

COMMISSIONED REVIEW

Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells

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Received 29 May 1991

Abstract. In the first part of this review, I discuss how we can predict the direct short-term effect of enhanced CO₂ on photosynthetic rate in C₃ terrestrial plants. To do this, I consider: (1) to what extent enhanced CO₂ will stimulate or relieve demand on partial processes like carboxylation, light harvesting and electron transport, the Calvin cycle, and end-product synthesis; and (2) the extent to which these various processes actually control the rate of photosynthesis. I conclude that control is usually shared between Rubisco (which responds sensitively to CO₂) and other components (which respond less sensitively), and that photosynthesis will be stimulated by 25–75% when the CO₂ concentration is doubled from 35 to 70 Pa. This is in good agreement with the published responses. In the next part of the review, I discuss the evidence that most plants undergo a gradual inhibition of photosynthesis during acclimation to enhanced CO₂. I argue that this is related to an inadequate demand for carbohydrate in the remainder of the plant. Differences in the long-term response to CO₂ may be explained by differences in the sink-source status of plants, depending upon the species, the developmental stage, and the developmental conditions. In the third part of the review, I consider the biochemical mechanisms which are involved in 'sink' regulation of photosynthesis. Accumulating carbohydrate could lead to a *direct* inhibition of photosynthesis, involving mechanical damage by large starch grains or Pi-limitation due to inhibition of sucrose synthesis. I argue that Pi is important in the short-term regulation of partitioning to sucrose and starch, but that its contribution to 'sink' regulation has not yet been conclusively demonstrated. *Indirect* or 'adaptive' regulation of photosynthesis is probably more important, involving decreases in amounts of key photosynthetic enzymes, including Rubisco. This decreases the rate of photosynthesis, and potentially would allow resources (e.g. amino acids) to be remobilized from the leaves and reinvested in sink growth to readjust the sink-source balance. In the final part of the review, I argue that similar changes of Rubisco and, possibly, other proteins are probably also involved during acclimation to high CO₂.

Key-words: CO₂ (enhanced); photosynthesis; sink regulation; sucrose synthesis; Rubisco.

1 Introduction

In the following review, I shall consider the potential impact of enhanced CO₂ on photosynthesis in terrestrial higher plants with C₃ metabolism. Enhanced CO₂ will stimulate the carboxylation reaction catalysed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Potentially, this allows higher rates of photosynthesis, and will provide more carbohydrate for plant growth. However, the rest of the plant may, for various reasons, be unable to utilize or store this additional carbohydrate. In this case, it is possible that long-term and indirect effects appear, in which feedback regulation leads to an inhibition of photosynthesis. Therefore, the planning and interpretation of experiments on enhanced CO₂ must clearly distinguish between the short- and long-term effects of CO₂. Within each timeframe, it is important to relate the results of CO₂-enhancement experiments to our basic understanding of photosynthetic metabolism, and how it is regulated.

2 What is the potential short-term effect of enhanced CO₂ on photosynthetic rate?

In the first part of the review, I shall consider the short-term effect of enhanced CO₂ on the rate of photosynthesis. To begin with, I will consider how CO₂ acts on Rubisco to increase the rate of carboxylation, and will then consider how an increased rate of carboxylation affects the subsequent reactions of photosynthesis.

2.1 Impact of increased CO₂ on Rubisco

The entry of CO₂ into photosynthetic metabolism is catalysed by Rubisco. This protein has a rather low turnover number and is, correspondingly, present at high concentrations in the leaf accounting for up to 50% of the total leaf protein (Andrews & Lorimer, 1987; Woodrow & Berry, 1988). It actually catalyses two reactions. One involves carboxylation of ribulose-1,5-bisphosphate (Ru1,5bisP), and produces two molecules

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of glycerate-3-P, whereas the other involves oxygenation of Ru1,5bisP and produces one molecule of glycerate-3-P and one molecule of glycollate-2-P.

Increased CO₂ stimulates the net rate of carboxylation for two reasons. Firstly, Rubisco has a relatively low affinity for CO₂ (Andrews & Lorimer, 1987; Woodrow & Berry, 1988) and is substrate-limited at atmospheric CO₂ and O₂ concentrations. Secondly, carboxylation is stimulated because increased CO₂ competes with O₂ and decreases oxygenation (Andrews & Lorimer, 1987).

The effect of increased CO₂ on the rates of carboxylation (V_c) and oxygenation (V_o) can be described in terms of the kinetic properties of the enzyme, as

$$V_c = W_c \frac{C_i}{C_i + K_m^c(1 + O_2/K_i^o)}$$

$$V_o = W_o \frac{[O_2]}{[O_2] + K_m^o(1 + C_i/K_i^c)}$$

where W_c and W_o are the maximum rates of carboxylation and oxygenation, C_i and O₂ are the concentrations of CO₂ and O₂ inside the leaf, K_m^c is the K_m for CO₂ (=27 Pa). K_i^o is the competitive inhibition constant for O₂ (=40 kPa), K_m^o is the K_m (O₂) (=40 kPa) and K_i^c is the inhibition constant of CO₂ for oxygenation (=27 Pa). Therefore, doubling the CO₂ concentration from 35 to 70 Pa should lead to an increase of V_c from 0.405 W_c to 0.604 W_c, and a decrease of V_o from 0.13 W_c to 0.067 W_c (assuming a typical gradient for diffusion through the stomata of about 7 Pa, and a ratio of W_c/W_o = 1.94 (Jorden & Ogren, 1984)).

The V_c/V_o ratio depends on the temperature (Andrews & Lorimer, 1987; Woodrow & Berry, 1988). The empirical values in the last paragraph are valid for 25°C. Oxygenation increases relative to carboxylation at higher temperatures. Temperature also alters the relative solubility of CO₂ and O₂. As a result, enhanced CO₂ has a progressively larger effect on the net rate of carboxylation when the temperature is increased.

2.2 Effects of increased Rubisco activity on photosynthetic rates

I shall now consider how these changes in the rates of carboxylation and oxygenation will interact with the other reactions involved in photosynthesis. Two general aspects need to be considered. Firstly, continued catalysis by Rubisco requires that one molecule of Ru1,5bisP is regenerated for every molecule used in the carboxylation or the oxygenation reaction. Therefore, an increased net rate of carboxylation will require increased Calvin cycle activity, and an increased supply of NADPH and ATP from the 'light' reactions in the thylakoid membranes. Secondly, an increased net rate of carboxylation will require an increased rate of end-product synthesis.

2.2.1 Rubisco limitation of photosynthesis. The simplest interaction occurs if photosynthesis is limited by Rubisco. Rubisco has a very high affinity for Ru1,5bisP (Andrews & Lorimer, 1987; Woodrow & Berry, 1988) and is often fully saturated *in vivo* (i.e. every active site contains a molecule of Ru1,5bisP; this is assumed in the above equations). Provided that an increased rate of Ru1,5bisP consumption can be matched by an increased rate of Ru1,5bisP production, Rubisco will remain Ru1,5bisP-saturated. In this case, the impact of increased V_c and decreased V_o on the net rate of assimilation (A) can be described (Farquhar & von Caemmerer, 1982) as

$$A = V_c - 0.5 V_o - RD$$

where RD is the rate of dark respiration.

2.2.2 'Ru1,5bisP-regeneration limitation' of photosynthesis. If carboxylation is occurring faster than Ru1,5bisP can be regenerated, the Ru1,5bisP concentration will decrease and Rubisco activity will be restricted, bringing the consumption and regeneration of Ru1,5bisP back into balance (see section 4.1. for a discussion of further regulation mechanisms which are involved). This is referred to as a 'Ru1,5bisP-regeneration limitation' of photosynthesis (Farquhar & von Caemmerer, 1982; Sharkey, 1985a). The rate of Ru1,5bisP regeneration could be controlled by many factors including (a) harvesting of light, (b) electron transport to NADPH, (c) ATP synthesis, or (d) the enzymes in the Calvin cycle which convert triose-phosphates to Ru1,5bisP.

Enhanced CO₂ still leads to an increased net rate of photosynthesis (at least in C₃ plants) when Ru1,5bisP regeneration limits the rate of photosynthesis for the following reason. Normally, a considerable portion of the available ATP, NADPH and Calvin cycle activity is involved in regenerating Ru1,5bisP which is subsequently oxygenated. ATP and NADPH are also required in photorespiration to salvage the 2-phosphoglycollate, and in the photorespiratory NH₃ cycle (Edwards & Walker, 1983). In enhanced CO₂, V_o is decreased, and less electron transport and Calvin cycle capacity is 'wasted'. The resulting stimulation of net photosynthesis can be estimated, based on the stoichiometries of the reactions involved (Farquhar & von Caemmerer, 1982).

For example, if photosynthesis is limited by the rate of whole chain electron transport to NADPH, net photosynthesis will be stimulated by enhanced CO₂ because less NADPH is required for photorespiration and NH₄⁺ reassimilation (and therefore becomes available to regenerate Ru1,5bisP). The net rate of electron transport limited assimilation can be described (Farquhar, von Caemmerer & Berry, 1980; Farquhar & von Caemmerer, 1982) as

$$A = \frac{J^e}{4 + 4(V_o/V_c)}$$

where J^e is the maximum rate of electron transport. If assimilation were 'limited' by ATP synthesis (Farquhar *et al.*, 1980; Farquhar & von Caemmerer, 1982).

$$A = \frac{J^a}{3 + 3.5(V_o/V_c)}$$

where J^a is the maximum rate of ATP synthesis. If the net assimilation were limited by the regeneration of Ru1,5bisP from triose-phosphate, then

$$A = \frac{J^c}{1 + (V_o/V_c)}$$

where J^c is the maximum capacity for conversion of triose-phosphate to Ru1,5bisP.

2.2.3 Limitation of photosynthesis by end product synthesis. The rate of photosynthesis can also be limited by the rate at which the immediate products of CO₂ fixation (phosphorylated intermediates) are converted into non-phosphorylated end-products (e.g. carbohydrate, amino acids and lipids). The major end-products in higher plants are sucrose and starch. Sucrose is synthesized from triose-phosphate in the cytosol (Stitt, Huber & Kerr, 1987a), and starch is synthesized in the chloroplast stroma (Preiss, 1982). If these reactions occur too slowly, phosphorylated intermediates will accumulate and the pool of P_i in the cytosol and chloroplast will be depleted. This eventually leads to an inhibition of photosynthesis because P_i is required in the chloroplast for ATP synthesis (Sharkey, 1985a; Stitt & Quick, 1989). This kind of limitation has also been termed triose-phosphate utilization (TPU) limitation (Sharkey, 1985a) or P_i-limitation (Walker & Sivak, 1986). In these conditions, increased rates of carboxylation or suppression of oxygenation and photorespiration merely generate excess electron transport and Calvin cycle capacity. These will allow high rates of photosynthesis during transients (because the pools of metabolites and P_i in the cytosol have a turnover time of up to 20–30 s, see Stitt & Große, 1988a) but they cannot be employed during steady state photosynthesis.

2.2.4 Estimated potential response to enhanced CO₂. Using the above equations, we can estimate the increase of A which would occur when CO₂ is increased, if photosynthesis is totally limited by a particular sub-process. An increase of the CO₂ concentration from 35 to 70 Pa at 25°C would increase A by 78% if Rubisco were limiting, by 23, 27 and 23% if electron transport, ATP synthesis or the regenerative phase of the Calvin cycle were limiting, and would be without effect if end-product synthesis were limiting (assuming a stomatal diffusion gradient of 7 Pa CO₂, and 21 kPa O₂). The actual increase would be in between these values if A was limited by more than one partial process. The potential increase would be larger at higher temperatures, and smaller at lower temperatures.

3 Which processes are responsible for the short-term control ('limitation') of photosynthesis?

3.1 Methods to identify control points in photosynthesis

Therefore, the short-term response of photosynthesis to

enhanced CO₂ will depend on which process(es) control the rate of CO₂ fixation. Therefore, I shall now consider what factors control the rate of photosynthesis at air levels of CO₂. I will first consider the experimental approaches which have been used.

3.1.1 Analysis of gas exchange responses. Most investigations have approached this question by measuring the CO₂- or light-responses of photosynthesis, and analysing them in terms of models of photosynthesis (Farquhar *et al.*, 1980; Farquhar & von Caemmerer, 1982; Sharkey, 1985a; Sage, 1990). For example, when irradiance is increased the net rate of assimilation rises in a near-linear way until it reaches a maximum ('light-saturated') value, after which it does not respond to further increases of the irradiance (Fig. 1A). The initial slope is interpreted as 'Ru1,5bisP-regeneration limited' (the low irradiance means the supply of ATP and NADPH is inadequate). The plateau was interpreted as 'Rubisco limitation' at ambient CO₂, and moderate temperatures. However, it later became apparent that the light-saturated rate in high CO₂ can also be 'limited' by the rate of end-product synthesis (Sharkey, 1985a; Walker & Sivak, 1986; Stitt & Quick, 1989). 'End-product synthesis limitation' can be distinguished from 'Rubisco limitation' by carrying out further experiments to show that A is not stimulated by further increases of the CO₂ concentration, or by decreasing the O₂ concentration from 21 to 2 kPa (Sharkey, 1985b; Sharkey *et al.*, 1986). It can also be diagnosed by imposing sudden changes of irradiance or the CO₂ concentration to see if oscillations and secondary induction transients occur (these are typically found during P_i- or end-product limited photosynthesis, see Walker & Sivak, 1986; Stitt & Quick, 1989).

The CO₂ response can be interpreted in an analogous manner (Fig. 1B). As the CO₂ concentration rises, the rate of photosynthesis increases in a near-linear manner, followed by a curvilinear region and then a plateau where photosynthesis is insensitive to further increases of CO₂. In the linear region, photosynthesis is limited by Rubisco, and there is a strictly linear relation between the amount of Rubisco in the leaf and the rate of photosynthesis (Woodrow & Berry, 1988; Evans, 1989; Stitt *et al.*, 1991b). In the curvilinear and plateau regions, further experiments (e.g. changing the O₂ concentration, or looking for oscillations, see above) can be carried out to check whether this is due to inadequate electron transport, Calvin cycle capacity or limitation by end-product synthesis (see above).

The interpretation of gas exchange data can be supported by measuring selected metabolites and enzymes. 'Endproduct synthesis limitation' is typically accompanied by high glycerate-3-P levels, low ATP/ADP ratios, and partial deactivation of Rubisco (Sharkey, 1989; Stitt & Quick, 1989). 'Rubisco-limitation' of photosynthesis is accompanied by high activation (carboxylation) of Rubisco, low glycerate-3-P levels, high ATP/ADP ratios and saturating Ru1,5bisP levels (1.5- to two-fold higher than the concentration of

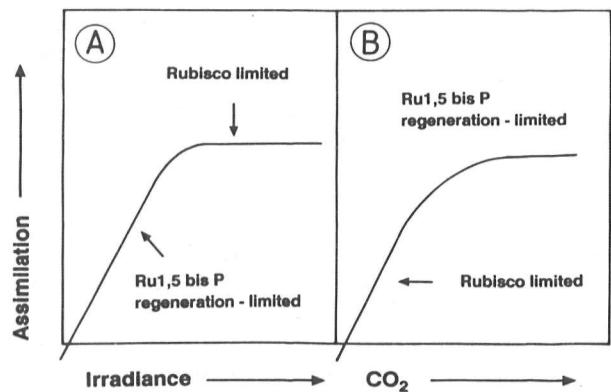


Figure 1. Idealized irradiance and CO₂ response of photosynthesis, and their interpretation in terms of the contribution of Rubisco and Ru1,5bisP regeneration rates to the control of photosynthesis: (A) increasing irradiance; and (B) increasing CO₂.

Rubisco active sites, see von Caemmerer & Edmonson, 1986; Sharkey, 1989). 'Ru1,5bisP-regeneration limited' photosynthesis is characterized by decreased activation of Rubisco and a relatively low Ru1,5bisP pool (note though that deactivation of Rubisco tends to prevent the Ru1,5bisP pool from falling too far, see Mott *et al.*, 1984; Sage, Sharkey & Seeman, 1988). Further analysis of metabolites, ATP and redox-states will indicate whether Ru1,5bisP regeneration is restricted by the supply of ATP or NADPH (i.e. the 'light' reactions) or by the Calvin cycle enzymes.

This approach, using modelling and measurements of gas-exchange and selected biochemical parameters, has the advantage that it can be used for a wide variety of species and conditions, and is obviously very useful for ecological studies. However, it also has some problems. In particular, although it may allow us to identify major controlling factors ('limitations'), it does not allow us to assess whether other minor factors are also contributing to control, nor does it readily allow analysis of situations in which control is shared between various enzymes. Although it has sometimes been assumed or asserted that photosynthesis is controlled by single 'limiting' factors (e.g. Farquhar & von Caemmerer, 1982; Sharkey, 1985a, 1989), this assertion is not easy to reconcile with the highly interactive and complex regulation of photosynthesis (Woodrow, 1986; Woodrow & Berry, 1988; Stitt & Quick, 1989), nor with the experimental evidence that control is shared between several enzymes and redistributes in a very flexible manner in other pathways (see section 3.2).

3.1.2 Control analysis using genetically manipulated plants. The concept of flux control coefficients (Kacser & Porteous, 1987) is an extremely useful tool for understanding how control is exerted in interactive biological systems. The ability of a particular enzyme, E_i, to control the flux, J, through a biochemical pathway is defined as the flux control coefficient,

$$C = \frac{dJ}{J} / \frac{dE_i}{E_i}$$

where dE_i/E_i is a fractional decrease in the amount of the enzyme (leaving all the other enzymes in the pathway unaltered) and dJ/J is the resulting fractional alteration in the steady-state flux through the pathway. This concept is quantitative, and can be applied in situations where control is shared between several enzymes. Recent technical advances now allow us to measure it.

The most direct way to measure flux control coefficients involves using isogenic reduced-activity mutants (Neuhaus *et al.*, 1989; Neuhaus & Stitt, 1990) or transgenic plants in which the expression of a single chosen enzyme is progressively decreased (Quick *et al.*, 1991; Stitt *et al.*, 1991b). This approach is illustrated in Fig. 2, using tobacco (*Nicotinia tabacum*) plants, in which the expression of Rubisco has been decreased by transforming them with 'antisense' rbc S sequences (the nuclear-encoded gene for the small subunit). The flux control coefficient, by definition, is the normalized slope of the J versus E relation in the range corresponding to the wildtype. In this example, Rubisco exerts considerable control (C→1) when photosynthesis is measured in 10Pa CO₂, little control in 33Pa CO₂ (C→0.1) and no control at all in 100Pa CO₂ (C→0).

In the past, it has been difficult to use this approach because of a lack of suitable mutants. As an alternative, highly-specific and tight-binding inhibitors were used to study electron transport and ATP synthesis in mammalian (Groen *et al.*, 1982) and plant (Padova, Dry & Wiskich, 1989) mitochondria, and photosynthetic electron transport in thylakoids (Heber, Niemanis & Dietz, 1988). Another more theoretical approach involves estimation of flux control coefficients from elasticity coefficients (see Kacser & Porteous, 1987, for the theoretical background; Woodrow, 1986; Woodrow & Mott, 1987; Woodrow, Ball & Berry, 1990, for examples of its application). However, both of these approaches involve assumptions and a direct approach using genetically manipulated plants is probably preferable. Antisense DNA technology (see e.g. Rodermel, Abbott & Bogorad, 1988) will allow the direct approach to be used with a far larger range of enzymes, and will revolutionize the study of metabolic regulation.

3.2 The distribution of control during photosynthesis

I shall now discuss the results which have been obtained using these approaches. I will first make some general comments about control in biological systems, and then discuss which factors control the rate of photosynthesis.

Experimental studies of mitochondrial ATP synthesis (Groen *et al.*, 1982), photosynthetic starch synthesis (Neuhaus *et al.*, 1989, 1990), photosynthetic electron transport (Heber *et al.*, 1988), and CO₂ entry into the leaf (Woodrow *et al.*, 1990) have led to two general and important conclusions. Firstly, control can be *shared* between several enzymes or processes in a pathway.

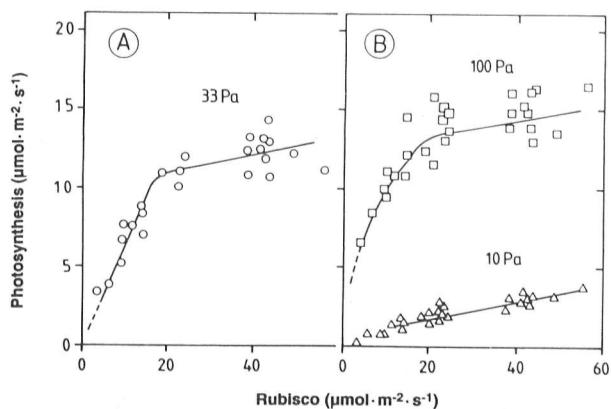


Figure 2. Experimental measurement of the flux control coefficient of Rubisco for photosynthesis. The amount of Rubisco was decreased by transforming the plants with antisense rbcS sequences (Rodermel *et al.*, 1988). The rate of photosynthesis was measured at enhanced (100 Pa), ambient (33 Pa), and decreased (10 Pa) CO₂. The flux control coefficient is given by the slope of the curve in the region immediately below the data points corresponding to the wildtype. (K. Fichtner, W.P. Quick, E.D. Schulze and M. Stitt, unpublished data.)

Secondly the distribution of control varies, depending on the conditions. Theoretical models, for example a simplified model of the Calvin cycle (Woodrow, 1986), lead to the same conclusions. The rate of CO₂ fixation is also controlled by several factors, whose contribution varies depending on the conditions. This conclusion is implicit in the interpretation of gas exchange responses discussed in section 3.1.1. It has recently received direct experimental confirmation in studies with transgenic plants. For example, Rubisco exerts considerable control in low CO₂, whereas it exerts little or no control in high CO₂ (see example in Fig. 2, also Stitt *et al.*, 1991b), or in limiting irradiance (Stitt *et al.*, 1991b).

These conclusions have important implications for the design of experiments to discover which factors control the rate of photosynthesis under growth conditions. If conditions are suddenly changed, it may be possible to generate a system in which one particular (usually, your favourite) process controls the rate of photosynthesis. This is obviously a useful experimental tool, if we want to study the mechanisms involved in regulation. However, we should not be misled into thinking that this is the normal or necessary state of affairs. If we want to know which enzyme(s) or process(es) normally control the rate of photosynthesis, we must be careful to carry out measurements in conditions which resemble those in which the plant is actually growing (i.e. the growth chamber or, if we are courageous enough, in the field).

Gas exchange studies indicate that, under growth conditions, many C₃ plants including cotton (*Gossypium hirsutum*) (Wong, 1979), bean (*Phaseolus vulgaris*) (von Caemmerer & Farquhar, 1981, 1984), wheat (*Triticum aestivum*) (Evans, 1986), pea (*Pisum sativum*) (Evans, 1987), spinach (*Spinacia oleracea*) (Brooks, 1986; Evans & Terashima, 1988), potato (*Solanum*

tuberosum), cabbage (*Brassica oleracea*) and *Chenopodium album* (Sage, Sharkey & Seeman, 1989) operate with an internal CO₂ concentration (C_i) which is close in the transition between the linear and the curvilinear region of their A/C_i response curve (Farquhar & von Caemmerer, 1982; Evans, 1989). This indicates that control may be shared between Rubisco and factors which determine the rate of Ru1,5bisP regeneration (see section 3.1.1). More recently, experiments with transgenic tobacco plants transformed with rbcS to decrease expression of Rubisco have provided direct evidence that Rubisco is not the only or major controlling factor for photosynthesis in growth conditions. Provided the tobacco plants were grown with adequate nitrogen (see below), one third of the Rubisco could be removed before it began to exert strong control on photosynthesis (Quick *et al.*, 1991). In the wildtype, a flux control coefficient of about 0.1 was estimated for photosynthesis in growth conditions (Stitt *et al.*, 1991b).

The remaining control is probably shared between CO₂ diffusion through the boundary layer and stomata (Woodrow *et al.*, 1990, Stitt *et al.*, 1991b), and biochemical factors affecting the rate of Ru1,5bisP regeneration. These include light harvesting and other aspects of the 'light reactions', as many plants are not light-saturated in growth conditions (see e.g. Farquhar & von Caemmerer, 1982; Sharkey, 1985a; Dietz, 1986; Evans, 1987; Stitt *et al.*, 1991b). It is not yet known whether the Calvin cycle enzymes ever restrict the rate of Ru1,5bisP regeneration.

The available evidence indicates that end product synthesis does not exert significant control over the rate of photosynthesis in growth conditions. For example, photosynthesis is O₂-sensitive and responds to increased CO₂ (Leegood & Furbank, 1986; Sage & Sharkey, 1987), oscillatory behaviour is rarely seen in growth conditions, and the studies of the A/C_i response cited above all found that the operating C_i in growth conditions was well below the plateau of the A/C_i response in saturating light (which is interpreted as the region in which photosynthesis becomes end-product limited, see section 3.1.1). However, end-product synthesis can become 'limiting' if the rate of photosynthesis is increased by 20–30%, or if the temperature is decreased below growth conditions (see below 8.2.4 for further discussion).

Summarizing, the short-term control of CO₂ fixation is shared between several factors, including Rubisco. It is interesting to note that nitrogen is used efficiently when control is shared in this way between the major proteins in a leaf (Woodrow & Berry, 1988; Evans, 1989). For the present purpose, it is not necessary to decide whether control is shared at each given point in time (which the reviewer believes to be the case), or whether it continually flips between one 'limitation' and another in response to small changes in the environment (which will always be occurring in nature). In either case, the time-averaged response of photosynthesis to increased CO₂ will be much smaller than that expected if Rubisco alone limits the rate of photosynthesis.

3.3 The control of photosynthesis in plants which have grown in, and adapted to, long-term environmental stresses

A sudden change of conditions frequently leads to an extreme or one-sided 'limitation' of photosynthesis. However, during prolonged exposure to a given environment or stress, there are often long-term changes in biochemistry, physiology and morphology, which ameliorate or remove the initial one-sided limitation. I shall briefly consider some examples of how control is distributed in plants which have acclimated to some selected environmental stresses, because this is frequently the case in natural conditions, and will obviously affect the potential short-term response of plants to enhanced CO_2 in the field.

3.3.1 Low nitrogen supply. Rubisco represents the largest single investment of nitrogen in the leaf (30–50% of the total protein). When plants are grown on low nitrogen they allocate proportionately less nitrogen to Rubisco, and more to the thylakoids (Terashima & Evans, 1988). The allocation of the nitrogen within the thylakoids remains unaltered (Evans & Terashima, 1988). This indicates that plants grown in low nitrogen might be more susceptible to limitation by Rubisco. Direct support for this idea is provided by experiments with tobacco plants transformed with antisense sequences to *rbcS*. The control strength of Rubisco increased from about 0.1 to 0.5 when tobacco was grown in low nitrogen (K. Fichtner, W.P. Quick, E.-D. Schulze and M. Stitt, unpublished data). Obviously, plants growing in low nitrogen 'optimize' their investment in Rubisco, and no longer contain the large 'excess' of Rubisco found in more favourable conditions (see above, section 3.2). This shift is brought about by redistributing about 10% of the total protein away from Rubisco. In agreement, Mächler *et al.* (1988) found that wheat seedlings grown in low nitrogen had decreased glycerate-3-P/triose-P ratios, increased ATP/ADP ratios and increased activation of Rubisco, as expected if photosynthesis were increasingly 'limited' by Rubisco.

Studies of the A/C_i response of several species growing in low nitrogen, including cotton (Wong, 1979) bean (von Caemmerer & Farquhar, 1981, 1984), wheat (Evans, 1986), spinach (Evans & Terashima, 1988) and *Chenopodium album* (Sage, Sharkey & Pearcy, 1990) showed a decreased initial slope as expected if Rubisco is decreased. However, since the transition from the linear to the curvilinear region occurred at the same C_i , these studies were interpreted as evidence that Rubisco and Ru1,5bisP regeneration capacity were decreased in parallel. It should be noted, though, that the transition cannot be unambiguously identified and a small shift of control towards Rubisco would probably not have been detected.

Growth is typically decreased in low nitrogen, and carbohydrate accumulates in the leaf, suggesting that photosynthesis could be directly limited by low rates of end-product synthesis. However, this is not always the

case. For example, in bean and cotton the operating C_i (i.e. the C_i found in growth conditions) lay closer to the plateau of the A/C_i curve (i.e. the region which is thought to represent end-product synthesis limitation) when they were grown in low nitrogen (Wong, 1979; von Caemmerer & Farquhar, 1984), but the opposite was found in *Chenopodium album*, and this species also became less O_2 -insensitive when it grew in low nitrogen (Sage *et al.*, 1990).

3.3.2 Low irradiance. When plants grow in low irradiance, nitrogen in Rubisco decreases relative to that in the thylakoids. Within the thylakoids, more nitrogen is invested in the light-harvesting proteins (Evans, 1989). This reallocation will tend to compensate for the low irradiance conditions, and maintain the balance between light harvesting and CO_2 fixation.

In agreement, when bean (von Caemmerer & Farquhar, 1981), pea or spinach (Evans, 1988) were grown in low light, analyses of the A/C_i curves showed that the initial slope decreased, but the C_i value at which the transition from the linear to the curvilinear region occurred was not greatly altered, indicating that the balance between Rubisco and Ru1,5bisP regeneration was being maintained even though less light was available. The transition also occurred at a C_i close to that found at ambient (35 Pa) CO_2 , indicating that control was shared between Rubisco and Ru1,5bisP regeneration. Recently, transgenic tobacco plants with decreased expression of Rubisco were grown at different light intensities, and it was shown that the control strength of Rubisco in air at the growth irradiance was always 0.1–0.2 (W.P. Quick, C. Labate, M. Laurer, E.-D. Schulze, D. Pankovic and M. Stitt, unpublished results), providing direct evidence that a balance is maintained between Rubisco and the other components of the photosynthetic apparatus over a wide range of growth irradiance.

3.3.3 Water stress. In the short-term, the major effect of water stress on photosynthesis is via stomatal closure. Reports of a direct 'non-stomatal' component can be attributed to problems in measuring C_i which arise when the stomata behave in a non-homogenous manner (Terashima *et al.*, 1988; Daley *et al.*, 1989). The contribution of the stomata to control of photosynthesis has been investigated by Woodrow *et al.* (1990). Following stomatal closure, C_i will also decrease. This can lead to Rubisco also exerting more control (Stitt *et al.*, 1991b), whereas the reactions needed for regeneration of Ru1,5bisP will exert little or no control. Less is known about long-term adaptation to water stress, but it is probable that the stomata continue to exert considerable control on photosynthetic rate.

3.3.4 Low temperature. Short-term exposure to low temperature leads to photosynthesis becoming 'limited' by the rate of end-product synthesis (see section 3.2, also Labate & Leegood, 1988). Several factors could be involved in this effect of temperature on end-product

synthesis, one being a temperature-dependent change in the properties of the cytosolic Fru1,6Pase such that it becomes more sensitive to inhibition by AMP and Fru2,6bisP (Stitt & Große, 1988b). However, within 24 h, barley (*Hordeum vulgare*) plants adjust to the low temperature, and 'escape' from this limitation (Labate & Leegood, 1988, 1989).

4 Response of photosynthetic rate to enhanced CO₂

4.1 Expectations, based on our knowledge of biochemistry

Summarizing the last sections, we have seen that plants growing at air levels of CO₂ are usually co-limited (a) by Rubisco, by (b) CO₂ entry into the leaf and (c) by various reactions in the thylakoids and, possibly, the chloroplast stroma which are required for the regeneration of Ru1,5bisP. This means that an increase of CO₂ will lead to an immediate stimulation of photosynthesis which lies between that expected if photosynthesis were limited by Rubisco (e.g. a 75% increase if CO₂ were increased from 35 to 70 Pa) and limited by factors affecting the regeneration of Ru1,5bisP (a 20–35% increase).

In a fluctuating environment, the stimulation obtained by enhanced CO₂ will also depend upon what is happening to the other environmental parameters. For example, enhanced CO₂ will be expected to have a larger effect at times when the irradiance level is temporarily high, because Rubisco will be exerting more control in these short-term conditions. Enhanced CO₂ will also have more effect at high temperature.

The short-term increment of photosynthesis will be attenuated if rising rates of photosynthesis (as a result of increased CO₂) lead to redistribution of control away from Rubisco towards other, less CO₂-responsive, processes. Transfer of control to other Calvin cycle enzymes or proteins in the thylakoid will reduce the gain until it just represents the benefit from the lower rate of photorespiration. Transfer of control to enzymes which are involved in sucrose and starch synthesis will mean that photosynthesis eventually does not respond to further increases of CO₂. Therefore, the maximum capacity for end-product synthesis represents a 'ceiling' above which the short-term rate of photosynthesis cannot rise. Attenuation due to redistribution of control away from Rubisco will depend on the precise conditions and species. It is important to realize that it will be more marked when large changes of the CO₂ concentration are suddenly imposed. For this reason, we should not assume that an increase from 35 to 70 or 100 Pa will always reflect the effect of smaller and more relevant increases of CO₂.

4.2 Experimental studies

4.2.1 The short-term responses.

There have been a vast number of experimental studies of the effect of enhanced CO₂ on photosynthesis. Cure & Adcock

(1986) have estimated from a review of many studies that, on average, photosynthesis is stimulated by 52% after doubling the CO₂ concentration. This empirical figure lies half way between the stimulation expected if photosynthesis were controlled by Rubisco, or by the rate of Ru1,5bisP regeneration. The gain varied between studies (see e.g. Mauney *et al.*, 1979; Kramer, 1981; Yelle *et al.*, 1989a; Huber *et al.*, 1984), as expected if the distribution of control depends on the species and the conditions.

In conditions where the maximum gain in photosynthetic rate is not achieved after increasing the CO₂ concentration, the activity of Rubisco will be decreased by internal feedback regulation (Sage *et al.*, 1988; Woodrow & Berry, 1988). Two mechanisms are involved: (a) Deactivation (decarboxylation) of Rubisco occurs, due to decreased activity of Rubisco activase. The precise mechanisms regulating Rubisco activase are not known, but could include regulation by the ATP/ADP ratio (Portis, 1990). This would allow Rubisco activation to be decreased when ATP consumption and production are out of balance (Brooks, Portis & Sharkey, 1988; Quick *et al.*, 1991). (b) In addition, the Ru1,5bisP pool may decrease below that required to saturate Rubisco binding sites; this is thought to occur when the total Ru1,5bisP concentration is less than 150% of the Rubisco active site concentration (von Caemmerer & Edmonson, 1986; Woodrow & Berry, 1988; Sharkey, 1989). In principle, Rubisco activity could also be decreased by an increase of carboxyarabitol-1-P (Berry *et al.*, 1987). However, there is no evidence to suggest that this mechanism operates during a response to enhanced CO₂.

4.2.2 Long-term suppression photosynthesis. However, a large number of studies have shown that this initial stimulation of photosynthesis decreases or disappears over a period of days or weeks (Kramer, 1981). For example, in tomato (*Lycopersicon esculentum*), an initial 30–40% stimulation declined to a residual stimulation of 2–8% after 6 weeks' growth in enhanced CO₂ (Yelle *et al.*, 1989a). In cotton, an initial stimulation of 50% decreased to 15% (Delucia, Sasek & Strain, 1985). Similar declines have been reported for cucumber (*Cucumis maximus*) (Peet, Huber & Patterson, 1986), rice (*Oryza sativa*) (Aoki & Yabuki, 1977), tobacco (Raper & Peedin, 1978) and soybean (*Glycine max*) (Havelka *et al.*, 1984a). In a literature survey, Cure & Adcock (1986) estimate that the stimulation decreased on average from 52 to 29% after acclimation to 70 Pa CO₂.

Consequently, enhanced CO₂ does not always lead to a large sustained increase of photosynthesis rate. Table 1 summarizes the rates of photosynthesis *at growth CO₂ concentration* for a selection of species after several weeks growth in air or in enhanced (70–100 Pa) CO₂. A small but significant increase was still found for potato (Sage *et al.*, 1989), soybean (Havelka *et al.*, 1984a; Clough, Peet & Kramer, 1981; Campbell, Allen & Bowes, 1988) and cotton (Wong, 1979). However,

enhanced CO₂ did not lead to a sustained increase of photosynthesis in bean *Chenopodium album* (Sage *et al.*, 1989), water hyacinth (*Eichhornia crassipes*) (Spencer & Bowes, 1986), tobacco (Thomas *et al.*, 1975), monoecious cucumber (Peet *et al.*, 1986) or tomato (Yelle *et al.*, 1989a). In cabbage (Sage *et al.*, 1989) and tobacco (Raper & Peedin, 1978), photosynthesis at growth CO₂ concentration was higher in plants growing in air than in plants growing at elevated CO₂. The effect of enhanced CO₂ also depended on the environmental conditions and plant age (see below).

These results raise the following questions. Firstly, what is responsible for the long-term suppression of photosynthesis in enhanced CO₂. Secondly, why is the response so variable? In the next part of the review, I shall argue that sink-source relations play an important role in these long-term changes, and will propose that species- or environmentally-dependent differences in the sink-source balance could explain much of the variability in the long-term responses.

5 Evidence that enhanced CO₂ leads to a change of the sink-source balance

Several different lines of evidence indicate that growth in enhanced CO₂ leads to a change in the sink-source balance of the plant. First, carbohydrate accumulates in the source leaves, as expected if the rate of photosynthesis exceeds the capacity of the sinks to utilize the photosynthate for growth. Secondly, variability in the response to CO₂ in different species, developmental stages or environmental conditions can be explained in terms of the differing 'sink strength' of the plants.

Thirdly, some of the morphological changes seen in enhanced CO₂ can be explained in terms of an increased supply of photosynthate which 'forces' the development of new sinks. I shall now discuss each of these lines of evidence in more detail.

Accumulation of carbohydrates in leaves has been seen in almost all studies of plant growth in enhanced CO₂, including cotton (Wong, 1990; Mauney *et al.*, 1979; Sasek, Delucia & Strain, 1985), soybean (Mauney *et al.*, 1979; Havelka *et al.*, 1984a; Huber *et al.*, 1984), sunflower (Mauney *et al.*, 1979), tomato (Madsen, 1968; Ho, 1977; Yelle *et al.*, 1989a), *Trifolium* (Cave, Tolley & Strain, 1981), monoecious cucumber (Peet *et al.*, 1986), tobacco (Thomas *et al.*, 1975), wheat (Havelka *et al.*, 1984b), and bean (Hoddinott & Jolliffe, 1987). In cotton, sunflower (*Helianthus annus*), soybean and clover (*Trifolium subterranean*) carbohydrate accumulated as starch, whereas in wheat and tomato it accumulated as soluble sugars. This reflects the normal storage strategy of these species.

The long-term response of photosynthesis and carbohydrate content to CO₂ often shows a developmental pattern, which can be related to the sink-source status of the plant. Enhanced CO₂ frequently leads to a larger stimulation of photosynthesis in young seedlings than in older plants, including soybean (Mauney, Fry & Quinn, 1978; Ackerson, Havelka & Boyle, 1984), cotton, sunflower (Mauney *et al.*, 1978; Radin *et al.*, 1987), and tomato (Ho, 1977). Significantly, rapidly-growing seedlings are often considered to be 'source-limited' (Baysdorfer & Bassham, 1985; Wardlaw, 1990) and can therefore utilize the additional carbohydrate resulting from enhanced CO₂ for growth. Correlations between the long-term response to enhanced CO₂ and 'sink'

Table 1. Stimulation of photosynthesis in mature leaves at growth CO₂ concentration in plants which have acclimatized to enhanced (70–100Pa) CO₂. The results are as a percentage of the rate found for plants growing in air (33–35 Pa CO₂)

Species	Photosynthesis rate (as a percentage of that for plants grown and measured in air)	References
Soybean	200	Clough <i>et al.</i> (1981)
	161	Havelka <i>et al.</i> (1984a)
	295	Campbell <i>et al.</i> (1988)
	153	Cure <i>et al.</i> (1989)
Potato	202	Sage <i>et al.</i> (1989)
Cotton (high N)	150	Wong (1979)
Vine	135	Kriedemann <i>et al.</i> (1976)
<i>Chenopodium album</i>	111	Sage <i>et al.</i> (1989)
Bean	102	Von Caemmerer & Farquhar (1984)
	104	Sage <i>et al.</i> (1989)
	120	Ho (1977)
	102	Yelle <i>et al.</i> (1989a)
Tomato	103	Besford <i>et al.</i> (1990)
	105	Peet <i>et al.</i> (1986)
	99	Spencer & Bowes (1986)
	78	Raper & Peedin (1978)
<i>Brassica oleracea</i>	77	Sage <i>et al.</i> (1989)
Cotton (low N)	78	Wong (1979)
Soybean (sinks removed)	100	Clough <i>et al.</i> (1981)

demand are also often seen during development in older plants. For example, when soybean (Ackerson *et al.*, 1984) and cucumber (Peet *et al.*, 1986) were grown in enhanced CO₂, there was a large accumulation of carbohydrate prior to flowering, and although this decreased during seed-filling, it was not depleted to low levels as usually happens in air.

These correlations during development should be interpreted with caution, because other factors are changing, apart from the sink-source balance. Additional support for the idea that the long-term response to enhanced CO₂ depends on the sink-source balance is provided by experiments which have compared plants with differing sink capacities. Whereas gynoecious cucumber (in which many seeds are set providing a large number of sinks) respond positively to enhanced CO₂ throughout their growth cycle (Kimball, 1983), there is a dramatic accumulation of carbohydrate and inhibition of photosynthesis in the older leaves of monoecious varieties (which have fewer sinks because only one or two seeds are set) (Peet *et al.*, 1986). Clough *et al.* (1981) manipulated the sink-source balance in soybean by trimming the pods immediately after pod set, and then exposing trimmed (low-sink) or untrimmed (high-sink) plants to normal or enhanced CO₂. The rate of photosynthesis declined faster and further in enhanced CO₂ in the low-sink plants than the high-sink plants. An interaction between the number of potential sinks and the response to enhanced CO₂ has also been noted by Kramer (1981) and Mauney *et al.* (1978), who point out that indeterminate plants like cotton, soybean and potato usually respond better to enhanced CO₂ than determinate plants like sunflower and tobacco.

The sink-source status of a plant can also be manipulated by altering the nutrient conditions. Wong (1979) grew cotton at high or limiting nitrogen and found that enhanced CO₂ had a larger inhibitory effect on photosynthesis in low nitrogen (when growth was slower and 'sink' demand would be less) than in high nitrogen. Cure, Israel & Rufty (1988) found that the response of photosynthesis to enhanced CO₂ decreased as soybean plants aged, and that this trend became apparent much earlier in plants growing on low nitrogen.

Some of the morphological changes during growth in enhanced CO₂ may be related to the increased availability of photosynthate, which leads to increased development of sinks, or the initiation of new sinks. For example, bushiness (the development of secondary shoots) could be a response to increased carbohydrate (Kramer, 1981; Mortensen, 1987). Increased tillering in cereals in enhanced CO₂ (Cloux *et al.*, 1987) could also be a response to increased carbohydrate, which has been reported to modify apical dominance (Wardlaw, 1990). Significantly, increased development of side-shoots has recently been observed in transgenic potato plants expressing *E. coli*-derived pyrophosphatase in the cytosol to increase partitioning towards sucrose and force more export (U. Sonnewald & L. Willmitzer, unpublished results). In other species, including *Citrus* (Koch *et al.*, 1986) and tropical trees (Oberbauer, Strain

& Fetcher, 1985), main shoot growth is increased. In pea cuttings, adventitious roots grew larger and had higher carbohydrate levels in enhanced CO₂ (Davis & Potter, 1989). The induction of flowering in *Pharbitis* in enhanced CO₂ might also, speculatively, be related to increased supply of carbohydrate (Hinklenton & Jolliffe, 1980).

Availability of carbohydrate will also enhance the development of nitrogen-fixing, nodules, which represent a considerable sink for carbohydrate. Indeed, enhanced CO₂ could have complex indirect effects on growth and photosynthetic rate in plants which develop nodules, because increased nodule development will increase supply of organic nitrogen to the plant. This might be one explanation for the positive response of soybeans to enhanced CO₂, and could be of considerable significance in natural conditions.

Increased availability of carbohydrate could also explain the increased rates of initiation and expansion of leaves (e.g. Kramer, 1981; Hinklenton & Jolliffe, 1980; Cure, Rufty & Israel, 1989). The increased leaf area will then interact with higher photosynthetic rates to increase the production of photosynthate even further. However, this effect is often counteracted at the whole plant level, either because self-shading occurs or because the rate of photosynthesis on a leaf area basis (see above) declines (Mauney *et al.*, 1978; Raper & Pedin, 1978; Bayersdorfer & Bassham, 1985; Oberbauer *et al.*, 1985; Cloux *et al.*, 1987; Poorter, Pot & Lambers, 1988). Non-productive accumulation of large amounts of storage carbohydrate which is not being turned over (see above) will also reduce the potential response of growth at the whole plant level (see Wong, 1990, for discussion).

Summarizing this section, enhanced CO₂ usually leads to an increased rate of photosynthesis and accumulation of carbohydrate. The plant could respond to this increased availability of carbohydrate in several different (and not mutually exclusive) ways. (a) Firstly, the rate of photosynthesis could be decreased. (b) Secondly, new temporary storage capacity can be opened up, both within the leaves, and in the stems along the transport pathway, and in pre-existent vegetative and reproductive sinks. Species may differ greatly in the extent to which this happens (see Wardlaw, 1990). (c) Thirdly, new sinks could be initiated and the growth of existing sinks accelerated to utilize the extra photosynthate. The extent to which sink metabolism responds to increased availability of carbohydrate will depend partly on genetic factors (e.g. determinate versus indeterminate growth, internal genetic constraints on the rate and extent of sink growth) and partly on environmental factors (e.g. nitrogen availability). This aspect will not be further discussed, because it lies outside the scope of this review. However, I believe that the identification of the molecular and biochemical factors, regulating the initiation and growth of sinks will be a crucial research field in the future if we are to understand sink-source interactions and the response of whole plant growth to enhanced CO₂.

I shall now discuss the evidence that 'sink' regulation of photosynthesis occurs, and will consider which biochemical mechanisms could be involved. I will return to experiments with enhanced CO₂ in the final section of the review, where I will ask whether the mechanisms which usually operate during sink-source adjustments in plants are also operating during long-term acclimation to enhanced CO₂.

6 The occurrence of 'sink' regulation of photosynthesis

A large number of investigations have shown that photosynthesis is inhibited when demand for photosynthate in the rest of the plant is decreased, and stimulated when demand in the rest of the plant is increased (Neales & Incoll, 1968; Geiger, 1976; Herold, 1980). The evidence includes studies of changes in demand during diurnal rhythms (Upmeyer & Koller, 1973; Kerr, Rufty & Huber, 1985; Stitt *et al.*, 1987a) and development (Chatterton, 1973; Flinn, 1974), as well as studies in which the sink-source relation was manipulated; for example, by removing sinks or inhibiting their metabolism (Nosberger & Humphries, 1965; King, Wardlaw & Evans, 1967; Fondy & Geiger, 1980; Shaw, Grange & Ho, 1986; Bagnall, King & Farquhar, 1988; Paul, Driscoll & Lawlor, 1991), by inhibiting export out of the leaf (Azcon-Bieto, 1983; Rufty & Huber, 1983; Shaw *et al.*, 1986; Blechschmidt-Schneider *et al.*, 1988), or by shading other leaves to remove competition from other sources (King *et al.*, 1967; Clough *et al.*, 1981; von Caemmerer & Farquhar, 1984; Rufty & Huber, 1983; Thorne & Koller, 1974). In studies where 'sink' regulation has not been seen, this can usually be explained (see Herold, 1980) because the demand by other sinks changed, or there was considerable 'buffering' by temporary storage pools along the transport pathway, or the plant was source-limited (e.g. during early growth, or during growth in low light).

However, the presence of a causal relationship between carbohydrate accumulation and the inhibition of photosynthesis is more controversial. Most of the evidence is based on correlations between carbohydrate levels and photosynthesis. For example, in studies of whole (unmanipulated) plants, the changes in photosynthesis rate often occur synchronously with changes in demand (Flinn, 1974; Chatterton, 1973; Upmeyer & Koller, 1973). Although this is consistent with a causal relation, it might also just reflect highly integrated regulation in intact systems (Geiger, 1976). Clearer evidence is provided in experiments where the sink-source relation was manipulated. In some studies, the rate of photosynthesis changed within hours (Azcon-Bieto, 1983; Clausen & Biller, 1977; Rufty & Huber, 1983; Herold, 1980; Foyer, 1988; Blechschmidt-Schneider, Ferrar & Osmond, 1989) and accompanied an accumulation of carbohydrate. However, some investigators have suggested that the short-term inhibition of photosynthesis can be caused by stomatal closure, and experiments should be designed to take account of this possibility (Setter, Brun & Brenner,

1980; Mayoral, Plaut & Reinhold, 1985). In other studies, the rate of assimilation only changes gradually over a period of days following a sink-source manipulation (see references in Geiger, 1976, also Herold & McNeil, 1979; Mayoral *et al.*, 1985; Shaw *et al.*, 1986; Sawada *et al.*, 1986; Plaut, Mayoral & Reinhold, 1987; Bagnall *et al.*, 1988). Carbohydrate levels were not monitored in many of these investigations, but when this was done it was often found that leaf carbohydrate also changed slowly and correlated reasonably with the inhibition of photosynthesis (Thorne & Koller, 1974; Herold & McNeil, 1979; Sawada *et al.*, 1986; Plaut *et al.*, 1987; Mayoral *et al.*, 1985). However, in some studies export and partitioning responded faster and more sensitively than the assimilation rate following a sink-source manipulation (see Geiger, 1976, 1979, 1986; Fondy & Geiger, 1980; Rufty & Huber, 1983; Shaw *et al.*, 1986; Plaut *et al.*, 1987). In these cases, carbohydrate accumulates rapidly in the leaf, whereas photosynthesis is only gradually inhibited.

There may be important species differences in the extent and timing of 'sink' regulation of photosynthesis. For example, Plaut *et al.* (1987) found that cucumbers, cotton and radish (*Raphanus sativus*) showed a strong inhibition within 1–3 d, whereas pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), bean and castor bean (*Ricinus communis*) showed a marked accumulation of carbohydrate but no inhibition of photosynthesis. Probably, soybean (Thorne & Koller, 1974; Rufty & Huber, 1983; Foyer, 1988), peanut (*Arachis hypogaea*) (Bagnall *et al.*, 1988), *Amaranthus edulis* (Blechschmidt-Schneider *et al.*, 1989), wheat (King *et al.*, 1967; Azcon-Bieto, 1983) and sunflower (Paul *et al.*, 1991) can be added to the group of 'sensitive' plants, whereas sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) (Geiger, 1986) and tomato (Ho, 1977; Shaw *et al.*, 1986) are less sensitive to a feedback inhibition. It may be significant that many of the most persuasive experiments for the absence of sink feedback on photosynthetic rate (Geiger, 1976; Geiger & Fondy, 1980; Shaw *et al.*, 1986) have carried out with sugar beet, bean or tomato.

The occurrence and extent of 'sink' regulation also depends on the growth conditions. For example, P_i-deficient spinach is rapidly inhibited when sucrose or glucose are fed to detached leaves (Foyer, 1988). Intuitively, we would also expect plants growing in low light to be less susceptible to sink-regulation, because growth in these conditions is likely to be source-limited.

Summarizing this section, sink-regulation of photosynthesis does occur, and it is often closely associated with or follows changes of carbohydrates in the source leaves. Although it is possible that additional factors (e.g. hormones) contribute, there is no compelling evidence at the moment that they are involved. Much of the controversy in the interpretation of sink-source experiments may arise because the underlying biochemical mechanisms do not always generate a simple negative correlation between photosynthetic rate and carbohydrate levels. For example, as will be discussed in

the next section, different feedback mechanisms could be involved, and these could act directly or indirectly. The inter- and intracellular sites of carbohydrate accumulation also vary. There may also be inherent differences in the susceptibility of photosynthesis to feedback inhibition depending on the species and the conditions. Therefore, for this reason, I shall now examine the potential mechanisms of feedback inhibition in more detail.

7 Mechanisms involved in the 'sink' regulation of photosynthesis

Coordination of 'sink' demand and photosynthetic capacity will be of crucial importance for plant growth, and it would be surprising if plants only have one mechanism or strategy for attaining this goal. In this section, I shall first discuss how starch or sucrose could act to *directly* inhibit the rate of photosynthesis. This can be viewed as a 'non-adaptive' response, because it will minimize or prevent carbohydrate accumulating further, but will not do anything to rectify basic imbalance of investment in the sinks and sources. Two different, but not mutually exclusive, mechanisms have been proposed whereby accumulating carbohydrate could directly inhibit the rate of photosynthesis. Firstly, large starch grains may physically disrupt the chloroplast. Secondly, high levels of carbohydrates could lead to a feedback inhibition of carbohydrate synthesis, with the result that photosynthesis is inhibited because P_i is not cycled rapidly enough. I shall then ask whether there is evidence for additional feedback mechanisms in which carbohydrate acts *indirectly*, by decreasing the levels of proteins and other components of the photosynthetic apparatus. These mechanisms can be viewed as an 'adaptive' response, because they will contribute to a readjustment of the sink-source balance by allowing nitrogen and other components to be remobilized from the leaves, and invested in sink growth.

7.1 Direct inhibition due to large starch grains

Large amounts of starch accumulate in the chloroplasts of some species. An apparent correlation between starch accumulation and inhibition of photosynthesis has been observed in soybean (Nafziger & Koller, 1976) and cotton (Mauney *et al.*, 1979; Sasek *et al.*, 1985). In *Trifolium subterraneum*, Cave *et al.* (1981) observed an apparent disruption of chloroplast structure, and proposed that mechanical damage was impairing the operation of enzymes and membranes. More recently, Grub & Mächler (1990) reported that the *in vivo* catalytic efficiency of Rubisco was decreased when starch accumulated (i.e. although Rubisco remained fully activated and Ru1,5bisP-saturated, photosynthesis was decreased at a given C_i value).

This hypothesis has been questioned because high levels of starch are frequently found in low nitrogen or enhanced CO₂, and a further small increase of starch

after a sink-source manipulation often leads to an inhibition of photosynthesis (e.g. Mauney *et al.*, 1979; Clausen & Biller, 1977). Another problem is that, in some leaves (e.g. soybean), a considerable portion of the starch may not be in the mesophyll cells. It is also possible that large starch grains have other effects; for example, the swollen and compressed cytoplasm might have an increased diffusive resistance to CO₂ (see Evans & Terashima, 1988, for a discussion of CO₂ diffusion). However, the major problem with the hypothesis is that the evidence consists of correlations, and this cannot prove that a causal relationship exists. Definitive proof will require a comparison of plants with different genetic capacities for starch synthesis. Yelle *et al.* (1989a) attempted this by comparing two different *Lycopersicon* species and found the inhibition of photosynthesis was just as large in the species which accumulated less starch. However, their experiment is inconclusive because near-isogenic lines must be used in such comparisons. This will be possible in the future, using genetically manipulated plants.

7.2 Direct inhibition of photosynthesis by low P_i due to feedback regulation of sucrose synthesis

Sucrose is synthesized in the cytosol from triose-phosphates, which are exported from the chloroplast via the phosphate translocator in exchange for P_i (Stitt *et al.*, 1987a; Stitt & Quick, 1989). It has been proposed that when sucrose synthesis is inhibited, there is an accumulation of phosphorylated metabolites in the cytosol and less P_i is recycled to the chloroplast, leading to an inhibition of photosynthesis (Herold, 1980). This hypothesis developed from experiments which showed that photosynthesis by isolated intact chloroplasts is inhibited if the P_i concentration in the surrounding medium is low (Edwards & Walker, 1983) and gained credence as a mechanism for regulating photosynthesis in leaves when it was realized that substances like mannose and 2-deoxyglucose inhibit photosynthesis because they are phosphorylated in the cytosol and sequester P_i as a non-metabolized sugar-phosphate (Herold, 1980). The notion that photosynthesis can be P_i-limited has had a large impact on research and the interpretation of data during the last 10 years, so I shall consider it in some detail. I will first discuss how sucrose synthesis is regulated in response to the accumulation of sucrose in the leaf. I will then discuss the evidence that a decreased supply of P_i to the chloroplast (a) stimulates the rate of starch synthesis and (b) inhibits the rate of photosynthesis in leaves.

7.2.1 Biochemical mechanisms for regulating sucrose synthesis.

The regulation of sucrose synthesis has been studied most extensively in spinach, where it has been shown that the key elements include (a) regulation of the cytosolic fructose-1,6-bisphosphatase (Fru1,6Pase) by the signal metabolite fructose-2,6-bisphosphate (Fru2,6bisP) and (b) regulation of sucrose phosphate synthase (SPS) by protein phosphorylation. These regulatory mechanisms increase the rate of sucrose synthesis in

response to rising rates of photosynthesis, and inhibit sucrose synthesis when sucrose accumulates in the leaf (Stitt *et al.*, 1987a, b; Stitt & Quick, 1989).

The first irreversible, or 'committed' reaction in the cytosol is catalysed by the Fru1,6Pase (Stitt *et al.*, 1987b). This enzyme is potently inhibited by Fru2,6bisP (Stitt, 1987, 1990). The concentration of Fru2,6bisP is controlled by the enzymes which are responsible for its synthesis and degradation, Fru6P,2-kinase and Fru2,6-Pase, respectively, and they, in turn, are regulated by metabolites to allow the Fru2,6bisP concentration to respond to the rate of photosynthesis and to the demand for hexose-phosphate (Stitt, 1987, 1990). In a feedforward loop, when photosynthesis increases, Fru2,6bisP is driven downwards because rising glycerate-3-phosphate and falling P_i inhibit its synthesis (Neuhaus *et al.*, 1990; Stitt, 1990). This activates the Fru1,6bisPase. The activation is amplified because the substrate (Fru1,6bisP) rises simultaneously (Stitt *et al.*, 1987b; Stitt, 1990). In a feedback loop, when the rate of hexose-phosphate production exceeds their use for sucrose synthesis, rising fructose-6-phosphate (Fru6P) leads to an increase of Fru2,6bisP, and Fru1,6Pase activity is decreased (Neuhaus *et al.*, 1989; Stitt, 1990).

The conversion of hexose-phosphate to sucrose involves three further irreversible reactions; SPS, sucrose-phosphatase and the hydrolysis of inorganic pyrophosphate (PP_i) (Stitt *et al.*, 1987a). Current research shows that SPS plays a major role in regulation. Sucrose-phosphatase is probably of less importance (Stitt *et al.*, 1987a; Krause & Stitt, 1991) and little is known about the regulation of PP_i turnover (Stitt, 1990).

SPS is regulated by an analogous balance of feedforward and feedback mechanisms to that described for Fru1,6Pase, but in this case, involving protein phosphorylation. When leaves are illuminated there is an apparent increase of SPS activity in rapidly-prepared extracts (Sicher & Kremer, 1984; Pollock & Housley, 1985; Stitt *et al.*, 1988; Kerr & Huber, 1987). When sucrose accumulates in the leaf or is supplied endogenously there is a decrease of apparent SPS activity (Stitt *et al.*, 1988; Foyer, 1990). In spinach, this reflects changes in the kinetic properties of SPS (Stitt *et al.*, 1988; Siegl & Stitt, 1990; Huber *et al.*, 1989b), and the V_{max} activity and amount of SPS protein in the leaf (Walker & Huber, 1989) remain constant. Inactivation is accompanied by increased incorporation of ³²P into a 120 kDa protein (Huber *et al.*, 1989a; Foyer, 1990) which has been shown to be SPS using monoclonal antibodies (Walker & Huber, 1989). Further evidence that SPS is regulated via protein phosphorylation is provided by two independent *in vitro* studies. (a) When partially purified spinach leaf SPS was incubated with [³²P]-ATP, ³²P was incorporated into the 120 kDa polypeptide and SPS activity decreased (Huber *et al.*, 1989a) (b) When a partially purified preparation of SPS in the low-affinity form (Siegl & Stitt, 1990) was incubated with highly purified rabbit muscle phosphoprotein phosphatase 2A, SPS was converted into a high-affinity form (Siegl *et al.*, 1990). This was prevented

by minute concentrations of okadaic acid and microcystin, which are known inhibitors of this class of protein phosphatases.

The protein kinase(s) and phosphatases involved in regulating SPS now need to be purified and characterized, in order to identify the precise signals regulating this phosphorylation cascade. When okadaic acid or microcystin were added to leaf extracts or fed into leaves they prevented activation of SPS, indicating that a protein phosphatase 2A is involved *in vivo* (Siegl, Stitt & Mackintosh, 1990). A protein of this type has recently been purified to homogeneity from *Brassica* leaves (Mackintosh & Cohen, 1989).

7.2.2 Interaction between feedforward and feedback regulation. Sucrose synthesis is therefore regulated by a balance between feedforward and feedback mechanisms. The interaction between these mechanisms will be crucial in determining whether an accumulation of sucrose leads to an inhibition of photosynthesis, or whether it leads to a change of partitioning (i.e. more starch and less sucrose is made), but is overridden before photosynthesis becomes inhibited.

Studies of the light-saturation responses of fluxes and metabolites in spinach leaves containing different amounts of sucrose showed that feedback regulation (deactivation of SPS and high Fru2,6bisP) leads to an alteration of partitioning in low light, without altering the rate of photosynthesis. In saturating light, the feedback inhibition is largely overridden, partitioning only changes slightly, and photosynthetic rate is maintained (Neuhaus *et al.*, 1990). Similar results were obtained in experiments with mutants of *Clarkia xantiana* which had decreased cytosolic phosphoglucose isomerase activity and increased levels Fru2,6bisP (Kruckeberg *et al.*, 1989; Neuhaus *et al.*, 1989), and in experiments in which okadaic acid and microcystin were added to spinach leaves to deactivate SPS (G. Siegl and M. Stitt, unpublished data).

These pathway responses can be understood in terms of the detailed properties of the enzymes. In high light, triose-phosphate and Fru1,6bisP increase and, since the cytosolic Fru1,6Pase has sigmoidal substrate kinetics, these are able to override Fru2,6bisP (Neuhaus *et al.*, 1989, 1990; see also Stitt *et al.*, 1987b; Stitt, 1989, for a detailed consideration of the kinetic interactions at the Fru1,6Pase). The deactivation of SPS in high light is overridden because hexose-phosphates probably increase to concentrations allowing the (phosphorylated) inactive form of SPS to show some activity (Siegl & Stitt, 1990; Neuhaus *et al.*, 1990). The action of glucose-6-phosphate as an allosteric activator (Doehlert & Huber, 1984) will also play an important role in overcoming the deactivation of SPS in these conditions.

Summarizing the last sections, sucrose synthesis is regulated by an interaction between 'feedforward' and 'feedback' regulation in spinach. This (a) allows partitioning to be altered when sucrose accumulates in the leaf but also (b) ensures that the feedback inhibition can be overridden if necessary at high rates of photosynthesis.

As a result, photosynthesis is not directly inhibited by accumulating sucrose. I have discussed this particular case in some detail to illustrate how a hierarchy of interacting regulation mechanisms can generate a very complex and flexible response at the pathway level.

7.2.3 Species-differences in the regulation of sucrose synthesis and the possible significance for the feedback regulation of photosynthetic metabolism. I shall now consider whether species-specific differences in the way in which sucrose synthesis is regulated could affect the susceptibility of photosynthesis to direct feedback inhibition. This section will be rather selective and speculative, because other species have not been studied in such detail as spinach.

Differences in the product-inhibition pattern of SPS provide one potential mechanism for changing the susceptibility of photosynthesis to feedback inhibition. Whereas spinach leaf SPS is not inhibited by sucrose or sucrose-6-phosphate (Stitt *et al.*, 1987a), SPS from pea, tobacco and peanut is sensitive to sucrose (Huber, 1981; Huber *et al.*, 1985). However, more detailed kinetic information is needed; for example, the sucrose would be more likely to lead to decreased rates of photosynthesis if it inhibited non-competitively because it would not be overridden by increased levels of substrates.

Differences in the mechanisms regulating SPS activation state, and their effects on the properties of SPS provide another way to alter the susceptibility of photosynthesis to direct feedback inhibition. Based on measurements in extracts, Huber *et al.* (1989) have proposed that three classes of SPS occur, which differ in whether they undergo activation and inactivation (presumably involving phosphorylation), and the effect that this has on their kinetics. These modes of regulation would all allow partitioning to be altered but differ in their potential as a mechanism to inhibit photosynthesis. Class II SPS, including spinach, sugar beet, bean and swiss chard (*Beta vulgaris* subsp. *cicla* L. Koch), are subject to phosphorylation, leading to changes of their kinetic properties. As discussed above, this will allow partitioning to be regulated while minimizing the risk that photosynthesis is inhibited. In contrast, inactivation of class I SPS in barley or *Zea mays* leads to an apparent decrease of V_{max} . Since this will not be overridden by increased metabolite levels, these species might be more susceptible to feedback inhibition when sucrose accumulates. In plants with a class III SPS, including soybean, tobacco, pea, cucumber, melon (*Cucumis melo*) and *Arabidopsis thaliana*, there is no activation or inactivation of SPS. At least in soybean, SPS also lacks allosteric activation by Glc6P or inhibition by P_i (Huber *et al.*, 1989b) and is subject to an endogenous diurnal rhythm (Kerr & Huber, 1987) which might be due to protein turnover. Here, again, it is likely that decreased SPS would ultimately lead to an inhibition of photosynthesis.

Another mechanism which has been proposed for feedback inhibition of photosynthesis involves invertase in the cell wall (Foyer, 1987, 1988). This would hydro-

lyze sucrose, and return glucose and fructose to the mesophyll cells. It has recently been shown that photosynthesis is inhibited in transgenic tobacco plants when yeast-derived invertase is expressed in their cell wall (von Schaewen *et al.*, 1990). A similar mechanism could also be imagined using a vacuolar invertase. It has recently been shown that many species which do not accumulate sucrose in their leaves contain invertase in their vacuole (Huber, 1989), and that these plants are more sensitive to feedback inhibition of photosynthesis (E.E. Goldschmidt and S.C. Huber, personal communication). Photosynthesis is also strongly inhibited in transgenic tobacco plants in which yeast-derived invertase is targeted to the vacuole (Sonnewald *et al.*, 1991).

Many other aspects of regulation could also be involved in determining whether direct feedback inhibition of sucrose synthesis inhibits the rate of photosynthesis. These could include the regulation of P_i movement across the chloroplast envelope and the tonoplast, and the detailed kinetic properties of the cytosolic Fru1,6bPase and enzymes involved in Fru2,6-bisP metabolism. Equally important will be the properties of enzymes within the chloroplast. For example, although ADP glucose pyrophosphorylase is evidently activated by a rising glycerate-3-phosphate P_i ratio (see below for more details) in a range where photosynthesis has not become P_i-limited in spinach, this need not necessarily be the case in other species.

Thus, sucrose synthesis is regulated by a sophisticated hierarchy of interacting mechanisms. In spinach, this regulation network operates to allow partitioning to respond to the accumulation of sucrose in the leaf, while minimizing the risk that photosynthesis is inhibited. In other species, there may be differences in the regulation mechanisms, which could alter their susceptibility to direct feedback inhibition of photosynthesis. In the future, detailed comparisons of fluxes, metabolites and enzyme kinetics will be needed to explore this possibility, and relate differences in biochemical regulation to strategies for allocation and growth at the whole plant level.

7.2.4 Does a decreased rate of sucrose synthesis lead to a P_i-limitation of photosynthesis? I shall now discuss the evidence that inhibition of sucrose synthesis affects chloroplast metabolism *in vivo* by changing the availability of P_i. I will first discuss the evidence that starch synthesis is stimulated *in vivo* by depletion of the metabolic P_i pool, and will then consider whether photosynthesis ever becomes P_i-limited as a result of a feedback-inhibition of sucrose synthesis.

When photosynthesis by isolated chloroplasts is inhibited by low concentrations of P_i in the medium, the stromal ATP/ADP ratio decreases. Glycerate-3-phosphate accumulates (Heldt *et al.*, 1977; Furbank, Foyer & Walker, 1987) because its reduction is particularly sensitive to the supply of ATP (Edwards & Walker, 1983). The activation state of Rubisco decreases (Heldt, Chon & Lorimer, 1978) and Ru1,5bisP increases (Heldt

et al., 1977; Furbank *et al.*, 1987). More research on the mechanism and regulation of Rubisco activase is needed, but it is possible that the deactivation of Rubisco could be a consequence of the low ATP/ADP ratio (Salvucci, 1989).

Starch synthesis in isolated chloroplasts is stimulated by low P_i because the increased glycerate-3-phosphate/ P_i ratio (Heldt *et al.*, 1977) activates ADP-glucose pyrophosphorylase (Preiss, 1982). There is reasonable evidence that a similar sequence of events occurs in leaves when sucrose synthesis is inhibited. Firstly, studies with various decreased-activity mutants have shown that ADP-glucose pyrophosphorylase is the most important control point in the pathway of starch synthesis (Neuhaus & Stitt, 1990). Second, the stimulation of starch synthesis in spinach leaves containing high levels of sucrose (Neuhaus *et al.*, 1990) or *Clarkia xantiana* mutants with low cytosolic phosphoglucose isomerase activity (Neuhaus *et al.*, 1989) is accompanied by an increase of glycerate-3-P. The sigmoidal kinetics of ADP-glucose pyrophosphorylase (Preiss, 1982) allow it to respond sensitively to relatively small changes of the metabolite levels.

The weak point in the evidence is the lack of measurements of stromal P_i in these conditions. Plants grown on normal nutrient media contain large amounts of P_i in the vacuoles (Loughman, Ratcliffe & Southan, 1989; Bligny *et al.*, 1990) and the stromal and cytosolic P_i are difficult to measure. However, Sharkey & Vanderveer (1989) recently used non-aqueous fractionation to show that the P_i concentration in the chloroplast decreases to under 1 mol m^{-3} conditions where end-product synthesis is limited. Free P_i may be even lower, because much P_i may be bound on Rubisco and thylakoids (Furbank *et al.*, 1987; Robinson & Giersch, 1987).

Since starch synthesis allows P_i to be recycled within the chloroplast, low external supplies of P_i will not necessarily lead to an inhibition of photosynthesis. Indeed, chloroplasts have a considerable ability to adjust to a lower supply of P_i without losing photosynthetic rate. This is possible because (a) glycerate-3-P reduction is a reversible reaction which can be driven by high ATP, or high NADPH and glycerate-3-phosphate (Edwards & Walker, 1983) and (b) there is considerable flexibility in the ability of the thylakoids to generate ATP and NADPH (Woodrow & Berry, 1988). This means that a chloroplast with a decreased external supply of P_i can maintain an increased glycerate-3-P/ P_i ratio and higher rates of starch synthesis, without this being accompanied by an inhibition of photosynthesis. This can be clearly observed in isolated chloroplasts (Heldt *et al.*, 1977) and leaves (see Neuhaus *et al.*, 1989, 1990, also section 7.2.2).

Various indirect approaches have been used to investigate whether photosynthesis in leaves ever becomes P_i -limited. These involve showing (a) that photosynthesis is O_2 -insensitive and does not respond to further increases of CO_2 (Sharkey, 1985b; Sharkey *et al.*, 1988), (b) that characteristic oscillations and induction transients occur (Walker & Sivak, 1986; Stitt &

Schreiber, 1988), (c) that photosynthesis is stimulated when P_i is supplied via the transpiration stream (Walker & Sivak, 1986; Leegood & Furbank, 1986), (d) that phosphorylated intermediates have accumulated and (e) that the ATP/ADP ratio decreases, glycerate-3-P remains high or increases, and Rubisco is deactivated, (as occurs in isolated chloroplasts, see above).

These criteria have been tested in mutants with decreased rates of sucrose or starch synthesis (Sharkey *et al.*, 1988; Neuhaus *et al.*, 1989; Neuhaus & Stitt, 1990), after adding inhibitors of sucrose synthesis (Stitt & Große, 1988; Stitt & Schreiber, 1988; Leegood *et al.*, 1988; Quick, Neuhaus & Stitt, 1988; Brauer, Sanders & Stitt, 1990; Brauer & Stitt, 1990), and during transients to low temperature or high light and high CO_2 when photosynthesis becomes limited by the rate of sucrose or starch synthesis (Leegood & Furbank, 1986; Labate & Leegood, 1988; Stitt & Große, 1988a, 1988b; Sharkey *et al.*, 1986). There is now ample evidence that photosynthesis can be P_i -limited *in vivo* when a sudden or large inhibition of end-product synthesis (due to low temperature, chemical inhibitors or removal or an enzyme) or a large stimulation of CO_2 fixation (due to saturating CO_2) leads to an imbalance between CO_2 fixation and sucrose or starch synthesis.

The kinds of experiments discussed in the last paragraph have frequently been invoked as support for the hypothesis that photosynthesis becomes P_i -limited when carbohydrate accumulates in the leaf. However, these manipulations are not comparable with 'natural' feedback regulation. They act in a different way, are anyway often extreme and 'unphysiological', and may disrupt or prevent the interaction between feedforward and feedback regulation which usually operates to adjust sucrose synthesis and photosynthesis to conflicting demands *in vivo* (see sections 7.2.1 and 7.2.2).

Unfortunately, very few experimental studies have directly asked whether photosynthesis becomes P_i -limited when carbohydrate accumulates in the leaf. The inhibition of photosynthesis in cold-girdled wheat leaves is accompanied by a loss of O_2 -sensitivity (Azcon-Bieto, 1983), and oscillations appear late in the photoperiod in P_i -deficient spinach (Foyer, 1988). However, O_2 sensitivity was not lost when photosynthesis was inhibited by cooling the roots of peanut (Bagnall *et al.*, 1988) or *Trifolium subterraneum* (Grub & Machler, 1990). Recently, von Schaewen *et al.* (1990) targeted yeast derived invertase to the cell wall of tobacco plants to interrupt apoplastic phloem loading (see below for more details). There was a gradual inhibition of photosynthesis coincident with accumulating carbohydrate, which was accompanied by a total loss of oscillatory behaviour (Stitt, von Schaewen & Willmitzer, 1991a). The small amount of data from studies of metabolism during 'sink' inhibition of photosynthesis is also inconsistent with a direct limitation by low P_i . In cold-girdled cotton and cucumber (Mayoral *et al.*, 1985; Plaut *et al.*, 1987), single-rooted soybean (Sawada *et al.*, 1989) and transgenic tobacco expressing invertase in the cell wall (Stitt *et al.*, 1991a), 'sink' inhibition of photosynthesis

was accompanied by a decrease of glycerate-3-phosphate. This contrasts with P_i-limited isolated chloroplasts, where the ratio increases (see above). Sawada *et al.* (1990) found that Rubisco was deactivated, but since the ATP/ADP ratio increased (Sawada *et al.*, 1989, 1990) this seems to involve a different mechanism than in isolated P_i-limited chloroplasts (see above). There are indications that low P_i could interact directly with Rubisco (Mächler & Nosberger, 1984; Parry *et al.*, 1985; Sawada *et al.*, 1990) but the details are not understood.

Summarizing this section, there is reasonable evidence that feedback regulation of sucrose synthesis leads to a stimulation of starch synthesis via shifts of P_i and metabolites. However, stimulation of starch synthesis can occur without photosynthesis being inhibited. In the examples where 'sink' inhibition of photosynthesis does occur, there is little direct evidence for P_i-limitation being involved. This deficit may be due to a lack of detailed biochemical studies, and will hopefully be rectified in the near future. However, the studies which are available indicate that additional mechanisms may be involved.

7.3 Indirect or 'adaptive' regulation of photosynthesis via decreases in the amount of Rubisco and other Calvin cycle enzymes

I shall now consider whether accumulation of carbohydrate has an indirect, longer-term, effect which involves changes in the amounts of key proteins which are required for photosynthesis. This can be viewed as an adaptation to a sink-source imbalance, because it would allow resources (e.g. amino-acids) to be remobilized from the leaves and invested in sinks (Geiger, 1976).

Potential mechanisms for long-term 'sink' regulation have received very little attention. However, they may be rather important. Gas exchange measurements in partially defoliated bean plants showed that the gradual increase of respiration in the remaining leaves was accompanied by an increase of the initial slope of the A/C_i curve, and an increase in the maximum rate of photosynthesis (von Caemmerer & Farquhar, 1984). This indicates that Rubisco and the capacity for Ru1,5-bisP regeneration have both increased. Direct measurement showed that Rubisco indeed increases gradually in beans after defoliation (von Caemmerer & Farquhar, 1984) and decreases in soybean when carbohydrate accumulates (Thorne & Koller, 1974; Crafts-Brandner, Salvucci & Egli, 1991).

Recent studies of transgenic tobacco plants expressing yeast-derived invertase in their cell wall provide strong evidence that accumulating carbohydrate can lead to a decrease of Rubisco and other Calvin cycle enzymes (von Schaewen *et al.*, 1990; Stitt *et al.*, 1991a). Invertase in the cell wall inhibits apoplastic phloem loading, because the sucrose is hydrolyzed to glucose and fructose which cannot be actively loaded into the phloem (Kallarackal & Komor, 1989). These plants showed a clear visual phenotype, losing chlorophyll progressively

from the tip as the leaves matured (von Schaewen *et al.*, 1990), following the developmental pattern of the sink-source transition (Turgeon, 1989). The areas of the leaf which were bleaching had a large accumulation of carbohydrate, especially soluble sugars (von Schaewen *et al.*, 1990). Measurement of metabolites showed that photosynthesis was limited by the rate at which triose-phosphate was converted to Ru1,5bisP, and by the rate of carboxylation (Stitt *et al.*, 1991a) and, in agreement, there was a dramatic decrease of Calvin cycle enzymes including Rubisco (Stitt *et al.*, 1991a). These results have been confirmed by supplying glucose to detached spinach leaves (Krapp, Quick & Stitt, in press). Rubisco protein had almost disappeared after 7 d, and the activity of other photosynthetic enzymes also declined. Measurements of chlorophyll fluorescence, ATP, NADP and metabolites showed that photosynthesis decreased because the conversion of triose-phosphate to Ru1,5bisP and the carboxylation of Ru1,5bisP had been inhibited. None of these changes occurred when detached leaves were supplied with water instead of glucose.

This decrease of photosynthetic proteins could, speculatively, be triggered by (a) shifts of metabolites or P_i which occur because carbohydrate has accumulated in the leaf, (b) by direct interactions of carbohydrates with a receptor, or (c) by physical changes in the leaf. More studies are needed to characterize the transduction pathway. Sheen (1990) has recently reported that the expression of reporter genes linked to several photosynthetic promoters (including rbcS) is inhibited by sucrose, glucose and fructose in a maize protoplast transient expression system, indicating that high levels of carbohydrate may act by decreasing gene expression. It is also possible that Rubisco is degraded more rapidly (Yamashita, 1987), or precipitates as an insoluble protein (Crafts-Brandner *et al.*, 1991) after sink-source manipulations.

Summarizing this section, relatively little attention has been paid to long-term 'adaptive' responses to high carbohydrate. However, there is evidence (a) that the levels of Calvin cycle enzymes decrease when carbohydrate accumulates and (b) that these changes can play a key role in inhibiting photosynthesis. These studies need to be extended in various whole plant systems, and the mechanisms have to be characterized. It is possible that regulation of photosynthetic enzymes levels by carbohydrate status could be involved not only in regulation of the sink-source balance, but also during acclimation of plants to a variety of factors including low nitrogen, high irradiance, low temperature and long days. In all of these treatments, one of the initial changes would be an increase of leaf carbohydrate.

8 Inhibition of photosynthesis by carbohydrate during acclimation to enhanced CO₂

In this last section, I shall discuss the mechanisms involved in inhibiting photosynthesis during acclimation to enhanced CO₂. In agreement with our conclusion in

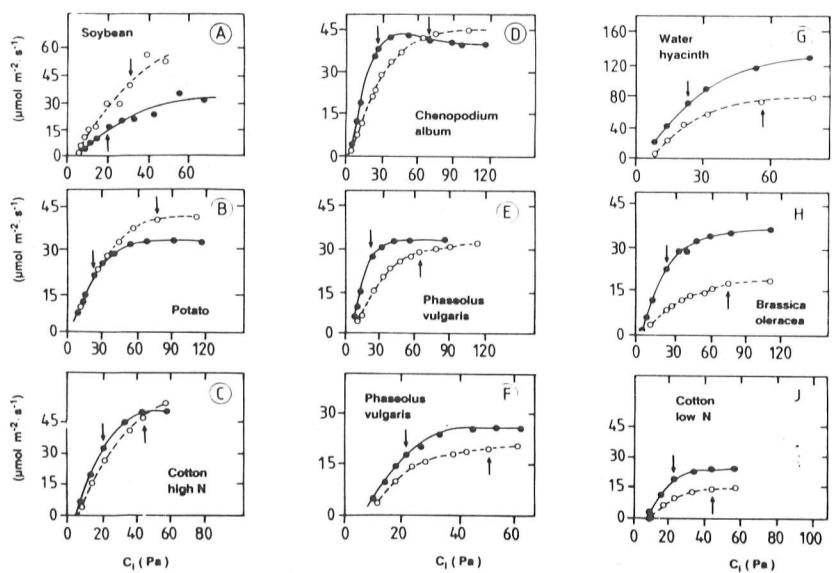


Figure 3. CO₂ response of photosynthesis in plants grown in air or acclimated to enhanced CO₂ concentrations: (A) soybean (Campbell *et al.*, 1988); (B) potato (Sage *et al.*, 1988); (C) cotton (Wong, 1979); (D) bean (von Caemmerer & Farquhar, 1984); (E) bean (Sage *et al.*, 1988); (F) *Chenopodium album* (Sage *et al.*, 1988); (G) water hyacinth (Spencer & Bowes, 1986); (H) *Brassica oleracea* (Sage *et al.*, 1988); and (J) cotton, grown in low nitrogen (Wong, 1979).

the previous section, we will see that there is little good evidence that photosynthesis becomes directly inhibited due to low P_i and, even if this does occur, it is probably only a transitory phenomenon. Long-term acclimation to enhanced CO₂ involves changes in the levels of proteins. Rubisco has received the most attention, but it is likely that many other proteins are also affected.

8.1.1 Changes in the CO₂-response curve. I shall first consider the evidence from gas exchange studies. Fig. 3 summarizes the CO₂-response of photosynthesis for several different species, after growing them for several weeks in air or enhanced CO₂. To remove additional effects due to stomatal conductance, the rate of photosynthesis is plotted against C_i.

In this particular study, soybean plants grown at enhanced CO₂ (Campbell *et al.*, 1988) had higher rates of photosynthesis than air-grown plants at all measuring-CO₂ concentrations (Fig. 3A). In potato (Sage *et al.*, 1988), photosynthesis was increased at high-measuring-CO₂ but not at low-measuring-CO₂ after acclimation to high CO₂ (Fig. 3B). In cotton grown with high nitrogen (Wong, 1979), bean (von Caemmerer & Farquhar, 1984); Sage *et al.*, 1988) and *Chenopodium album* (Sage *et al.*, 1988), photosynthesis was decreased at low measuring-CO₂ and unaltered or marginally increased in high-measuring CO₂ after acclimation to enhanced CO₂ (Fig. 3C-F). For cotton grown in low nitrogen (Wong, 1979), water hyacinth (Spencer & Bowes, 1986) and *Brassica oleracea* (Sage *et al.*, 1988), photosynthesis was decreased over the entire CO₂ range after acclimation to enhanced CO₂ (Fig. 3G-J). In all of these studies, photosynthesis was measured at irradiances and temperatures which were similar to those in which the plants had grown.

Despite this variability, two general trends emerge. Firstly in eight out of the 10 species, the initial slope of the A/C_i response is decreased when plants are grown at high CO₂. Secondly, plants grown in enhanced CO₂ have, relatively speaking, a poorer response when photosynthesis is measured at low CO₂ than at high CO₂. A third general point emerging from the studies summarized in Fig. 3 is that the O₂-sensitivity of photosynthesis (measured at 35 Pa CO₂) was retained or even increased in plants which had acclimated to enhanced CO₂ (Kriedeman, Sward & Downton, 1976; Spencer & Bowes, 1986; Sage *et al.*, 1989).

These trends are the opposite of what should happen if photosynthesis becomes P_i-limited due to accumulation of carbohydrate (see section 7.2). Instead, the decrease of the initial slope of the A/C_i response suggests that Rubisco has decreased (see section 3.1). The rather variable response at high C_i could be due to differences in the extent to which other photosynthesis proteins needed for Ru1,5bisP regeneration are changing.

8.1.2 Biochemical studies of acclimation to enhanced CO₂. I shall now discuss the evidence that Rubisco and other photosynthesis enzymes decrease. Table 2 compares the changes of Rubisco activity (assayed after incubation in high pH, Mg²⁺ and HCO₃⁻ to ensure the enzyme was fully activated) and photosynthetic rate after acclimation to enhanced CO₂ in 10 species. Rubisco activity decreased in eight species, with the reduction varying between 10 and 15% in bean (Sage *et al.*, 1989) and 50% in tomato (Besford, Ludwig & Withers, 1990). In all of these species, photosynthesis was also inhibited. Care is needed in interpreting some of the earlier measurements, because they were made before

the tight-binding inhibitor carboxyarabinitol-1-P was discovered. However, recent studies have directly quantified the number of active sites (Sage *et al.*, 1989) and have shown using antibodies that Rubisco protein changes (Besford, 1990).

There is considerable variety in the response of Rubisco to enhanced CO₂. However, this response broadly mirrors the effect on the rate of photosynthesis. For example, photosynthesis and Rubisco both remained high in potato (Sage *et al.*, 1989) and both increased in this particular study with soybean (Campbell *et al.*, 1988). In other studies with soybean, photosynthesis and Rubisco were both reported to decrease (Thorne & Koller, 1976; Delucia *et al.*, 1985). This variability is rather difficult to understand if Rubisco is responding directly to CO₂. However, it can easily be explained, once we hypothesize that Rubisco is decreasing in response to a change of the sink-source relation in the whole plant brought about by the enhanced CO₂. As already discussed, the effect on sink-source relations could be quite variable and will depend on many factors. For example, if enhanced CO₂ acts indirectly via sink-effects on Rubisco, we can understand why Rubisco often remains high or increases when young leaves (Porter & Grodzinski, 1986; Koch *et al.*, 1986; Yelle *et al.*, 1989a; Besford *et al.*, 1990) and young seedlings (Hinklenton & Joliffe, 1988; Peet *et al.*, 1985) acclimate to enhanced CO₂. In all of these cases, it is likely that 'sink' regulation of photosynthesis will be weak or inoperative. Young leaves operate as 'sinks' anyway, and young seedlings (see section 5) are often source-limited.

The experiments discussed in the last section showed that several other photosynthetic enzymes also decrease when carbohydrates accumulate in the leaf, in addition

to Rubisco. Most studies of acclimation to enhanced CO₂ have unfortunately been restricted to Rubisco. However, other enzymes do also decrease, including carbonic anhydrase (Porter & Grodzinski, 1960), 3-phosphoglycerate kinase and NADP-glyceraldehyde-3-phosphatase (Besford & Hand, 1988; Besford, 1990). Therefore, the question arises, whether the decrease of Rubisco is playing a major role in the acclimation to enhanced CO₂. Strictly, we need to demonstrate (a) that the decrease of Rubisco is large enough to account for the inhibition of photosynthesis, (b) that Rubisco is fully activated, (c) that the substrates (Ru1,5bisP and C_i) both increase and (d) that the product (glycerate-3-P) decreases. Unfortunately, there have been no detailed studies of metabolic regulation during acclimation to enhanced CO₂, so the following discussion will be restricted to the first two criteria.

In cotton (Wong, 1979), vine (Kriedeman *et al.*, 1976), cucumber (Peet *et al.*, 1985) and tomato (Yelle, 1989b; Besford *et al.*, 1990) the decrease of Rubisco is large enough to account for the observed inhibition of photosynthesis. In tomato, the activation state of Rubisco increased (Yelle *et al.*, 1989b; Besford *et al.*, 1990) and detailed comparison of the rate of photosynthesis, C_i, and Rubisco activity provided strong evidence that the decrease of Rubisco is an important determinant of photosynthetic rate (Besford *et al.*, 1990). Rubisco activation state was also increased when photoautotrophic *Nicotiana plumbaginifolia* calli were grown in enhanced CO₂ (Rey, Eymery & Pettier, 1990).

In other species including bean (von Caemmerer & Farquhar, 1984; Sage *et al.*, 1989), *Chenopodium album* (Sage *et al.*, 1989), *Brassica oleracea* (Sage *et al.*, 1989) and water hyacinth (Spencer & Bowes, 1986), the inhibition of photosynthesis was larger than the decrease

Table 2. Changes of Rubisco activity and loss of photosynthetic rate in different species during acclimation to enhanced CO₂

Species	Decrease of Rubisco (as a percentage of control grown at 35 Pa CO ₂)	Inhibition of photosynthesis (as a percentage of control grown at 35 Pa CO ₂)		References
		35 Pa CO ₂	100 Pa CO ₂	
Tomato	50	n.d.*	n.d.*	Yelle <i>et al.</i> (1989a)
Tomato	50	35	40	Besford <i>et al.</i> (1990)
<i>Brassica oleracea</i>	42	60	50	Sage <i>et al.</i> (1986)
Water hyacinth	39	50	50	Spencer & Bowes (1986)
<i>Vitis vinifera</i>	55	51	63	Kriedman <i>et al.</i> (1976)
Cucumber	40	40	n.d.*	Peet <i>et al.</i> (1986)
Cotton (high N)	36	14	n.d.*	Wong (1979)
Cotton (low N)	60	45	n.d.*	Wong (1979)
<i>Chenopodium album</i>	24	33	0	Sage <i>et al.</i> (1989)
Bean	26	32	37	von Caemmerer & Farquhar (1984)
Bean	12	45	10	Sage <i>et al.</i> (1989)
Potato	4	0	30†	Sage <i>et al.</i> (1988)
Soybean	0	n.d.*	n.d.*	Havelka <i>et al.</i> (1984a)
Soybean	0	40†	50†	Campbell <i>et al.</i> (1988)

*n.d.: not determined

†Stimulation of photosynthesis at enhanced CO₂

in Rubisco. Sage *et al.* (1989) found that the activation state of Rubisco was lower in enhanced CO₂ than in air, indicating that Rubisco was being inactivated in response to a restriction elsewhere in photosynthesis. Close examination of Fig. 3 reveals that most species acclimated to enhanced CO₂ operate close to the plateau of the A/C_i response in growth conditions. This again indicates that there may often be co-limitation by further partial processes, in addition to Rubisco, after acclimation to enhanced CO₂. The site(s) of the additional co-limitation(s) are not known, and there are several possibilities. The regeneration of Ru1,5bisP from triose-phosphates was strongly inhibited during 'sink' regulation of photosynthesis in transgenic tobacco plant (see section 8.3), and similar changes could also be involved during acclimation to enhanced CO₂. There might also be effects on electron transport or light harvesting; for example, expression of the Chl a/b binding protein promoter was decreased by soluble carbohydrates in a maize protoplast transient expression system (Sheen, 1990). Plants in enhanced CO₂ may also have decreased rates of sucrose or starch synthesis which could exacerbate the effect of a decrease of Rubisco protein. For example (see section 7.2) low P_i may inhibit Rubisco activase by decreasing the ATP/ADP ratio, and may also have other more direct and, as yet, not properly understood effects on Rubisco activity (see section 7.3). This would exacerbate the effect of a decrease in the amount of Rubisco. Leaves of some species accumulate large amounts of starch in enhanced CO₂, which (see section 7.2.1) might directly inhibit photosynthesis (Nafziger & Koller, 1976; Sasek *et al.*, 1985; Delucia *et al.*, 1985).

Summarizing this section, I suggest that the acclimation of photosynthesis to enhanced CO₂ can be understood in terms of the mechanisms which usually operate during the 'sink' regulation of photosynthesis in higher plants. During this long-term 'adaptive' process, direct P_i-limitation does not make a major or sustained contribution. Instead, Rubisco and, probably, a variety of other photosynthetic proteins decrease. In the future, it will be important to monitor the expression and levels of a wider range of proteins during acclimation to enhanced CO₂. It will also be important to carry out detailed metabolic studies of this process, both in wildtype plants and in plants which have been genetically manipulated to change their sink-source balance.

8.1.3 Concluding remarks. In this review, I have considered the potential response of photosynthetic fluxes to enhanced CO₂ and attempted to relate this to our understanding of the short- and long-term regulation of photosynthesis. The response to enhanced CO₂ is likely to vary considerably. In the short-term, the response depends on what controls the current rate of photosynthesis. Considerable advances have been made in understanding the short-term regulation of photosynthesis, and in identifying and quantifying factors which control the rate of photosynthesis.

In the long-term, the ability of the leaf to maintain

higher photosynthetic rates will depend upon the sink-source status of the whole plant and how this is regulated. In turn, this will vary considerably depending upon the species, its *habitus* and physiology, and the environmental conditions. Therefore, physiological and biochemical mechanisms which regulate sink-source interactions will be an important target for research in the future. Such studies will provide insights into the long-term response of plants to enhanced CO₂. Equally, molecular and biochemical studies of plants growing at enhanced CO₂ will provide an important tool to probe and understand sink-source interactions during plant development and during adaptation to other environmental changes.

Acknowledgments

The author's work described in the review was supported by the Deutsche Forschungsgemeinschaft. I am grateful to Dr W.P. Quick, A. Krapp and Dr M. Paul for criticisms of the manuscript, and to Frau E. Bischofsberger for typing it.

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