

Importance of leaf versus whole plant CO₂ environment for photosynthetic acclimation

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ABSTRACT

The reduction of photosynthetic capacity in many plants grown at elevated CO₂ is thought to result from a feedback effect of leaf carbohydrates on gene expression. Carbohydrate feedback at elevated CO₂ could result from limitations on carbohydrate utilization at many different points, for example export of triose phosphates from the chloroplast, sucrose synthesis and phloem loading, transport in the phloem, unloading of the phloem at the sinks, or utilization for growth of sinks. To determine the relative importance of leaf versus whole plant level limitations on carbohydrate utilization at elevated CO₂, and the possible effects on the regulation of photosynthetic capacity, we constructed a treatment system in which we could expose single, attached, soybean leaflets to CO₂ concentrations different from those experienced by the rest of the plant. The single leaflet treatments had dramatic effects on the carbohydrate contents of the treated leaflets. However, photosynthetic capacity and rubisco content were unaffected by the individual leaflet treatment and instead were related to the whole plant CO₂ environment, despite the fact that the CO₂ environment around the rest of the plant had no significant effect on the total non-structural carbohydrate (TNC) contents of the treated leaflets. These results necessitate a re-evaluation of the response mechanisms to CO₂ as well as some of the methods used to test these responses. We propose mechanisms by which sink strength could influence leaf physiology independently of changes in carbohydrate accumulation.

Key-words: *Glycine max*; Fabaceae; acclimation; carbohydrates; carbon dioxide; photosynthesis; rubisco; soybean.

INTRODUCTION

Elevated CO₂ increases photosynthetic rates in the short term (minutes to hours) but over the long term (days to weeks) photosynthetic capacities often decrease in response to elevated CO₂ [for a review, see Griffin & Seemann (1996)]. Photosynthetic acclimation to CO₂ may result from feedback inhibition of photosynthetic enzyme

synthesis when carbohydrate utilization does not match demand (Stitt 1991). A carbohydrate feedback signal could be generated in response to a limitation in carbohydrate utilization at many different points; export of triose phosphates from the chloroplast, sucrose synthesis and phloem loading, transport in the phloem, unloading of the phloem at the sinks, or carbohydrate utilization for growth of sinks. Studies of carbohydrate allocation in plants tend to assume that one or another of these factors constitutes the major limitation (Farrar 1996). However, control theory suggests that the most efficient utilization of resources results when a plant adjusts the capacities of each limiting step so that the overall process is equally limited by all factors (Farrar 1996). In this case, we would expect that both whole plant and leaf level limitations to carbohydrate utilization would contribute to carbohydrate feedback in plants grown at elevated CO₂.

Most tests of the carbohydrate feedback hypothesis have applied rather drastic and unnatural treatments to increase the carbohydrate content of leaves, for example feeding sugars to leaves or intact plants (Krapp, Quick & Stitt 1991; Krapp *et al.* 1993; van Oosten & Besford 1994; van Oosten, Wilkins & Besford 1994; Winters *et al.* 1994; Jones, Lloyd & Raines 1996), cold girdling of petioles (Krapp *et al.* 1993; Krapp & Stitt 1995), heat girdling of petioles (Goldschmidt & Huber 1992), and transforming plants to express invertase in the apoplast (Stitt, von Schaewen & Willmitzer 1990; Krapp *et al.* 1993). All of these treatments resulted in reduction of photosynthetic gene expression and/or enzyme synthesis in response to increases in carbohydrate concentrations. However, these systems cannot be used to determine the relative importance of the various potential limitations to carbohydrate utilization occurring normally within a plant.

To test the relative limitation to carbohydrate utilization imposed by factors within single leaves versus factors in the rest of the plant, and the consequences of these limitations for photosynthetic acclimation, we constructed a treatment system in which we could expose single soybean leaflets to CO₂ concentrations different from those experienced by the rest of the plant. We measured changes in leaf carbohydrate contents as well as changes in photosynthesis and rubisco content. The results suggest that the regulation of photosynthetic capacity and carbohydrate utilization at elevated CO₂ is more complicated than is often assumed.

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METHODS

Plant material and growth conditions

Soybean (*Glycine max* cv. 'Williams') were planted in a 50:50 (vol:vol) mixture of topsoil and sand in 45 cm high black plastic pots (3 dm³ total volume). The plants were grown in controlled environment chambers ('Ecopods', Desert Research Institute, Reno, NV, USA). Two chambers were used, one controlled to 250 p.p.m. CO₂ and the other to 1000 p.p.m. CO₂. Temperatures were controlled to 28 °C day and 18 °C night. Relative humidity was not controlled and averaged 60% at midday. The chambers had clear glass tops and the plants received natural sunlight with a photon flux density (PFD) of 15–25 mol m⁻² d⁻¹.

Leaf and plant treatments

Leaf treatment chambers (Fig. 1) were designed which allowed us to treat single leaflets with CO₂ concentrations independent from the rest of the plant. Each pair of chambers consisted of two Plexiglas boxes (12 × 12 × 12 cm) connected at their corners so that the two side leaflets of a soybean trifoliate leaf could be enclosed in separate chambers while the central leaflet remained outside the chambers. Chamber temperature was maintained near that of ambient air by circulating water from a thermostatted water bath through a water jacket built into the bottom of each chamber. A small fan (model V60TL, Micronel Corp., Vista, CA, USA) in the bottom of each chamber provided air circulation.

Each replication of the experiment consisted of paired treatment and control leaflets on one low CO₂ and one high CO₂ grown plant. The treatment and control leaflets were the opposite side leaflets of a single trifoliate leaf. The

treatment leaflets received a CO₂ concentration opposite to that experienced by the rest of the plant, whereas the control leaflets received the same CO₂ concentration as the rest of the plant. Soybean plants grown in the two Ecopods were used after 6–8 weeks' growth. Leaves selected for the treatments were as similar as possible developmentally. The second youngest fully expanded leaf was selected on each plant. The plants were the same age and the CO₂ treatments had remarkably little effect on whole plant development. Although high CO₂ treatment did increase total dry mass of the plants, they varied by less than one node in terms of the number of mainstem leaves at the time of the experiments. The plants had six to 10 nodes (this varied between replications of the experiment but not between plants in any particular replication) and were flowering and setting fruit but had not completed vegetative growth. Over a 10 d period, one side leaflet received air pumped at 5 dm³ min⁻¹ (pump model N010 STI, KNF Neuberger, Trenton, NJ, USA) from within the same Ecopod and the other leaflet received air pumped from the other Ecopod. There were four replicates over time using different plants.

Gas exchange measurements

CO₂ assimilation was measured with an open system gas exchange apparatus (LiCor 6400, LiCor Inc, Lincoln, NE, USA) equipped with the standard leaf chamber and the CO₂ injector system (model 6400-01, LiCor) for control of CO₂ concentrations. The light source for all measurements was a tungsten halogen projector lamp (model ENH, 120 V 250 W, Radiac Inc, Japan) reflected off of a 45° cold mirror.

The response of assimilation to intercellular CO₂ concentration was measured at PFDs of 1200–1500 μmol m⁻²

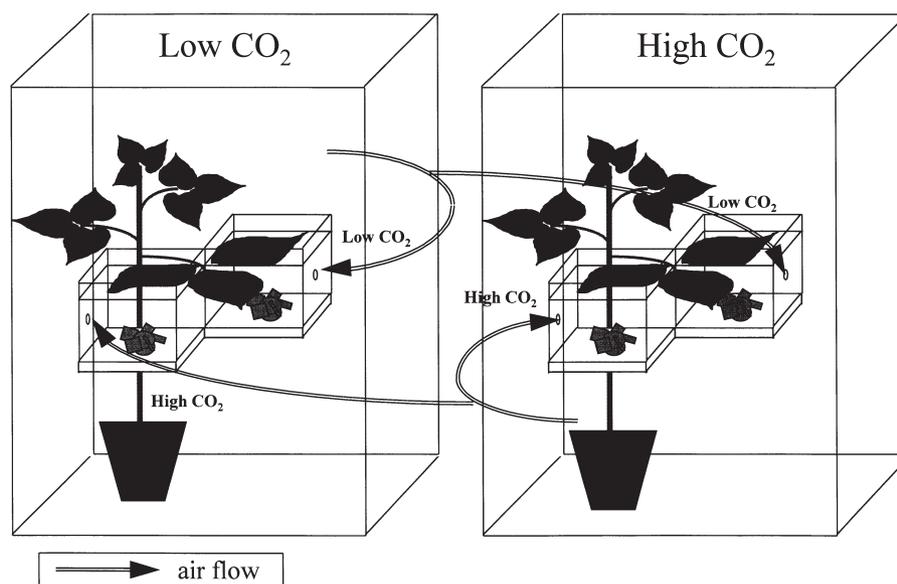


Figure 1. Diagram of the experimental apparatus for exposing soybean leaflets to CO₂ concentrations different from those around the rest of the plant.

s⁻¹ which were sufficient to saturate photosynthesis. Leaf temperature was 28 °C and water vapour concentration was 30 ± 2 mmol mol⁻¹. The leaves were initially allowed to equilibrate for 30 min at 350 p.p.m. CO₂, then the CO₂ concentration was reduced to ≈ 80 p.p.m. and subsequently increased in eight steps to ≈ 1000 p.p.m. allowing 3–4 min for equilibration at each CO₂ concentration. The measured responses of assimilation to intercellular CO₂ concentration (*A/c_i* curves) were fitted to a photosynthesis model (Farquhar, von Caemmerer & Berry 1980) for estimation of carboxylation capacity (*V_{cm}*).

Leaf composition

Leaf tissue samples were collected between 1500 h and 1600 h on the final day of treatment. The leaflets were detached from the petiole, the midrib excised and the lamina divided into four portions. Three of these were rapidly frozen in liquid nitrogen and stored at -80 °C for carbohydrate and rubisco analyses. The fourth portion was weighed to determine fresh mass, area was measured with an area meter (model 3000, LiCor). It was then dried at 60 °C for 48 h before determination of dry mass. Total rubisco content was measured as described by Evans & Seemann (1984) and carbohydrates were measured using the technique of Hendrix (1993).

RESULTS

Treatment of one leaflet on a 250 p.p.m. CO₂ grown plant with 1000 p.p.m. CO₂ approximately doubled the total non-structural carbohydrate (TNC) content of that leaflet relative to the control leaflet remaining at 250 p.p.m. CO₂ (Fig. 2). Conversely, treatment of a leaflet on a 1000 p.p.m. CO₂ grown plant with 250 p.p.m. CO₂ reduced the TNC content of that leaflet by approximately half relative to the control leaflet remaining at 1000 p.p.m. CO₂. At the end of the 10 d treatment period, the TNC content of the leaflets receiving 250 p.p.m. CO₂ was about the same regardless of whether the leaf was on a plant exposed to 250 or 1000 p.p.m. CO₂ (Fig. 2). The leaflets receiving 1000 p.p.m. CO₂ also had similar TNC contents. These changes were not due to changes in leaf thickness because the dry mass (after subtraction of TNC) and water content per unit leaf area were not significantly affected by the treatments (data not shown).

In contrast to the TNC results, the single leaflet treatments had no significant effect on *V_{cm}* (Fig. 2). *V_{cm}* in leaflets on the 250 p.p.m. CO₂ grown plants was about double that of leaflets on the 1000 p.p.m. CO₂ grown plants but it did not change with leaflet CO₂ treatment. Total rubisco content also did not respond to the local leaflet CO₂ environment (Fig. 2).

The lack of a response of *V_{cm}* and rubisco to the local leaflet environment, in contrast to the response of TNC, could represent either control by signals from the rest of the plant or an inability to readjust in response to the change in environment. To address the latter possibility we

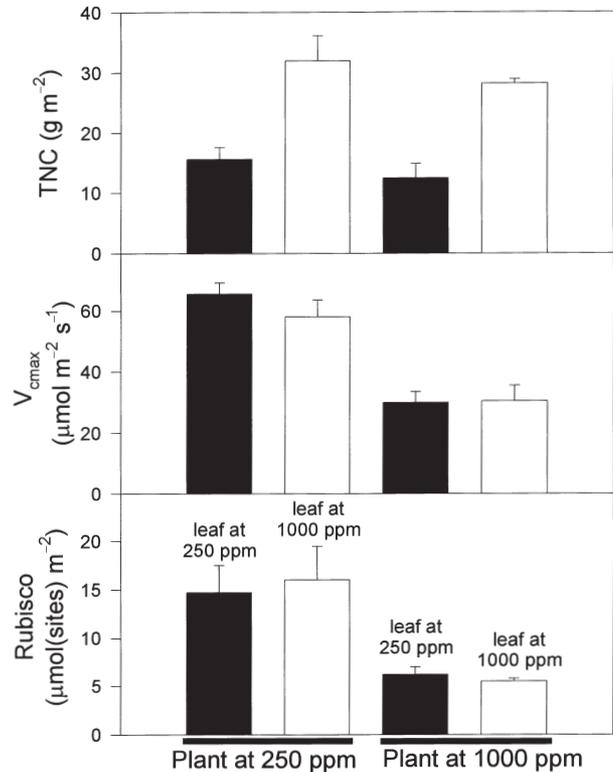


Figure 2. Responses of total non-structural carbohydrates (TNC), carboxylation capacity (*V_{cm}*, calculated from the response of assimilation to intercellular CO₂ concentration) and total rubisco protein (expressed as the number of active sites), after 10 d treatment, for soybean leaflets on plants grown in 250 p.p.m. CO₂ (left two bars) or 1000 p.p.m. CO₂ (right two bars) and exposed to either 250 p.p.m. CO₂ (filled bars) or 1000 p.p.m. CO₂ (open bars).

transferred whole plants between the two CO₂ environments and measured the response of *V_{cm}* of leaves of similar age to those used in the single leaflet treatments. *V_{cm}* of a leaf on a plant transferred from 1000 to 250 p.p.m. CO₂ increased to match the value of a control 250 p.p.m. plant within 6 d, demonstrating that the leaves maintained the capacity for adjustment (Fig. 3). Similarly, *V_{cm}* declined in a leaf on a plant transferred from 250 to 1000 p.p.m. CO₂. Consequently, we conclude that feedback inhibition of *V_{cm}* is primarily a function of the whole plant environment and is independent of the local CO₂ environment.

Changes in TNC were dominated by changes in starch content that accounted for more than 95% of TNC (Fig. 4, note difference in scales). The soluble sugar contents appeared to follow the same general pattern as starch but there are some important differences. Whereas starch content was about double for leaves receiving 1000 p.p.m. as opposed to 250 p.p.m. CO₂, soluble sugar contents were similar when the leaf and plant received the same CO₂ (far left and far right bars). Soluble sugar contents were greater in leaflets exposed to 1000 p.p.m. CO₂ when the leaflet was attached to a plant receiving 250 p.p.m. than for one

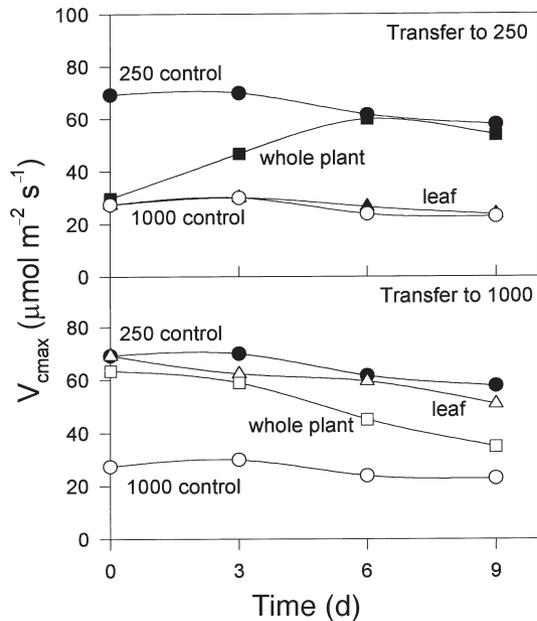


Figure 3. Comparison of the response of carboxylation capacity (V_{cmax} , calculated from the response of assimilation to intercellular CO_2 concentration) in soybean leaflets when the whole plant was transferred to a new CO_2 environment (squares), when only the individual leaflet was exposed to a new CO_2 environment (triangles) or when no transfer was made (closed circles, 250 p.p.m. CO_2 ; open circles, 1000 p.p.m. CO_2).

attached to a plant receiving 1000 p.p.m. CO_2 . Conversely, soluble sugars were reduced in a 250 p.p.m. CO_2 leaflet when the leaflet was attached to a 1000 p.p.m. CO_2 plant.

The differences in soluble sugar contents could be explained by differences in actual photosynthetic rates of the leaves (A_{growth} , measured at the growth CO_2 concentration and midday PFD, Fig. 5). Because the photosynthetic capacities of the leaflets did not respond to the single leaflet CO_2 treatments, actual photosynthetic rates were greater for leaflets receiving a given CO_2 concentration when the leaflet was attached to a plant growing in 250 p.p.m. than in 1000 p.p.m. CO_2 . This appeared to explain the greater overall soluble sugar contents for leaves on the 250 as opposed to 1000 p.p.m. CO_2 plants.

DISCUSSION

Treatment of single leaflets with elevated CO_2 , while the rest of the plant remained at ambient CO_2 , resulted in carbohydrate accumulation in these leaflets to levels similar to those in leaflets on whole plants exposed to elevated CO_2 . However, photosynthetic capacity, as determined by both the initial slope of the response of assimilation to intercellular CO_2 concentration and the leaf content of rubisco, was not affected in the single leaflet exposed to elevated CO_2 , although it was substantially reduced in similar leaflets on whole plants exposed to elevated CO_2 . These results necessitate a re-evaluation of

the response mechanisms to CO_2 as well as some of the methods used to test these responses. Several recent studies have reported the responses of single branches or portions of leaves exposed to elevated CO_2 (Barton, Lee & Jarvis 1993; Körner & Würth 1996; Kellomaki & Wang 1997; Teskey 1997; Wang & Kellomaki 1997). Results from these experiments should be interpreted with caution if the effect of the whole plant environment on branch or leaf responses is not known. Our results may suggest a reason why none of the single branch elevated CO_2 treatments (Barton *et al.* 1993; Kellomaki & Wang 1997; Teskey 1997; Wang & Kellomaki 1997) resulted in reductions in photosynthetic capacity. Carbohydrate contents were not measured in the branch studies, but Körner & Würth (1996) found increased TNCs in portions of leaves exposed to elevated CO_2 , consistent with our results.

Our results provide insights into the regulation of carbon partitioning in plants. We predicted that leaf level processes would represent at least part of the limitation to carbohydrate utilization. The increases in carbohydrates in leaves exposed to elevated CO_2 while the rest of the plant remained at low CO_2 appear to confirm this prediction.

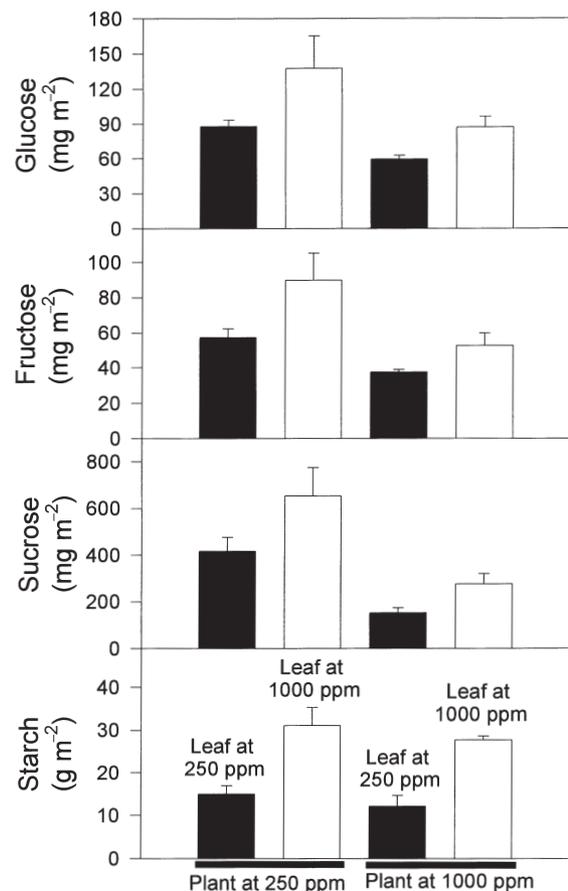


Figure 4. Responses of soluble sugars and starch, after 10 d treatment, for soybean leaflets on plants grown in 250 p.p.m. CO_2 (left two bars) or 1000 p.p.m. CO_2 (right two bars) and exposed to either 250 p.p.m. CO_2 (filled bars) or 1000 p.p.m. CO_2 (open bars).

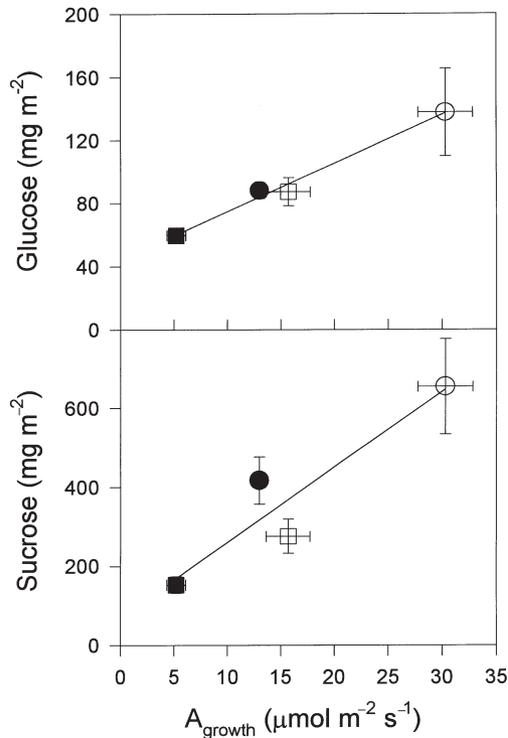


Figure 5. Leaf glucose and sucrose contents as a function of the photosynthetic rate at the treatment CO₂ concentration and midday photon flux density (A_{growth}) for leaflets exposed to 250 p.p.m. CO₂ (filled symbols) or 1000 p.p.m. CO₂ (open symbols) and attached to plants growing in 250 p.p.m. CO₂ (circles) or 1000 p.p.m. CO₂ (squares).

Starch was the primary storage carbohydrate and showed the most dramatic response to CO₂ treatment. Starch synthesis is primarily limited by the activity of ADP-glucose pyrophosphorylase, which is activated by 3-phosphoglycerate and is inhibited by inorganic phosphate (Preiss 1982). Thus, starch synthesis could be stimulated by factors that limit triose phosphate export from the chloroplast and/or that limit recycling of phosphate back to the chloroplast. Morin, André & Betsche (1992), working with clover, found that starch accumulation increased when photosynthesis was increased by elevated CO₂ but not when photosynthesis was increased a similar amount by an increase in light. This result suggests that the capacity to utilize the extra carbohydrate production for growth and maintenance was not limiting. The authors concluded that increased starch content at high CO₂ was a result of reduced photorespiration and consequently reduced phosphate regeneration in the chloroplast rather than a limitation in carbohydrate utilization by the rest of the plant. Starch accumulation in leaves exposed to low O₂, which reduces photorespiration, also supports this conclusion (Madore & Grodzinski 1984). An effect of reduced photorespiration on starch accumulation could explain why starch contents of leaves on high CO₂ plants almost always

increase even when photosynthetic capacities have been reduced to the extent that there is little difference in actual photosynthetic rates.

In addition to the short-term direct effects of elevated CO₂ on starch accumulation, there might be longer term acclimation in the capacities for carbohydrate utilization. The gene transcripts for ADP-glucose pyrophosphorylase have been shown to increase following transfer of tomato plants to elevated CO₂ (van Oosten *et al.* 1994) and following cold girdling of spinach leaves (Krapp & Stitt 1995). If this resulted in increased ADP-glucose pyrophosphorylase enzyme concentrations it might further stimulate starch synthesis at elevated CO₂.

For a wide range of C₃ species, final biomass production of elevated CO₂ grown plants is on average 41% greater than that for ambient CO₂ grown plants (Poorter 1993). Because of compounding effects during growth, such an increase can be produced by an increase of only a few percentage points in instantaneous relative growth rate, even though in the short term elevated CO₂ increases photosynthetic rates by 50% or more. This lower than expected growth response results both from reductions in photosynthetic capacity per unit leaf area and reductions in leaf area ratio for elevated CO₂ plants. In our experiments, leaf mass per unit area increased 36% (data not shown), due mainly to increases in starch content. Carbohydrates not invested in new growth tend to decrease relative growth rates because they do not contribute to compounding. It has generally been assumed that carbohydrate accumulation in leaves grown at elevated CO₂ was a result of limited sink demand when growth was limited by factors such as nitrogen supply (Conroy & Hocking 1993), temperature (Hofstra & Hesketh 1975) and/or the maximal rates of cell division and expansion in meristems (Kinsman *et al.* 1996). However, our results suggest that limitations in the processing of carbohydrates within leaves may be just as important a factor in limiting the export and utilization of increased carbohydrate production, and, thus, limiting relative growth rate, at elevated CO₂.

Just as the accumulation of starch suggests a limitation in the export of triose phosphates out of the chloroplast, the strong correlation between sucrose content and photosynthetic rates under the treatment conditions (Fig. 5) suggests that there was also a significant resistance in the sucrose export pathway. Similar correlations between sucrose concentrations and photosynthetic rate/export have been reported for soybean (Thorne & Koller 1974; Fader & Koller 1983), tomato (Ho 1976), *Salvia splendens* (Jiao & Grodzinski 1996) and sugar beet (Servaites & Geiger 1974). In addition, Grodzinski, Jiao & Leonardos (1998) measured 21 species and found a general correlation between export rate and total ethanol soluble sugars. However, these correlations were mostly generated from treatments that imposed rapid changes in photosynthetic rates. Similar relationships may not hold for plants growing under constant conditions. If only the two control treatments in our experiments are considered, the 1000 p.p.m.

CO₂ leaf (open square in Fig. 5) had a slightly higher photosynthetic rate but a lower sucrose concentration than the 250 p.p.m. CO₂ leaf (closed circle in Fig. 5). This result suggests that in the long term plants may be able to adjust their export capacity to match their average rates of photosynthesis, and, in fact, Cure, Ruffy & Israel (1991) found that export rates of soybean leaves were able to adjust to increased carbohydrate production following a transfer to elevated CO₂.

Although photosynthetic capacity did not respond to bulk leaf carbohydrate contents, this does not by any means rule out carbohydrates as a signal for reduction of photosynthetic capacity. One possibility is that the carbohydrate contents we measured at midday are not representative of carbohydrate concentrations at other times of the day. However, in experiments similar to those reported here, we have found that carbohydrate contents of elevated CO₂ treated leaflets on low CO₂ grown plants are still higher than those of the low CO₂ treated leaflets when both are measured in the predawn period (D. Sims & J. Seemann, unpublished results). Alternatively, because carbohydrates are often highly partitioned between subcellular compartments (Heineke *et al.* 1994; Moore, Palmquist & Seemann 1997), bulk carbohydrate contents may not be an adequate measure of subcellular concentrations. However, attempts to correlate cytosolic hexose concentrations with changes in photosynthetic gene expression have also been unproductive (Moore *et al.* 1997). A lack of correlation between photosynthetic responses and sugar concentrations could be explained if photosynthetic capacity responds to the rate of hexose metabolism by hexokinase rather than the absolute hexose concentrations (Jang & Sheen 1994; Koch 1996). The extent of reduction in photosynthetic capacity at elevated CO₂ has been shown to correlate with the activity of acid invertase, which may determine the rate of sucrose cycling and, thus, the flux through hexokinase (Goldschmidt & Huber 1992; Moore *et al.* 1998).

The linkage between cellular responses and changes in sink demand in the whole plant remains unclear. Changes in sink demand might affect phloem carbohydrate concentrations, but there is little direct evidence for this. Based on theoretical considerations there are several reasons to believe that in fact there may not be a good coupling between sink demand and leaf phloem sucrose concentrations. First, very small proportional changes in sucrose concentration can result in large changes in the flux of solutes through the phloem. A pressure gradient of only 0.04 MPa m⁻¹ is required to maintain observed rates of flow in the phloem, whereas total osmotic potential in the phloem is often greater than 1 MPa (Nobel 1983). Consequently, large changes in sink demand could be met by small changes in sucrose concentration gradients. Second, changes in the rate of phloem loading resulting from changes in photosynthetic production may have significant effects on phloem sucrose concentrations independent of sink demand. Magnuson, Goeschl & Fares (1986), working with ¹¹C tracers, obtained results that

suggest that phloem sugar concentrations change in response to diurnal changes in phloem loading and changes in CO₂ concentration. Third, changes in water potential gradients between sinks and sources may require large changes in phloem osmotic concentration gradients to maintain flux even when there is no change in sink demand (Boersma, Lindstrom & Childs 1991). Fisher (1978), using a negative staining technique to measure phloem osmotic concentrations in soybean, found large osmotic potential gradients between leaf and root phloem that were similar to those predicted to be required to maintain flux based on the resistance of the phloem and the water potential gradient between the roots and leaves.

Smith & Milburn (1980) suggested that phloem loading responds not to the concentration of sugars in the phloem but rather to the phloem turgor pressure. This conclusion is supported by the demonstration of direct turgor effects on the activity of sucrose transporters in phloem (Daie 1987) and sink tissues (Wyse, Zamski & Tomos 1986). Phloem unloading and growth of sinks results in a reduction in phloem turgor pressure which can be rapidly transmitted through the phloem to the source tissues (Watson 1976). That this actually results in changes in cellular physiology in leaves is only a hypothesis, but we hope that it will stimulate further research. The potential transduction mechanisms for a signal between the sites of phloem loading and the mesophyll cells are also unclear. It may be that changes in phloem loading in response to phloem turgor pressure result in changes in the flux of sugars out of mesophyll cells and that this serves as a signal. However, this is rather hard to reconcile with the results of our current experiments. Alternatively, as proposed by Lucas *et al.* (1996), there may be other signal molecules which traffic between the mesophyll cells and the phloem to keep photosynthetic production and phloem loading in balance.

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