Review article

Integration of photosynthetic acclimation to CO$_2$ at the whole-plant level

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Abstract

Primary events in photosynthetic (PS) acclimation to elevated CO$_2$ concentration ([CO$_2$]) occur at the molecular level in leaf mesophyll cells, but final growth response to [CO$_2$] involves acclimation responses associated with photosynthate partitioning among plant organs in relation to resources limiting growth. Source–sink interactions, particularly with regard to carbon (C) and nitrogen (N), are key determinants of PS acclimation to elevated [CO$_2$] at the whole-plant level. In the long term, PS and growth response to [CO$_2$] are dependent on genotypic and environmental factors affecting the plant's ability to develop new sinks for C, and acquire adequate N and other resources to support an enhanced growth potential. Growth at elevated [CO$_2$] usually increases N use efficiency because PS rates can be maintained at levels comparable to those observed at ambient [CO$_2$] with less N investment in PS enzymes. A frequent acclimation response, particularly under N-limited conditions, is for the accumulation of leaf carbohydrates at elevated [CO$_2$] to lead to repression of genes associated with the production of PS enzymes. The hypothesis that this is an adaptive response, leading to a diversion of N to plant organs where it is of greatest benefit in terms of competitive ability and reproductive fitness, needs to be more rigorously tested.

The biological control mechanisms which plants have evolved to acclimate to shifts in source–sink balance caused by elevated [CO$_2$] are complex, and will only be fully elucidated by probing at all scales along the hierarchy from molecular to ecosystem. Use of environmental manipulations and genotypic comparisons will facilitate the testing of specific hypotheses. Improving our ability to predict PS acclimation to [CO$_2$] will require the integration of results from laboratory studies using simple model systems with results from whole-plant studies that include measurements of processes operating at several scales.

Abbreviations: CAM, crassulacean acid metabolism; FACE, Free-Air CO$_2$ Enrichment; Pi, inorganic phosphate; LAR, leaf area ratio (m^2 g^{-1}); LWR, leaf weight ratio (g g^{-1}); NAR, net assimilation rate (g m^{-2} d^{-1}); PS, photosynthetic; RGR, relative growth rate (g g^{-1} d^{-1}); R:S, root/shoot ratio; rubisco, ribulose bisphosphate carboxylase/oxygenase; RuBP, ribulose bisphosphate; SLA, specific leaf area (m^2 g^{-1}); SPS, sucrose phosphate synthase; WUE, water use efficiency (g biomass g H$_2$O^{-1}).

Keywords: carbon dioxide, photosynthesis, acclimation, nitrogen, partitioning, source–sink relations, plant growth, leaf area, leaf morphology, roots, root:shoot ratio

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Introduction

Leaf mesophyll cells are the primary sites of carbon (C) assimilation and control of gene expression for ribulose bisphosphate carboxylase/oxygenase (rubisco) and other photosynthetic (PS) enzymes. In recent years, the use of simple model systems, such as cell suspension cultures and transgenic plantlets varying in rubisco level, has provided exciting new insights regarding C metabolism and PS gene expression at the molecular and cellular levels (Sheen 1994; Koch 1996). The results from these studies have helped to explain why the concentration of the key PS enzyme, rubisco, as well as nitrogen (N), often decline when plants are exposed to elevated [CO₂] and carbohydrates accumulate in leaf mesophyll cells.

Photosynthetic C fixation is a key process by which plants sense and respond to changes in atmospheric [CO₂]. However, growth responses to [CO₂] at the whole-plant level can be buffered against changes in cellular level PS capacity by direct and indirect effects of [CO₂] on respiration (Amthor 1991; Drake et al. 1997), shifts in C use efficiency, and the effectiveness of photosynthate partitioning among plant organs in relation to resources limiting growth (Gifford et al. 1984; Chapin et al. 1990; Luo et al. 1997). A recent review (Körner 1996) found that in 13 of 14 field experiments, the PS stimulation from elevated [CO₂] was much larger than the growth stimulation. It is becoming increasingly clear that source–sink interactions, particularly with regard to C and N, are key determinants of growth response and PS acclimation to elevated [CO₂] (Gifford 1992). A primary objective of this review is to consider the implications of cellular and leaf level responses to [CO₂] on whole-plant source–sink interactions in relation to PS acclimation. This review encompasses studies in which genotypic or environmental factors affecting source- sink interactions and partitioning have modified long-term PS response to [CO₂].

Observed acclimation responses to elevated CO₂

Photosynthetic acclimation as defined in this paper refers to any adjustment in the C acquisition system that may develop over time in plants grown continuously in elevated compared to a control [CO₂]. As such, 'acclimation' is not confined to leaf level processes expressed per unit leaf area or per unit leaf mass. The proposed mechanisms of PS acclimation to [CO₂] operate at a range of temporal and spatial scales (Table 1). At the ecosystem level, adjustments to C fixation occur over all timescales from minutes to centuries. At the whole-plant level we are concerned with a subset of that range of scales from minutes to weeks.

One initial consequence of a step increase in atmospheric [CO₂], occurring within minutes, is an accumulation of phosphorylated sugar intermediates in the pathway of sucrose synthesis. This can lead to a shortage of inorganic phosphate (Pi) in the chloroplast for ATP synthesis and RuBP regeneration, and thus constrain the stimulatory effect of elevated [CO₂] on C assimilation (Socias et al. 1993; Sage & Reid 1994). Such 'end-product inhibition' of PS is not normally a long-term regulator of PS metabolism, however, because the plant eventually adjusts concentrations of PS enzymes and other factors to bring C assimilation and utilization into balance (Stitt 1991).

Inactivation of rubisco is another short-term response to elevated [CO₂] that has sometimes been reported (e.g. Sage et al. 1989). The mechanisms of this response are poorly understood. This is not considered a long-term or efficient acclimation response since the inactivated rubisco sequesters N and other resources that could possibly be used more effectively elsewhere in the plant. In some longer term CO₂ enrichment studies (e.g. Xu et al. 1994), increased rubisco activation has been observed.

Over the timescale of hours to days, when PS stimulation due to high [CO₂] results in increased levels of specific carbohydrates (e.g. glucose or sucrose) in the leaves, expression of genes transcribing for rubisco or other PS enzymes can be repressed (Sheen 1994; Koch 1996). This is now recognized as an important regulation mechanism affecting the magnitude of PS stimulation by elevated [CO₂] in the long term.

Also on the timescale of hours to days, starch may accumulate to such an extent in the leaf that mechanical damage to the thylakoids occurs (Carmi & Shoma 1979; Wulff & Strain 1982; DeLucia et al. 1985). This usually is confined to studies where [CO₂] increases were imposed abruptly, or where genetic or other environmental factors constrained C sink capacity (Wolfe 1994).

Over days to weeks, PS and growth response to [CO₂] are dependent on the plant's ability to develop new sinks or expand the storage capacity or growth rate of existing sinks (Arp 1991; Farrar & Williams 1991; Wolfe 1994). Also over weeks, there is usually an adjustment of leaf area. These whole-plant growth and C and N partitioning responses to elevated [CO₂] alter the rate of production and utilization of photosynthate, and thereby feedback to affect C metabolism and PS gene expression at the leaf or chloroplast level.

Growth and partitioning responses to [CO₂], together with carbohydrate regulation of gene expression, form the core of long-term mechanisms of PS acclimation to elevated [CO₂]. They are discussed in more detail below.

Table 1 Responses to elevated [CO$_2$] affecting photosynthetic acclimation in relation to temporal and spatial scale

Response to [CO$_2$]	Temporal Scale	Spatial Scale
Triose phosphate accumulation in mesophyll cells; shortage of Pi for ATP synthesis	minutes, hours	organelle, cell
Inactivation of rubisco	minutes, hours	organelle, cell
Mechanical damage to thylakoids due to excessive starch accumulation	hours, days	organelle
Glucose, sucrose accumulation in source leaves; decreased expression of genes transcribing for rubisco, other enzymes	hours, days	cell, leaf
Shifts in sink organ and leaf area development; altered C and N allocation patterns; morphological changes	days, months	organ, whole-plant
Shifts in cycling and availability of N, water, other soil resources	months, years	ecosystem

Shifts in photosynthetic capacity and leaf level resource allocation

Rubisco and leaf N concentration. A reduction in active rubisco (determined by either biochemical assay or leaf gas exchange) is one of the most commonly described acclimation responses to prolonged exposure to elevated [CO$_2$] (Lawlor & Mitchell 1991; Gunderson & Wullschleger 1994; Sage 1994; Drake *et al.* 1997). Reductions in PS enzymes other than rubisco have also been reported (Nie *et al.* 1995). The reverse, an 'upregulation' of PS, has sometimes been observed (Campbell *et al.* 1988; Idso *et al.* 1991), and any successful theory of acclimation to [CO$_2$] must be able to explain this phenomenon. Arp (1991) and Sage (1994) provided evidence to suggest that the magnitude of downward acclimation may be partially dependent on pot size and the volume for root growth. However, other work found acclimation of carboxylation capacity to be independent of pot size when the correlated nutrient supply was taken into account (McConnaughy *et al.* 1993; Barrett & Gifford 1995a). In addition, even under field conditions with unrestricted root growth some level of downward acclimation is often observed (Lawlor & Mitchell 1991; Gunderson & Wullschleger 1994; Drake *et al.* 1997).

Despite a reduction in rubisco, C3 plants grown at high [CO$_2$] can maintain a higher PS rate than plants grown at low [CO$_2$] when measured at their respective growth [CO$_2$]. In a theoretical analysis using the biochemical model developed by Farquhar *et al.* (1980), Webber *et al.* (1994) calculated that at 25 °C, rubisco activity or quantity could be decreased by 30% at twice ambient [CO$_2$] (700 μmol mol^{-1}) before it would exert an equivalent limitation to that which it imposed for the same leaf at current [CO$_2$] (350 μmol mol^{-1}).

Correlated with the reduction in PS enzymes, during downward PS acclimation to elevated [CO$_2$] there is a reduction in leaf N concentration (Conroy 1992). This would be expected since rubisco alone typically comprises 25–50% of leaf N (Evans & Seemann 1989). A number of

papers (e.g. Bowes 1991; Stitt 1991; Stitt & Schulze 1994; Webber *et al.* 1994; Xu *et al.* 1994) have suggested that reduction in rubisco content at elevated [CO$_2$] to a level at which rubisco and RuBP regeneration are equally colimiting PS, represents a physiological re-optimization of the N distribution away from the PS apparatus and toward systems to acquire other resources such as light and minerals, and/or expand sink capacity for photosynthates. Stitt (1991) and Webber *et al.* (1994) suggested that the redistribution of N in [CO$_2$]-acclimating plants is an adaptive mechanism that adjusts PS (i.e. 'source') capacity to sink capacity in order to maintain whole-plant source–sink balance. The adaptive value of a reduction in rubisco as an acclimation response to elevated [CO$_2$] has yet to be rigorously tested in terms of plant-plant competition or genetic fitness, and will likely depend on other environmental conditions, particularly soil N availability (see below).

Sucrose and starch synthesis. Sucrose and starch concentration generally increase in the leaves of plants grown at above- ambient [CO$_2$] (Long & Drake 1992; Gunderson & Wullschleger 1994; Wolfe 1994), and this in part explains the decrease of leaf N concentration – a dilution effect. The review of the literature by Long & Drake (1992) found that the carbohydrate increase tended to be less pronounced in plants grown in large or unrestricted rooting volumes, but it was still apparent. This response is associated with whole-plant source–sink relations, and has implications for cellular level events affecting PS acclimation to [CO$_2$]. The biochemistry of the regulation of carbohydrate metabolism at the leaf level is relatively well understood, and is briefly reviewed here in relation to plant acclimation to [CO$_2$].

Carbon assimilation via the Calvin cycle and starch synthesis occur in the chloroplast, while sucrose is synthesized in the cytosol. The latter requires triose phosphate export from the chloroplast in a strict counterexchange for Pi import into the chloroplast. This exchange

is catalysed by the phosphate translocator (Flügge & Heldt 1991). Triose phosphates are converted to sucrose in the cytosol, releasing Pi which then returns to the chloroplast in exchange for more triose phosphate. Thus, Pi functions as a link between the cytosol and chloroplast.

When sucrose demand by sinks external to the leaf mesophyll does not keep pace with C assimilation and sucrose synthesis, sucrose accumulates in the cytosol. High levels of sucrose inhibit the key enzyme in sucrose synthesis, sucrose phosphate synthase (SPS). The mechanism for this feedback inhibition is not yet completely understood, but probably involves phosphorylation and dephosphorylation of a serine residue on the protein, leading to inactivation and activation of the enzyme, respectively (Huber & Huber 1992). Low sink relative to source capacity can also lead to accumulation of fructose-2,6 bisphosphate, an intermediate in sucrose synthesis that acts as an inhibitor of another enzyme in the chain of events leading to sucrose synthesis, fructose-1,6 bisphosphatase (Stitt & Quick 1989).

Slowed sucrose synthesis, by the feedback mechanisms described above, results in accumulation of Pi in the cytosol and a decrease in Pi within the chloroplast. The depletion of ATP that results from low Pi leads to an accumulation of glycerate-3-phosphate within the chloroplast. The consequential increase in the glycerate-3-phosphate:Pi ratio activates ADP-glucose pyrophosphorylase, the key enzyme in the pathway of starch synthesis (Stitt & Quick 1989). This is the mechanism by which elevated $[CO_2]$ leads to increased starch accumulation within the chloroplast.

A stimulation of starch synthesis when sucrose accumulates under elevated $[CO_2]$ can function as an acclimation response of adaptive value for two reasons: (i) the Pi released within the chloroplast during starch synthesis partially buffers the plant from the initial 'end-product inhibition' of PS associated with lack of Pi import from cytosol to chloroplast (Stitt 1993); and (ii) the accumulated starch functions as a mechanism of storing C and thus, at least temporarily, expanding sink capacity. However, in some extreme cases the expansion of starch granules in high $[CO_2]$-grown plants may cause mechanical damage to the thylakoids and long-term reduction in PS capacity (Carmi & Shoma 1979; Wulff & Strain 1982; DeLucia *et al.* 1985). This is not thought to be a common mechanism of PS downward acclimation, but might occur in genotypes with particularly low sink capacity, or when environmental factors such as low temperature (see below) limit growth and rate of C utilization.

Specific leaf area and leaf morphology. Many CO_2 experiments have found that the increase in leaf area in high $[CO_2]$-grown plants is proportionally less than the increase in

leaf weight, so that specific leaf area (SLA, leaf area per unit leaf dry weight) declines. This also leads to a decline in leaf area ratio (LAR, leaf area per unit total plant dry weight) if the proportion of total biomass allocated to the leaves (LWR, leaf weight ratio) is unchanged or declines (Acock & Pasternak 1986; Wolfe 1994). Some of this is associated with accumulation of leaf carbohydrates at elevated $[CO_2]$, but differences in leaf structural dry matter are usually equally important. The relative magnitude of the decrease in leaf N per unit leaf mass at high $[CO_2]$ (associated with reduced expression of PS genes) vs. changes in the leaf area:leaf mass ratio (i.e. SLA) determines whether leaf N (and presumably rubisco) increase or decrease on a *per unit leaf area* basis. Luo *et al.* (1994) suggested this may be an important factor in explaining the observed variation in PS acclimation to elevated $[CO_2]$ when measured on a per unit leaf area basis.

The reduction in SLA in plants grown at elevated $[CO_2]$ is often correlated with an increase in leaf thickness, and, in some cases, morphological changes. Extra palisade layer development (Mousseau & Enoch 1989), overall increase in mesophyll cell size (Thomas & Harvey 1983; Conroy *et al.* 1986) and increase in internal surface area for CO_2 absorption (Radoglou & Jarvis 1990a) have been reported in high $[CO_2]$-grown plants. Such morphological changes will tend to compensate for, or partially obscure, any biochemical downward acclimation of PS.

Stomatal density is another morphological feature that may respond to the CO_2 environment. There is some evidence in the fossil and old herbarium record for a decrease in stomatal density with increases in atmospheric $[CO_2]$ (Woodward 1987; Paoletti & Gelini 1993; Van der Burgh *et al.* 1993). However, not all such evidence is supportive of that trend (Körner 1988). Results from growth experiments have also varied, with stomatal density decreasing (Oberbauer *et al.* 1985; Paoletti *et al.* 1993), increasing (Gaudillere & Mousseau 1989), or not changing (Thomas & Harvey 1983; Oberbauer *et al.* 1985; Mousseau & Enoch 1989; Radoglou & Jarvis 1990b) when plants were grown at elevated $[CO_2]$. So we cannot yet generalize regarding stomatal density response to $[CO_2]$.

Shifts in whole-plant C and N allocation

$[CO_2]$ effects on cellular- and leaf-level processes eventually manifest themselves at the whole-plant level as shifts in C and N allocation and plant morphology. These whole-plant acclimation responses directly impact growth potential and productivity, and also have a substantial impact on subsequent molecular level events at the primary mesophyll sites by affecting sink capacity and capacity for acquisition of C, N, and other resources.

Leaf area development. Plants grown at twice ambient [CO$_2$] are sometimes larger, with greater leaf area, than plants grown at ambient [CO$_2$] levels. In some cases the increase in leaf area at high [CO$_2$] has been found to be a neutral allometric consequence of accelerated plant development rather than a direct [CO$_2$] effect on leaf expansion or maximum size of leaves (Tolley & Strain 1984; Conroy et al. 1986; Radoglou & Jarvis 1990a; Berryman et al. 1993). In some experiments leaf area has decreased at elevated [CO$_2$] (Mousseau & Enoch 1989; Norby & O'Neill 1991). This may occur when N supply is nonoptimal (Lutze 1996). Recent field studies with cotton and wheat (Pinter et al. 1996), rice (Rowland-Bamford et al. 1991), and in a prarie ecosystem (Ham et al. 1995) have shown no clear evidence of an increase in leaf area index with elevated [CO$_2$].

Even when the rate of leaf area development is increased in plants grown under elevated [CO$_2$] conditions, the ratio of leaf area to total plant weight, LAR, will be reduced in high [CO$_2$]- grown plants if LWR remains relatively constant or declines (Norby et al. 1992; Berryman et al. 1993; Wolfe 1994) because leaves are thicker with lower SLA, as discussed above. Badger (1992) showed that, at the whole-plant level, this can counteract the stimulatory effect of elevated [CO$_2$] on net assimilation rate (NAR), such that relative growth rate (RGR) is less enhanced by [CO$_2$] enrichment than if the LAR response did not occur. This is based on the assumption of classical growth analysis that RGR is the product of NAR and LAR (i.e. RGR = NAR × LAR).

A reduction in LAR at elevated [CO$_2$] may represent an adaptive acclimation mechanism (operating at the leaf and canopy, as opposed to cellular, level) by which the plant adjusts the balance between C assimilation and utilization. This interpretation is supported by experiments in which transgenic tobacco plants with lower leaf rubisco have higher LAR, partially counter- balancing the lower PS per unit leaf area (Quick et al. 1991). Another view is that the reduction in LAR at elevated [CO$_2$] is simply an expression of the normal decline of LAR as plants increase in size, high [CO$_2$] developing faster and thus being bigger at any point in time. Lutze and Gifford (in prep.) showed by allometric analysis that leaf area was reduced by elevated [CO$_2$] when data were plotted with respect to plant size rather than plant age.

It is important to note that the experiments by Badger (1992) cited above were on young plants before canopy closure. Growth analysis data must be interpreted cautiously in studies comparing [CO$_2$] treatments when canopy closure has occurred in one treatment but not the other. An initially faster growth rate of elevated [CO$_2$] plants can lead to more rapid canopy closure, which will reduce NAR because of mutual leaf shading, and subsequently cause a decline in RGR. Also, SLA, and

therefore LAR, will be affected by [CO$_2$] effects on the proportion of sun vs. shade leaves as the canopy closes. Low irradiance tends to increase SLA (McMillen & McClendon 1983). For these reasons, lack of a positive RGR response to elevated [CO$_2$] does not necessarily indicate downward PS acclimation to [CO$_2$] (Gifford et al. 1997).

Roots and root:shoot (R:S) ratio. Since under elevated [CO$_2$] less N can be invested in the leaves while maintaining PS rates (see above), the plant could potentially increase N investment in roots relative to shoots. Such a response could be adaptive if it increased the sink capacity for photoassimilates and/or increased the roots' ability to acquire limiting soil resources, especially N. A theoretical cost-benefit analysis (Hilbert et al. 1991), calculating the optimal leaf N concentration and R:S ratio that maximizes RGR during the exponential growth phase, concluded that reduced leaf N concentration in response to elevated [CO$_2$] is adaptive in most circumstances. This analysis explicitly considers the tradeoff during early growth (prior to significant mutual leaf shading) between producing a large amount of leaves (increasing LAR) vs. growing leaves of high PS capacity (increasing NAR). Since elevated [CO$_2$] increases photosynthesis, at least initially, greater biomass partitioning to the roots would be necessary in order to acquire sufficient N to maintain leaf N concentration and associated PS capacity. Since resources allocated to roots are diverted from shoot growth (lowering LAR), this will decrease RGR unless NAR is increased sufficiently by the increased availability of N from the roots. Thus, this cost-benefit analysis describes how, at the whole-plant level, RGR at high [CO$_2$] can be higher when PS downward acclimation occurs, because the marginal cost of maintaining high PS capacity at elevated [CO$_2$] is greater than the marginal benefit. This analysis, however, assumes that N uptake by roots is primarily controlled by soil N availability. To the extent that elevated [CO$_2$] may influence root physiological activity or energy available for uptake processes, the optimal responses may differ from those predicted.

Models simulating C and N partitioning (e.g. Thornely 1977) generally all predict increased R:S ratio whenever shoot specific activity (g C assimilated per g shoot biomass) increases. Thus, elevated [CO$_2$] leads to increased R:S in these models. Hilbert & Reynolds (1991) present a partitioning model that also considers variable allocation of N to proteins in the shoots and assumes balanced root:shoot activity. In response to elevated [CO$_2$], this model predicts increased R:S ratios and decreased leaf N concentration.

The experimental data of [CO$_2$] effects on the R:S total biomass or C ratios show no clear trend. While 87% of

studies that measured root mass described increases in the amount of root at elevated [CO_2], only 41% of those reporting R:S found an increase in that dry matter ratio (Rogers *et al.* 1994); that ratio declined in 20% of examples. An increased biomass R:S ratio under high [CO_2] is sometimes observed for herbaceous dicots (Tognoni *et al.* 1967; Patterson & Flint 1980; Kriedemann & Wong 1984), while several studies with cereals (Cure & Acock 1986) and forest species (Rogers *et al.* 1994) suggest these species show little response. Eamus & Jarvis (1989) found no evidence of an increase in R:S of temperate tree saplings grown at elevated [CO_2], although in some cases R:S declined. Insufficient data are available to group species confidently with regard to R:S response to [CO_2], and indeed there may never be a generalization possible.

It has also not yet been clearly established experimentally that increased N allocation to roots is matched by increased C allocation. For example, Lutze and Gifford (in preparation) have recorded for the grass *Danthonia richardsonii* that allocation of N to root was increased under elevated [CO_2] and this effect increased with increasing N-deficiency. The shift in allocation of C to roots under elevated [CO_2] was less than that of N. Moreover, that shift in C allocation was explained, via allometric analysis, entirely by [CO_2] effects on plant size with which R:S ratio for C was related irrespective of [CO_2] treatment. Perhaps the failure of a clear picture to emerge about [CO_2] effects on R:S ratio for C, and even for N, is that it is not the mass of root which is functionally important in the context of re-optimization of N deployment under elevated [CO_2]. Of greater importance may be the surface area of root, the nutrient uptake kinetics per unit area of root (Lutze and Gifford, in preparation) and the rate of fine root turnover, which may increase under elevated [CO_2] (Norby 1994).

Mechanisms of [CO2] effects on partitioning. We are beginning to understand some of the mechanisms of [CO_2] effects on C and N allocation at the cellular level, such as effects on sucrose vs. starch synthesis, and effects on expression of PS genes, as discussed above. In contrast, although it is quite clear that elevated [CO_2] also alters C and N partitioning at the whole-plant level, we know relatively little about the mechanisms involved.

There is evidence that plant hormones act as long-distance signals modulating growth of various sink organs and thereby play an important role in regulating partitioning and R:S ratio (review: Brenner 1987). However, whether elevated [CO_2] and plant carbohydrate status can alter the synthesis, transport or operation of plant hormones is not known (Stitt & Schulze 1994). Sucrose itself, which tends to accumulate under elevated [CO_2], may not just be a substrate and transporter of C, but may also be involved in the control of the expression

of genes regulating sink organ growth (Farrar & Williams 1991; Koch 1996). Sucrose, or other carbohydrates, could also modulate growth by affecting enzyme activity rather than enzyme production. Data presented by Huber *et al.* (1992) suggest that carbohydrate supply can alter the activity of the key enzymes in C and N metabolism, SPS and nitrate reductase, by a reversible post-translational mechanism involving protein phosphorylation.

Elevated [CO_2] effects on plant water relations, via stomatal closure or sugar accumulation, is another mechanism by which [CO_2] can alter partitioning patterns (Farrar & Williams 1991; Stitt & Schulze 1994). Shifts in carbohydrate accumulation within leaves and among plant organs associated with long-term exposure to elevated [CO_2] affects water potential gradients within and among plant organs, and thus could alter turgor pressures, expansive growth of specific organs, and C flux. Spollen & Sharpe (1991) showed that maintenance of root growth in water-stressed maize is due to a combination of regulation of osmotic potential and cell wall elasticity.

Factors affecting whole-plant acclimation

Many aspects of plant acclimation to elevated [CO_2] may be explained by a source–sink conceptual model in which the capacity for C assimilation is up- or down-regulated depending on the balance between supply and utilization of photosynthates. This model is applicable to a range of spatial and temporal scales. Lack of sufficient 'sink capacity' for incoming photosynthate leads to a negative feedback effect and downward acclimation of PS capacity. Conversely, increased supply of carbohydrates under elevated [CO_2] could potentially expand sink capacity, resulting in a positive feedback effect on C assimilation (i.e. upregulation of PS capacity). Genetic and environmental factors can directly affect PS capacity and thus the nature of acclimation to [CO_2]. Genetic and environmental factors can also *indirectly* affect PS acclimation response by their *direct* effects on sink capacity. These issues are discussed in more detail below.

Genetic factors and plant developmental stage

Genotypic variation in PS pathway is perhaps the most obvious example of a genotypic factor affecting acclimation to [CO_2]. Photorespiration losses are less, and therefore potential benefit from increasing [CO_2] is less, in C4 and CAM species compared to C3 species because the Calvin cycle is spatially (C4) or temporally (CAM) isolated. C3 plants which rely exclusively on rubisco and the Calvin cycle have the most to gain, potentially, in terms of carbohydrate supply from the inhibition of photorespiration with CO_2. Since a shift in carbohydrate

supply triggers many of the acclimation responses to [CO₂], we would expect acclimation to be more profound for C3 plants compared to species with the C4 or CAM PS pathways. Numerous studies have compared C3 and C4 species and documented that the benefit from a doubling of [CO₂] in terms of C assimilation and growth is significantly less in C4 plants (Kimball 1983; Cure & Acock 1986; Wolfe 1994). Acclimation responses, however, such as shifts in PS gene expression or partitioning have not been carefully assessed in these comparison studies. There is less data available for CAM species. The magnitude of beneficial effects on CAM plants is highly dependent on environmental conditions (Nobel *et al.* 1994), and is probably associated with Calvin cycle activity. The substantial positive growth responses of C4 species to elevated [CO₂] in many experiments (Poorter 1993) may be associated with imposed or inadvertant water deficits and the advantage of lower stomatal conductance and improved water use efficiency in high [CO₂] grown plants (Samarakoon & Gifford 1995).

Within the C3 species, genotypic variation in sink capacity for photosynthates is likely a very important factor in determining the magnitude and nature of acclimation responses to [CO₂]. Studies with cucumber (Peet *et al.* 1986) showed that gyneocious varieties that have a higher fruit load than moneocious varieties show a more positive response to CO₂ enrichment. The up-regulation, as opposed to downregulation, of PS in citrus grown at elevated [CO₂] was attributed in part to a high sink capacity associated with an indeterminate growth habit (Idso *et al.* 1991). Earlier, Kramer (1981) postulated that indeterminate species will tend to respond more positively to increased [CO₂] than determinate types. In a comparison of the growth response of 156 species to elevated (CO₂], Poorter (1993) concluded that inherently fast-growing C3 species exhibit a stronger [CO₂] response than slow-growing species. However, in their review of the literature on woody perennials, Gunderson & Wullschleger (1994) did not find a clear correlation between inherent growth capacity and the direction or magnitude of PS acclimation to [CO₂]. Therefore, an unequivocal conclusion about the [CO₂] responsiveness of genotypes in relation to inherent growth capacity is not yet possible.

There is some potential for genetically engineering crop plants for increased sink capacity (Lawlor 1995), and perhaps consequentially, response to [CO₂]. Sink capacity of some cereals appears to be under the influence of relatively few genes (Gale & Yossefian 1985). Genetic manipulation to increase expression of the key C metabolism enzyme, SPS, in tomato, led to an increase in C flux to sucrose rather than starch, and a significant increase in total biomass production (Foyer *et al.* 1995).

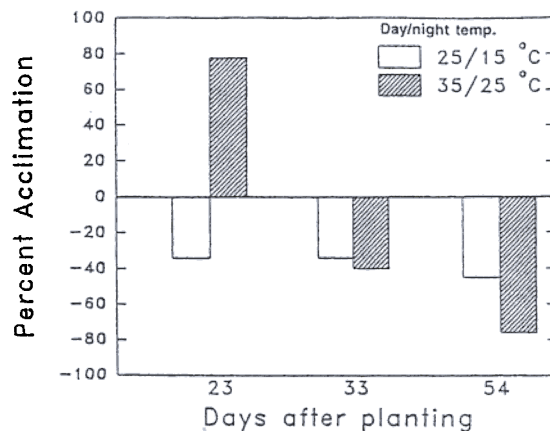Source–sink relations change with plant developmental

Fig. 1 Effect of growth stage and growth temperature on percentage photosynthetic (PS) acclimation to elevated [CO₂] in bean (*Phaseolus vulgaris*). Percent acclimation was calculated as the difference in PS rate (measured at 350 μmol mol⁻¹ [CO₂]) between plants grown at 700 and 350 μmol mol⁻¹ [CO₂], relative to the rate of plants grown and measured at 350 μmol mol⁻¹ [CO₂]: [(PS₇₀₀ − PS₃₅₀)/PS₃₅₀] × 100. Downward acclimation (a negative value), often reflects a reduction in concentration of PS enzymes in high [CO₂]- grown plants. It should be noted that even with downward acclimation, plants grown at elevated [CO₂] can maintain a higher PS rate than plants grown at ambient [CO₂] when measured at their respective growth [CO₂] (see text). In this particular study, high temperature stimulated early vegetative growth, leading to upregulation of 700-grown plants at 23 days after planting, but impaired reproductive development, leading to greater downward acclimation at high compared to optimum temperature at later growth stages.

stage, and so we would expect that growth stage may also influence PS acclimation response to [CO₂], as reported by Xu *et al.* (1994) for pea and soybean. This is illustrated in Fig. 1, showing data from an experiment (J. Jifon and D. Wolfe, unpublished) with bean (*Phaseolus vulgaris*). At the early vegetative growth stage, during rapid exponential growth and high C utilization rate, there was less carbohydrate accumulation in the leaves, and PS response to [CO₂] was less repressed than at the latest growth stage. Early vegetative growth was particularly stimulated in the warm (35/15 °C) temperature regime, and PS capacity was upregulated at elevated [CO₂] during that period.

Environmental factors

Nutrient availability. Elevated [CO₂] increases availability of C for growth, but sustained growth enhancement at above-ambient [CO₂] requires an increased supply of other material elements needed for construction and maintenance of plant cells. Nutrient deficiencies may reduce beneficial effects from elevated [CO₂] on an absolute basis, but on a relative basis the beneficial effect from increasing [CO₂] may be no less under nutrient-

limited than under nutrient-abundant conditions if elevated [CO_2] improves the efficiency of nutrient utilization or acquisition from recalcitrant sources.

Available N currently limits the productivity of many ecosystems (Linder & Rook 1984). This could be exacerbated under elevated [CO_2]. However, plant N productivity (g dry weight increase per unit plant N content) may increase under elevated [CO_2] (Hilbert *et al.* 1991; Pettersson & McDonald 1994) because of the potential for maintaining PS rates at a level similar to those observed at current ambient [CO_2], but with a reduced investment in leaf N.

Does N availability affect acclimation to [CO_2]? Results from experiments in which both [CO_2] and N supply were varied have not been consistent. There is often, but not always, greater downward acclimation of PS in low compared to high N environments. Wong (1979) observed greater downward acclimation of PS in cotton at low N supply in one short-term study, but there was no evidence of a N effect in a second short-term experiment by the same investigator with the same species (Wong 1990). Sage *et al.* (1989) found no evidence of greater downward acclimation at low compared to high N supply in *Chenopodium album*, and Radoglou *et al.* (1992), working with *Phaseolus vulgaris*, found no downward acclimation at elevated [CO_2] at either low or high N supply. In a three month study with *Ledum* species from the Alaskan tundra, Oberbauer *et al.* (1986) did observe a N effect, with a larger downregulation at elevated [CO_2] at low compared to high N supply. In contrast, in a long-term study with wheat where both N and [CO_2] were varied, Mitchell *et al.* (1993) found no evidence for downward acclimation.

In a study with pea (Riviere-Rolland *et al.* 1996) rubisco activity and quantity, and mRNA transcript levels associated with rubisco synthesis, were unaltered by growth at elevated [CO_2] at high N, but declined at low N, depending on the degree of N deprivation. Their data indicated a threshold value for N status, above which rubisco was not downregulated at elevated [CO_2]. This suggests that some of the variation in results between experiments may be due to distinctions in the severity of N deprivation imposed. Genotypic variation in sink capacity and inconsistency in experimental protocol may also explain lack of agreement between experiments with regard to N supply effect on plant acclimation to [CO_2].

A conceptual model summarizing some of the basic assumptions regarding optimization of C and N allocation in relation to plant acclimation to elevated [CO_2] is shown in Fig. 2. With a low supply of soil N there will be less N available for root and shoot sinks, and this will reduce the capacity of these sinks for utilizing C. This will reduce the C flux (as sucrose) from source leaves to roots and shoots and exacerbate the accumulation of carbohydrates

in the leaves. This leads to greater feedback inhibition of PS via repression of PS gene expression and reduced Pi cycling. Repression of PS genes results in less N allocation to the PS apparatus and more N available for shoots or roots. This could partially counteract further downward acclimation processes by increasing sink capacity and possibly increasing root growth and root acquisition of more N.

Optimization of N allocation as depicted in Fig. 2 may be important, and a driving force for natural selection, in environments where N is growth-limiting. In intensive agriculture, where fertilizer N can be supplied as needed by the crop, downward acclimation of PS at elevated [CO_2] may be considered an undesireable trait that counters the objectives of maximizing yields. An alternative strategy would be to breed crops which maintain current PS capacity and leaf N concentration at elevated [CO_2], and which have a greater RuBP regeneration capacity. This is a daunting breeding goal because of the many biochemical processes involved, and multigenic nature of control of RuBP regeneration capacity.

Most CO_2 studies that have considered the impact of soil resources have focused on N, with relatively little attention paid to other essential nutrients. Usually, beneficial effects from elevated [CO_2] are less on an absolute basis when nutrients are limiting, whether the element in deficient supply is N or something else. Goudriaan & de Ruiter (1983) found no positive growth response to CO_2 enrichment in P-deficient *Lolium perenne* plants. Conroy *et al.* (1986) observed substantial dysfunction of the PS apparatus at low phosphorus (P) supply in *Pinus radiata*, and no benefit from elevated [CO_2]. In contrast, Tissue *et al.* (1993) observed greater relative benefit from elevated [CO_2] at low compared to high P in *Pinus taeda*. The complexity of acclimation to [CO_2] under different levels of P nutrition was exemplified in cotton (Barrett & Gifford 1995a). For low enough P levels, complete acclimation to [CO_2] did occur and there was no growth response to [CO_2]. However, as with N-deficiency, it was concluded from the evidence that P deficiency caused downregulation of photosynthesis by influencing source–sink balance in favour of supply (Barrett & Gifford 1995b). More research is needed to determine the influence of nutrients other than N on plant acclimation to [CO_2], and mechanisms of their effects.

Temperature. Theoretical treatments (Long 1991; Gifford 1992) based on the Farquhar *et al.* (1980) model of leaf photosynthesis suggest that PS rate of C3 species would show little benefit from CO_2 enrichment when temperature is low (e.g. < 15 °C). Some long-term field experiments have indeed found very little PS response to elevated [CO_2] in cold environments, such as in the arctic tundra (Billing *et al.* 1984; Tissue & Oechel 1987). Reviews
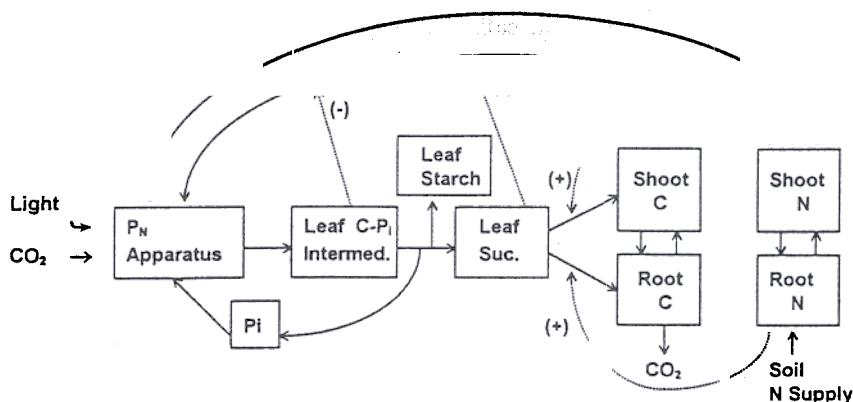
Fig. 2 A conceptual model summarizing some of the basic assumptions regarding optimization of C and N allocation in relation to plant acclimation to elevated [CO₂]. Solid lines represent material flow (e.g. C, N); dashed lines represent control or influencing factors. With a low supply of soil N there will be less N available for root and shoot sinks, and this will reduce the capacity of these sinks for utilizing C. Smaller shoot and root N pools will result in less C flux (as sucrose) from source leaves to roots and shoots, and exacerbate the accumulation of carbohydrates in the leaves that occurs with elevated [CO₂]. In the short term, accumulation of sucrose and phosphorylated sugar (C-Pi) intermediates can lead to 'endproduct inhibition' by slowing cycling of inorganic phosphate (Pi) needed for photosynthesis. A more important long-term regulation mechanism is the repression of photosynthetic (PS) genes associated with accumulation of phosphorylated sugars and sucrose. By this mechanism an increase in sugar pools negatively affects N allocation to the PS apparatus, lowers leaf N concentrations, and leads to more N available for shoots or roots. This could partially counteract further downward acclimation processes by increasing sink capacity and increasing root growth and subsequent N acquisition. The validity of this hypothesis remains controversial, and to the extent that such a shift in N allocation occurs under elevated [CO₂], the adaptive value in terms of impact on reproductive fitness has not been rigorously tested.

of the literature (Kimball 1986; Rawson 1992; Wolfe 1994) indicate that while, in general, the trend is for greater PS and growth stimulation by elevated [CO₂] as temperatures increase, there are exceptions and considerable variation in response.

There are several examples of perplexingly strong PS or growth responses to elevated [CO₂] at low temperature. For example, a 3-year open-topped chamber study in Finland (Wang et al. 1996) found an up-regulation of PS capacity in Scots pine at temperatures as low as 6 °C. Elevated [CO₂] increased the cold adaptability of Scots pine PS and led to a decrease rather than the theoretical increase in the optimum temperature for PS. By contrast, at high temperature (30 °C) PS down-regulated in the high [CO₂] chambers. A wheat crop grown in elevated [CO₂] from germination at 11 °C to maturity at 18 °C exhibited a 20% increase in the efficiency of conversion of intercepted radiation into dry matter, in response to doubled [CO₂], with no correlation with the progressive increase in temperature through the season (Gifford & Morison 1993).

Greer et al. (1993) found a diversity of CO₂ × temperature interaction responses in grasses. In some species elevated [CO₂] increased the optimum temperature for PS (e.g. Lolium perenne, Agrostis capillaris), for others there was no change (e.g. Lolium multiflorum), and yet others a decrease in optimum temperature (e.g. Dactylis glomerata). The acclimated response of PS (absolute as well as relative) to doubled [CO₂] for L.

multiflorum was greater at 12 °C than at 18 or 28 °C. The positive PS response to elevated [CO₂] observed by Greer et al. (1993) at low as well as high temperatures was not translated into the growth response, however. The RGR was similar between [CO₂] treatments at all temperatures, and the average ratio of final dry weight of C3 species at 700 μmol mol⁻¹ [CO₂] to those at 350 varied from 0.13 to 0.72 between 12 and 28 °C.

The distinction between model predictions of temperature effects on PS response to [CO₂] and actual measured responses, and the distinction between PS and growth response sometimes observed, may be explained in part by long-term partitioning and morphological acclimation to temperature as well as acclimation to [CO₂]. For example, low temperatures tend to increase leaf N per unit leaf area (Wolfe & Kelly 1992), decrease SLA (Dale 1965; Kapitsimadi 1988; Wolfe 1991; Wolfe & Kelly 1992) and increase R:S ratio (Cooper 1973; Wolfe 1991). The effect of increasing temperature on leaf N is similar to the effect of increasing [CO₂] (see above), while the effects of increasing temperature on SLA and R:S ratio are opposite to that observed in some studies when [CO₂] is increased (see above). If a global warming accompanies the increase in atmospheric [CO₂], interactions such as these may become important.

Temperature also affects PS response to [CO₂] by altering the C utilization rate of growing organs. In the nonstress temperature range of 20–35 °C, the metabolism of sinks rises with temperature (Farrar & Williams 1991),

and the magnitude of diurnal change is also small compared to changes in other environmental variables such as temperature and light. It is not clear therefore that there has been sufficient selection pressure for plants to have evolved 'adaptive' acclimation responses to [CO$_2$]. However, it is possible that plants have evolved acclimation responses to shifts in source–sink C balance caused by a variety of other factors, and this may be relevant to acclimation to [CO$_2$].

A major challenge for whole-plant [CO$_2$] research in the future is to formulate testable hypotheses regarding 'optimal' acclimation responses for various environments and for various functional groups. Another research priority will be a more thorough evaluation of genotypic variation in those acclimation responses to [CO$_2$] that are determined to be adaptive within specific environmental contexts. Integration of results from laboratory studies using simple model systems with whole-plant experiments incorporating environmental and genotypic manipulation will be a useful strategy for elucidating acclimation mechanisms.

At a more fundamental level, we need a better understanding of physiological mechanisms determining initiation and growth potential of plant organs, and C and N allocation. For example, hormonal signals undoubtedly play a role in modulating resource allocation and growth response to [CO$_2$], but little research has been conducted to determine whether carbohydrate status alters the synthesis or function of plant hormones.

Acknowledgements

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