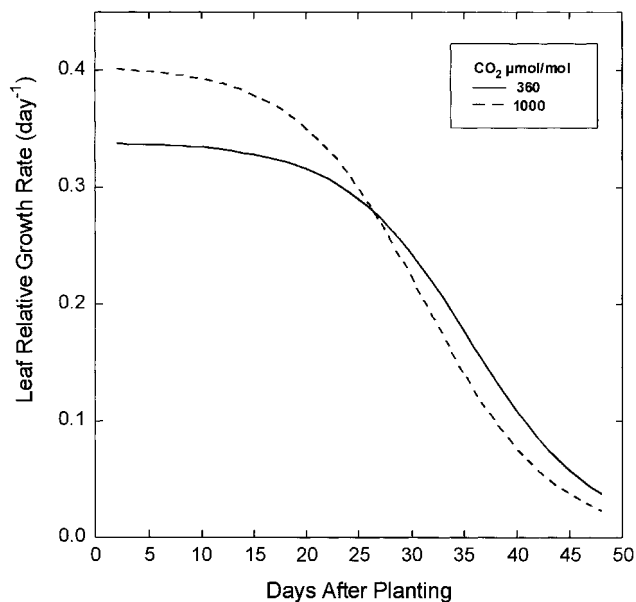


Figure 2. The relative growth rate (RGR) of leaves was initially greater in plants grown at elevated CO_2 , but declined to similar rates as plants initiated flowering. RGR was calculated using the Richards function parameters as described.

of plants under both treatments began to decline to similar values.

The root weight ratio (RWR), defined as the proportion of root DW to whole plant DW, varied with time and CO_2 concentration (Fig. 3 A). The RWR was initially low, then peaked at the time of the initiation of the inflorescence, about 26 or 30 days for plants grown in elevated or low CO_2 , respectively. However, if RWR was plotted versus plant DW (Fig. 3 B) then no

difference was observed – only the time dependent changes in RWR were apparent.

The specific leaf area (SLA), defined as the leaf area per unit of leaf DW, was lowered by elevated CO_2 when compared on a time basis (Fig. 4 A), although about one-third of this difference was negated when compared on a leaf DW basis (Fig. 4 B). If the leaf area per unit of cell-wall DW (specific “wall” area, SWA) was compared versus time (Fig. 5 A) or leaf DW (Fig. 5 B), then only the time dependent effects, independent of plant DW, were apparent. These data indicate that allocation of wall material was not greatly altered by growth at elevated CO_2 , if you take into account the accelerated rate of development. Furthermore, the composition of the cell wall, as determined using sequential extraction, showed differences between CO_2 treatments when compared on a time basis (data not shown) but not on a leaf DW basis (Fig. 6). Despite many differences in the fractionation procedure, the proportions of the fractions we obtained (Fig. 6) were similar to the values deduced from Zablackis et al. (1995): 48 % pectin, 27 % cross-linking glycan, 8 % acetic/nitric acid soluble, and 16 % crystalline cellulose.

The activity of UDP-Glc dehydrogenase in leaves was increased by elevated CO_2 concentration in the early stages of growth (Fig. 7). This response was similar to that of RGR (Fig. 2) and is more easily seen by plotting the activity of UDP-Glc dehydrogenase against RGR (Fig. 8). The linear response ($r^2 = 0.83$) indicates that there is a very good correlation between these two factors. Some complications with these measurements were observed when the plants began the transition to flowering. Extracts obtained from the forming floral shoot apices, which included much more meristematic tissue compared to leaves, were about 16-fold greater than those in slowly expanding leaves (1620 ± 126 vs. 100 ± 9 nmole $\text{min}^{-1} \text{g}^{-1}$ FW; mean \pm SE). This indicates that there is a much higher con-

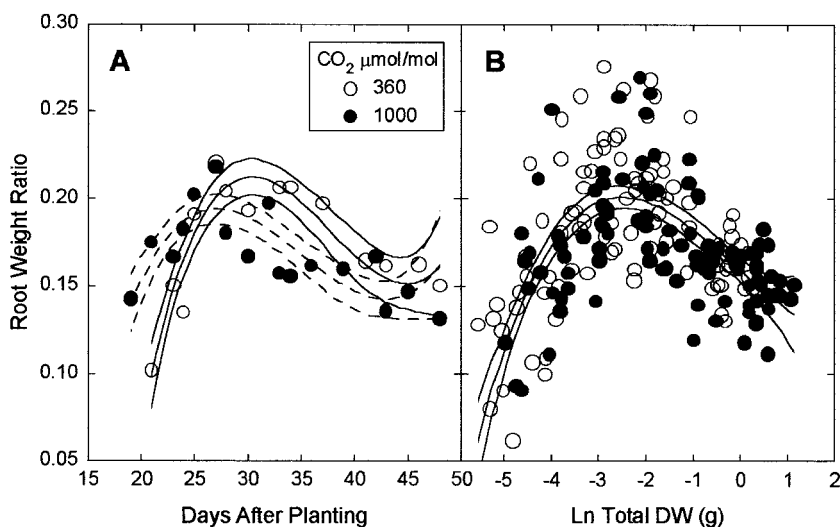


Figure 3. The root weight ratio (RWR) was initially greater in plants grown at elevated CO_2 . The ratio peaks at about the time of formation of the floral apex, d 30 or 25 for plants grown at low or elevated CO_2 , respectively (A). When plotted versus leaf DW, the lines could not be distinguished (B). The lines were fitted by third order polynomials. Error bars are 95 % confidence values.

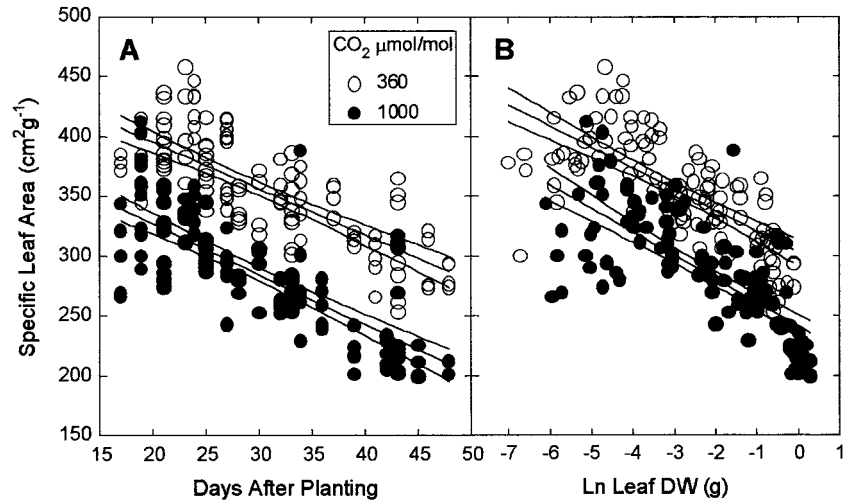


Figure 4. The specific leaf area (SLA) was lower for plants grown at elevated CO₂ (A). About one-third of this difference was negated when SLA was compared based on leaf DW (B). Lines were fitted by linear regression with 95% confidence intervals.

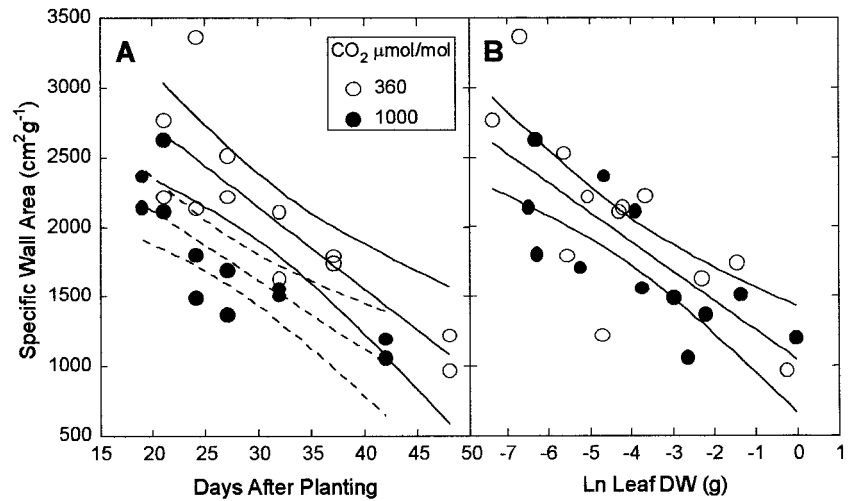


Figure 5. The specific “wall” area (SWA) was lower for plants grown at elevated CO₂ when compared vs. time (A). However, this difference was negated when compared vs. leaf DW (B). Lines were fitted by linear regression with 95% confidence intervals.

centration of enzyme in the very early stages of organ formation compared to more slowly expanding leaves.

Discussion

Analysis of RGR can pinpoint the critical developmental phase when plant responses to the environment are greatest. In *Arabidopsis* the RGR was most affected by atmospheric CO₂ concentration during the first three weeks of growth; after this time the differences in RGR between ambient and elevated CO₂ treated plants disappeared (Fig. 2). If one were to only examine these plants after 3 weeks for physiological or biochemical responses to CO₂, then the critical response to CO₂ may be missed – only the cumulative effects will be apparent. Other researchers have observed that the effects of CO₂ on growth are greatest in the early developmental stages of *Arabidopsis* (Eckardt et al. 1997), rice (Jitla et al. 1997,

Makino et al. 1997), wheat (Robertson and Leech 1995), tobacco (Masle et al. 1993), and poplar (Radoglou and Jarvis 1990).

In this study the increased RGR in the first few weeks of growth could account for most of the accelerated developmental effects observed. For example, the RWR was equal at the onset of flowering, and the DW was equal when bolting commenced although the timing of these events was offset by several days. For these reasons we compared our data on a DW basis and found few differences between CO₂ treatments for many growth parameters. Indeed it has been stated that the main effect of CO₂ on growth and allocation of carbon is in a changed timing of development (Eamus and Jarvis 1989).

One effect of elevated CO₂ not fully accounted for by the altered rate of development was the change of SLA. Most C₃ plants grown at elevated CO₂ do not entirely redistribute the increased DW from photosynthesis into increased leaf area; this results in reduced SLA and LAR (Badger 1992) through

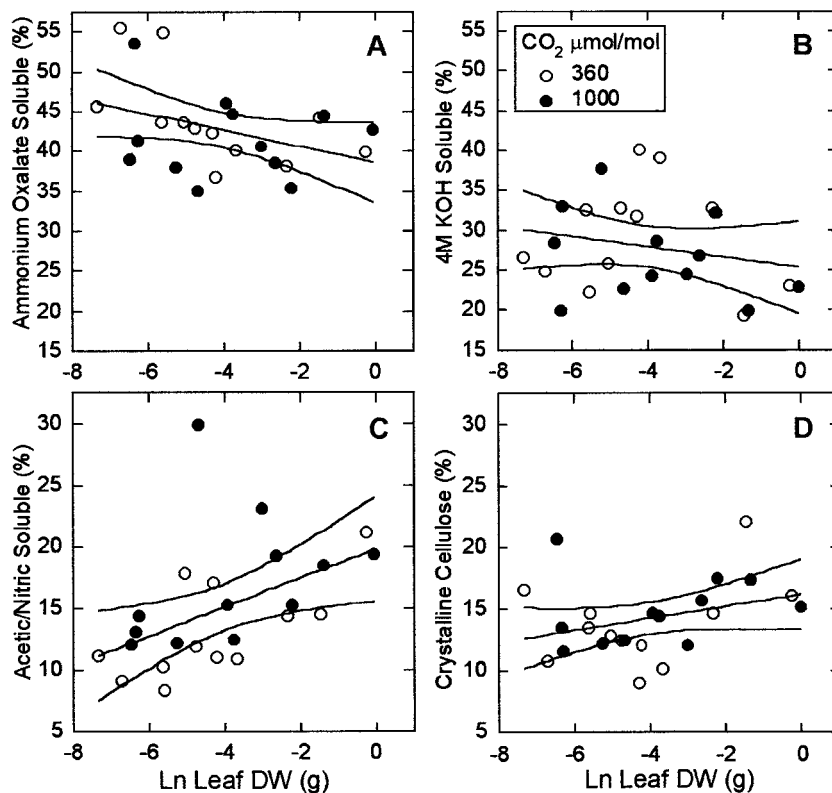


Figure 6. As the plants grew, the proportion of pectin (AO soluble), and cross-linking glycan (KOH soluble) decreased relative to an increase in α -cellulose (A/N soluble plus crystalline cellulose). The concentration of CO₂ had no measurable effect on cell wall composition when compared based on plant size. Lines were fitted by linear regression with 95% confidence intervals.

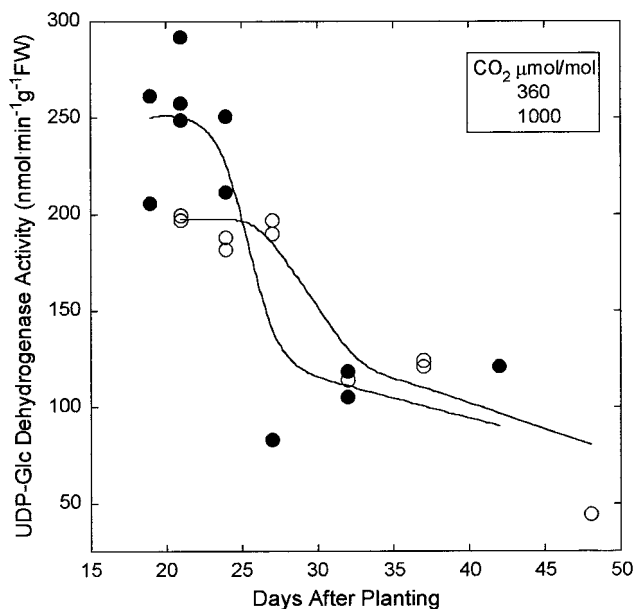


Figure 7. The activity of UDP-Glc dehydrogenase in leaves was greatest in young plants, especially those grown at elevated CO₂. Activity declined in a fashion similar to the decline in RGR (Fig. 2). Lines were fitted by interpolation.

the accumulation of starch and soluble sugars (Poorter et al. 1997). Similarly in this study, the SLA of *Arabidopsis* grown at elevated CO₂ was reduced throughout development (Fig. 4) even when the difference in developmental rate was considered. More importantly, on the basis of leaf area per unit cell wall (SWA), there was no effect of CO₂ when the different rates of development were taken into account (Fig. 5). In other words, elevated CO₂ did not significantly change the amount of wall carbon per unit leaf area when equivalent developmental plant ages were considered. Because the SWA was only affected by developmental rate and because there was a greater accumulation of starch and soluble sugar in plants grown at elevated CO₂ (data not shown), any increased allocation of carbon to the cell wall (from elevated CO₂) could lead to increased growth. However, we also observed that alterations in cell wall composition were developmentally related. Increased growth would have to occur before any developmental changes that rigidify the wall.

Growth and the physical properties of the cell wall can be experimentally altered by promoting or disrupting the synthesis or secretion of cell wall materials. Auxin stimulates hemicellulose secretion and cell wall extensibility (Kutschera and Briggs 1987); whereas, ABA and salinity inhibit hemicellulose secretion and cell expansion (Wakabayashi et al. 1991,

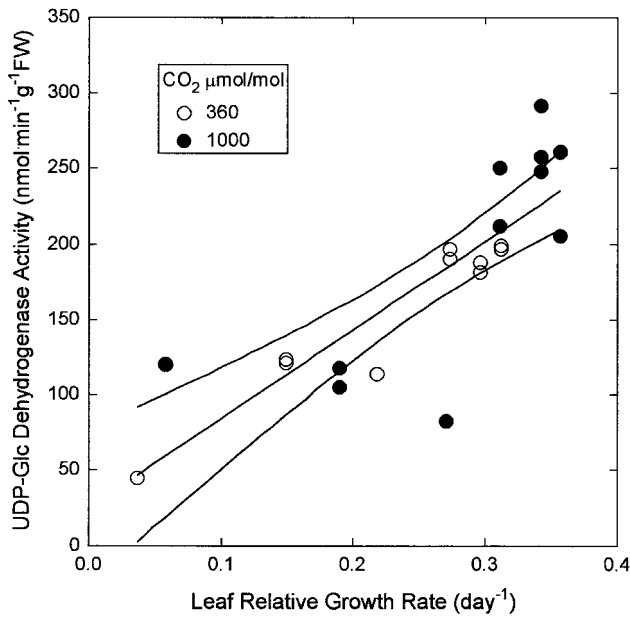


Figure 8. The activity of UDP-Glc dehydrogenase in leaves was positively correlated with the RGR of leaves, irrespective of the concentration of CO₂. Lines were fitted by linear regression with 95% confidence intervals ($r^2=0.83$).

Zhong and Läuchli 1988, 1993). Furthermore, treatment with monensin (U. Kutschera and W. R. Briggs 1987) or Brefeldin-A (Schindler et al. 1994) inhibits secretion and cell wall extensibility by disrupting vesicle transport. It is possible that elevated CO₂ enhances growth and cell wall properties through increased cell wall synthesis. Therefore, regulation of substrate concentrations for cell wall synthesis could affect the ability of the plant to respond to CO₂.

In support of this argument, there was a good correlation of the effect of elevated CO₂ on RGR with the effect of elevated CO₂ on the activity of UDP-Glc dehydrogenase (Figs. 2, 7, and 8). Because the majority of the non-glucosyl sugars of the cell wall are derived from the interconversion of UDP-Glc beginning with the activity of UDP-Glc dehydrogenase, regulation of this activity could be critical in the synthesis of xyloglucan and other growth relevant matrix polysaccharides. Dalessandro and Northcote (1977b) hypothesized that UDP-Glc dehydrogenase could be a controlling step in the formation of UDP-Xyl and cell wall synthesis in pea seedlings because the activity was low compared to the activity of UDP-GlcUA decarboxylase. Amino et al. (1985b) also concluded that UDP-Glc dehydrogenase was a probable regulatory step in cell wall synthesis because the level of UDP-GlcUA correlated with both the changes in UDP-Glc dehydrogenase activity and the increased cell wall synthesis during the G1 phase of synchronous cell cultures (S.-I. Amino et al. 1985a). Furthermore, the enzyme activity was shown to increase in hypocotyls and suspension cultured cells of French bean with a concomitant increase in wall synthesis and differentia-

tion (D. Robertson et al. 1995). In addition, the expression of mRNA for UDP-Glc dehydrogenase was shown to be greatest in the growing portions of leaves and roots of soybean (R. Tenhaken and O. Thulke 1996). In the present study we observed that the activity per FW was 16-fold greater in the shoot meristem during the transition to flowering compared to the activity in leaves. Thus, UDP-Glc dehydrogenase activity appears to have an important role in the early developmental phases of growth and its influence may occur very early in the formation of new sink tissues.

References

- Abdul-Baki AA, Ray PM (1971) Regulation by auxin of carbohydrate metabolism involved in cell wall synthesis by pea stem tissue. *Plant Physiol* 47: 537–544
- Amino S-I, Takeuchi Y, Komamine A (1985a) Changes in enzyme activities involved in formation and interconversion of UDP-sugars during the cell cycle in a synchronous culture of *Catharanthus roseus*. *Physiol Plant* 64: 111–117
- Amino S-I, Takeuchi Y, Komamine A (1985b) Changes in intracellular UDP-sugar levels during the cell cycle in a synchronous culture of *Catharanthus roseus*. *Physiol Plant* 64: 197–201
- Badger MR (1992) Manipulating agricultural plants for a future high CO₂ environment. *Aust J Bot* 40: 421–429
- Brummell DA, Hall JL (1985) The role of cell wall synthesis in sustained auxin-induced growth. *Physiol Plant* 63: 406–412
- Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J* 3: 1–30
- Carpita NC, Kanabus J (1987) Extraction of starch by dimethyl sulphoxide and quantitation by enzymatic assay. *Anal Biochem* 161: 132–139
- Cosgrove DJ (1997) Relaxation in a high-stress environment: The molecular bases of extensible cell walls and cell enlargement. *Plant Cell* 9: 1031–1041
- Dalessandro G, Northcote DH (1977a) Changes in enzyme activities of nucleoside diphosphate sugar interconversions during differentiation of cambium and xylem in pine and fir. *Biochem J* 162: 281–288
- Dalessandro G, Northcote DH (1977b) Possible control sites of polysaccharide synthesis during cell growth and wall expansion of pea seedlings (*Pisum sativum* L.). *Planta* 134: 39–44
- Delmer DP, Amor Y (1995) Cellulose biosynthesis. *Plant Cell* 7: 205–215
- Drionch A, Faye L, Staehelin LA (1993) The plant Golgi apparatus: a factory for complex polysaccharide and glycoproteins. *Trends Biochem Sci* 18: 210–214
- Eamus D, Jarvis PG (1989) The direct effects of increase in the global atmospheric CO₂ concentration on natural and commercial temperate trees and forests. *Adv Ecol Res* 19: 1–55
- Eckardt NA, Snyder GW, Portis AR Jr, Ogren WL (1997) Growth and photosynthesis under high and low irradiance of *Arabidopsis thaliana* antisense mutants with reduced ribulose-1,5-bisphosphate carboxylase/oxygenase activase content. *Plant Physiol* 113: 575–586
- Edelmann H, Bergfeld R, Schopfer P (1989) Role of cell-wall biogenesis in the initiation of auxin-mediated growth in coleoptiles of *Zea mays* L. *Planta* 179: 486–494

- Epstein E (1994) The anomaly of silicon in plant biology. *Proc Natl Acad Sci USA* 91: 11–17
- Ferris R, Taylor G (1994) Increased root growth in elevated CO₂: biophysical analysis of root cell elongation. *J Exp Bot* 45: 1603–1612
- Gibeaut DA, Carpita NC (1994) Biosynthesis of plant cell wall polysaccharides. *The FASEB Journal* 8: 904–915
- Gibeaut DM, Hulett J, Cramer GR, Seemann JR (1997) Maximal biomass of *Arabidopsis thaliana* using a simple, low-maintenance hydroponic method and favorable environmental conditions. *Plant Physiol* 115: 317–319
- Jitta DS, Rogers GS, Seneweera SP, Basra AS, Oldfield RJ, Conroy JP (1997) Accelerated early growth of rice at elevated CO₂ – Is it related to developmental changes in the shoot apex? *Plant Physiol* 115: 15–22
- Kutschera U, Briggs WR (1987) Rapid auxin-induced stimulation of cell wall synthesis in pea internodes. *Proc Natl Acad Sci USA* 84: 2747–2751
- Makino A, Harada M, Sato T, Nakano H, Mae T (1997) Growth and N allocation in rice plants under CO₂ enrichment. *Plant Physiol* 115: 199–203
- Masle J, Hudson GS, Badger MR (1993) Effects of ambient CO₂ concentration on growth and nitrogen use in tobacco (*Nicotiana tabacum*) plants transformed with an antisense gene to the small subunit of ribulose-1,5-bisphosphate carboxylase oxygenase. *Plant Physiol* 103: 1075–1088
- McCann MC, Roberts K, Wilson RH, Gidley MJ, Gibeaut DM, Kim J-B, Carpita NC (1995) Old and new ways to probe plant cell-wall architecture. *Can J Bot* 73: S 103–S 113
- Mcqueen-Mason SJ, Fry SC, Durachko DM, Cosgrove DJ (1993) The relationship between xyloglucan endotransglycosylase and *in-vitro* cell wall extension in cucumber hypocotyls. *Planta* 190: 327–331
- Penning de Vries FWT, Brunsting AHM, van Laar HH (1974) Products, requirements and efficiency of biosynthesis: a quantitative approach. *J Theoret Biol* 45: 339–377
- Poorter H, Van Berkel Y, Baxter R, Den Hertog J, Dijkstra P, Gifford RM, Griffin KL, Roumet C, Roy J, Wong SC (1997) The effect of elevated CO₂ on the chemical composition and construction costs of leaves of 27 C₃ species. *Plant Cell Environ* 20: 472–482
- Radoglou KM, Jarvis PG (1990) Effects of CO₂ enrichment on four poplar clones. I. Growth and leaf anatomy. *Ann Bot* 65: 617–626
- Rafi MM, Epstein E, Falk RH (1997) Silicon deprivation causes physical abnormalities in wheat (*Triticum aestivum* L.). *J Plant Physiol* 151: 497–501
- Ranasinghe S, Taylor G (1996) Mechanism for increased leaf growth in elevated CO₂. *J Exp Bot* 47: 349–358
- Ray PM (1987) Principles of plant cell expansion. In: Cosgrove DJ, Knievel DP (eds) *Physiology of Cell Expansion During Plant Growth*. The American Society of Plant Physiologists, Rockville, pp 1–17
- Robertson D, McCormack BA, Bolwell GP (1995) Cell wall polysaccharide biosynthesis and related metabolism in elicitor-stressed cells of French bean (*Phaseolus vulgaris* L.). *Biochem J* 306: 745–750
- Robertson EJ, Leech RM (1995) Significant changes in cell and chloroplast development in young wheat leaves (*Triticum aestivum* cv. Hereward) grown in elevated CO₂. *Plant Physiol* 107: 63–71
- Schindler T, Bergfeld R, Hohl M, Schopfer P (1994) Inhibition of Golgi-apparatus function by Brefeldin-A in maize coleoptiles and its consequences on auxin-mediated growth, cell-wall extensibility and secretion of cell-wall proteins. *Planta* 192: 404–413
- Taiz L (1984) Plant cell expansion: regulation of cell wall mechanical properties. *Annu Rev Plant Physiol* 35: 585–657
- Taylor G, Ranasinghe S, Bosac C, Gardner SDL, Ferris R (1994) Elevated CO₂ and plant growth: cellular mechanisms and responses of whole plants. *J Exp Bot* 45: 1761–1774
- Tenhaken R, Thulke O (1996) Cloning of an enzyme that synthesizes a key nucleotide-sugar precursor of hemicellulose biosynthesis from soybean: UDP-glucose dehydrogenase. *Plant Physiol* 112: 1127–1134
- Updegraff DM (1969) Semimicro determination of cellulose in biological materials. *Anal Biochem* 32: 420–424
- Venus J, Causton D (1979) Plant growth analysis: the use of the Richards function as an alternative to polynomial exponentials. *Ann Bot* 43: 623–632
- Wakabayashi K, Sakurai N, Kuraishi S (1991) Effects of abscisic acid on the synthesis of cell-wall polysaccharides in segments of etiolated squash hypocotyl. II. Levels of UDP-neutral sugars. *Plant Cell Physiol* 32: 427–432
- Zabackis E, Huang J, Muller B, Darvill AG, Albersheim P (1995) Characterization of the cell-wall polysaccharides of *Arabidopsis thaliana* leaves. *Plant Physiol* 107: 1129–1138
- Zhong H, Läuchli A (1988) Incorporation of [¹⁴C]glucose into cell wall polysaccharides of cotton roots: effects of NaCl and CaCl₂. *Plant Physiol* 88: 511–514
- Zhong H, Läuchli A (1993) Changes of cell wall composition and polymer size in primary roots of cotton seedlings under high salinity. *J Exp Bot* 44: 773–778