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RISING ATMOSPHERIC CARBON DIOXIDE: Plants FACE the Future*

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■ **Abstract** Atmospheric CO_2 concentration ($[CO_2]$) is now higher than it was at any time in the past 26 million years and is expected to nearly double during this century. Terrestrial plants with the C_3 photosynthetic pathway respond in the short term to increased $[CO_2]$ via increased net photosynthesis and decreased transpiration. In the longer term this increase is often offset by downregulation of photosynthetic capacity. But much of what is currently known about plant responses to elevated $[CO_2]$ comes from enclosure studies, where the responses of plants may be modified by size constraints and the limited life-cycle stages that are examined. Free-Air CO_2 Enrichment (FACE) was developed as a means to grow plants in the field at controlled elevation of CO_2 under fully open-air field conditions. The findings of FACE experiments are quantitatively summarized via meta-analytic statistics and compared to findings from chamber studies. Although trends agree with parallel summaries of enclosure studies, important quantitative differences emerge that have important implications both for predicting the future terrestrial biosphere and understanding how crops may need to be adapted to the changed and changing atmosphere.

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INTRODUCTION

Atmospheric CO_2 concentration ($[CO_2]$) has risen at an accelerating pace since the start of the Industrial Revolution. For the 1000 years prior to the Industrial Revolution $[CO_2]$ was stable at about 270 μmol mol^{-1}; today $[CO_2]$ is approximately 38% higher at 372 μmol mol^{-1}, and by the middle of this century it is predicted to reach 550 μmol mol^{-1} and to surpass 700 μmol mol^{-1} by the end of the century (169). This is not just an issue of the future. Today's crops and natural vegetation are growing at an elevated $[CO_2]$ level that has not been experienced by terrestrial vegetation for 26 million years (164). Understanding how plants respond and might be adapted to a future increase in $[CO_2]$ will also help us understand how they are currently responding and how they may have adapted to the increase that has already occurred. The effects of increases in $[CO_2]$ on the physiology and development of plants has been the subject of much research over the past 20 years and has been the subject of many detailed reviews (40, 155, 194). Studies of how plants respond to these projected future levels of $[CO_2]$ began in earnest some 30 years ago, initially with glasshouse and controlled environment studies. As scientific understanding increased, the need to test findings and hypotheses under truly open-air field conditions became increasingly apparent, leading to the development of a new technology, Free-Air Carbon dioxide Enrichment (FACE). Here, we examine the theory of plant responses to rising $[CO_2]$ and how FACE is altering this understanding.

How Do Plants Sense and Respond to Rising $[CO_2]$ in the Short Term?

Here we show that despite many potential points in metabolism effected by $[CO_2]$, there is clear evidence only for effects on Rubisco and stomatal movement in the range of $[CO_2]$ that are relevant to global change (i.e., 270–1000 μmol mol^{-1}). Plants can only perceive a change in atmospheric concentration through tissues that are exposed to the open air. With the exception of some reproductive organs,

only the photosynthetic organs of the plant have direct contact with the atmosphere. The protective cuticle of higher-plant leaves and other photosynthetic organs means that only the inner surfaces of the guard cells of stomata and the mesophyll can directly sense a change in atmospheric [CO_2]. Although many steps in metabolism utilize or respond to CO_2, the only sites where there is convincing evidence for a response in the concentration range of relevance (240–1000 μmol mol^{-1}) are Rubisco and a yet undefined metabolic step affecting stomatal aperture.

Although many steps in metabolism use or may be modulated by CO_2 or HCO_3^-, Rubisco is at current [CO_2] substrate limited by its principal substrate, CO_2. Thus only Rubisco has the potential to meaningfully respond to increasing [CO_2] and also function as a key metabolic step with sufficient regulatory control that a change in reaction rate would alter the flux through a major metabolic pathway. At a physiological level, C_3 photosynthesis, dark respiration, and stomatal conductance have all been reported to respond to instantaneous elevation of [CO_2] (40). Earlier studies reported a decrease of ca. 10% to 20% in respiratory CO_2 evolution in response to an instantaneous elevation of [CO_2] from 372–700 μmol mol^{-1} (reviewed in 28, 39). Two difficulties in explaining this decrease have been: (*a*) the absence of a metabolic step with adequate sensitivity and control to explain this decrease (57), and (*b*) understanding how an increase in external [CO_2] is sensed within the mesophyll when the stomata are closed. More recent analyses show that these apparent decreases in CO_2 evolution in the dark are likely an artifact of the measuring systems used, due to adsorption and absorption of CO_2, and leakage of CO_2, both via chamber seals and via the intercellular air spaces of leaves (7, 8, 90, 91). The alternative approach of determining respiration by measuring O_2 uptake, which escapes these limitations, has demonstrated a complete lack of any sensitivity of dark respiration to changes in [CO_2] from 0–2000 μmol mol^{-1} for a wide range of species (33).

The direct increase in photosynthesis due to elevation of [CO_2] results from two properties of Rubisco of terrestrial C_3 plants. (*a*) The K_m of the enzyme for CO_2 is close to the current atmospheric concentration, so elevated [CO_2] increases the velocity of carboxylation. (*b*) CO_2 competitively inhibits the oxygenation reaction, which produces glycolate leading to photorespiration. This latter effect is particularly important because it increases the efficiency of net carbon CO_2 uptake by decreasing photorespiratory CO_2 loss and diverting ATP and NADPH (generated by the light reactions) away from photorespiratory metabolism to photosynthetic assimilation. Thus, because the efficiency of net photosynthesis increases, rate increases regardless of whether other factors limit gross photosynthetic rate (117, 120). Assuming the average specificity and K_m for CO_2 and O_2 for Rubisco from terrestrial plants, and a constant intercellular versus external [CO_2] (c_i/c_a), one can calculate the increases in net CO_2 uptake that would result from an increase in atmospheric [CO_2] (11, 117). For a leaf temperature of 25°C, the increase in atmospheric [CO_2] from today's 372 μmol mol^{-1} to 550 μmol mol^{-1} by the middle of this century would increase Rubisco-limited and ribulose bisphosphate (RuBP)-limited photosynthesis by 36% and 12%, respectively, and the predicted increase

to 700 μmol mol^{-1} by the end of the century would cause respective increases of 63% and 18%.

Although the short-term response of C_3 photosynthesis to increased [CO_2] may be closely predicted from Rubisco's properties, mystery still surrounds the mechanism by which stomatal aperture responds to variation in [CO_2] (141). A simple phenomenological model, which holds that stomatal conductance is linearly proportional to the product of assimilation rate and humidity, and inversely proportional to [CO_2], is highly effective in predicting stomatal response to [CO_2] in the absence of water and humidity stress (19). However, the mechanism by which the stomata sense [CO_2], and where in the leaf [CO_2] is sensed, is unclear. From gas exchange measurements, Mott (142) deduced that stomatal conductance corresponds to c_i not c_a, thus explaining the remarkable constancy of c_i that is often observed. By simultaneous modulated fluorescence measurement it was recently shown that whole-chain photosynthetic electron transport (J_{PSII}) within the guard cell corresponds to changes in c_i and not c_a (109), which is consistent with observations of turgor and Cl^- efflux (65). Buckley et al. (19) recently showed that stomatal response to [CO_2] can be accurately modeled by assuming that ATP availability governs turgor, and that ATP levels in photosynthesizing guard cells follow the same factors governing the level in the mesophyll. This is consistent with the observation that J_{PSII} in guard cells tracks responses of J_{PSII} in the mesophyll (109). However, if stomatal aperture is responding to [CO_2] via photosynthesis in the guard cells, as implied by these studies, a further mechanism must also be operating because stomata in an isolated epidermis can respond to decreased [CO_2] in the dark. Mutations that make *Arabidopsis thaliana* stomatal movements abscisic acid (ABA) insensitive (*ABI1* and *ABI2*) also make them CO_2 and Ca^{2+} insensitive, indicating that these stimuli converge on or are close to the *ABI1* and *ABI2* gene products (214). Although most angiosperms, including C_4 plants, show a progressive decrease in stomatal conductance (g_s) with an increase in [CO_2], there are exceptions. Conifers, as a group, appear insensitive to variation in [CO_2], as do *Fagus* sp. among angiosperms (184).

Do the decreases in g_s in elevated [CO_2] offset the increases in A? For a constant A, any decrease in g_s will cause an increase in c_a-c_i. From the response of A to c_i it is possible to deduce the decrease in A that results from the decline in [CO_2] across the stomata (i.e., c_a–c_i). Figure 1 shows this response and the effect of g_s on c_i. For leaves in the current atmospheric [CO_2] of 372 μmol mol^{-1}, if $A = 0$ then $c_i = c_a$. This is illustrated by the intercept of the left-hand dotted yellow line and the x-axis in Figure 1. As A increases, c_i declines linearly and in inverse proportion to g_s; the point at which this line (the supply function) intercepts the response curve of A to c_i (solid black line; the demand function) gives the operating c_i. If there were not a diffusive barrier (i.e., $g_s = \infty$), c_i would equal the external [CO_2], as indicated by the vertical dashed yellow line originating from 372 μmol mol^{-1} on the x-axis. If A (marked on Figure 1) is the actual rate at the actual c_i, the limitation imposed by the stomata (l) is given by $(A^o - A)/A^o$ (in this example 0.136). At elevated [CO_2] g_s is assumed to be decreased to half the

value at the current ambient [CO_2], following the expectation that g_s is inversely proportional to [CO_2] and that c_i/c_a remains constant. This is represented by the more negative slope of the dotted blue line originating from the x-axis at 700 μmol mol^{-1}. However, because the slope of the supply function ($\delta A/\delta c_i$) diminishes with an increase in c_i, stomatal limitation (l) in this example is just 0.035; i.e. despite partial closure, the limitation that the stomata place on photosynthesis is diminished at elevated [CO_2]. If there was no decrease in stomatal aperture and therefore conductance, how much greater would the increase in A be on doubling [CO_2]? For the same example, if we hold g_s constant on doubling [CO_2], then l would be 0.02, compared to 0.035. Extrapolating from Figure 1, decrease in g_s lowers A by only 1.5%. But, because transpiration is linearly proportional to g_s, it lowers the loss of water vapor by 50%. Assuming that the stomata respond to rising [CO_2] to maintain a constant (c_i/c_a), decreased stomatal conductance in elevated [CO_2] causes a negligible offset of the increase in the photosynthetic rate, but greatly decreases transpiration and thus greatly increases water use efficiency. This analysis assumes stomatal behavior is unaffected by growth at an elevated [CO_2], an issue that we examine below.

The forms of PEP carboxylase that catalyze the primary carboxylation of C_4 photosynthesis have a K_m for [HCO_3^-], which means that the carboxylation reaction is normally near "CO_2" saturation. Only when the CO_2 supply is strongly restricted due to decreased stomatal and/or mesophyll conductance can a direct response of photosynthesis to increasing [CO_2] occur in C_4 plants. However, because stomatal conductance decreases in roughly inverse proportion to the increase in [CO_2], as in C_3 plants, water loss decreases. Thus, even in C_4 plants, photosynthesis and production may be indirectly increased through improved water status (54, 110).

In sum, in the short term C_3 land plants appear to sense and respond directly to rising [CO_2] exclusively through direct effects of increased carboxylation by Rubisco and decreased stomatal opening. Figure 2 shows the implications of these changes to plant growth, as a first approximation. For a plant growing in isolation, increased [CO_2] by increasing efficiency of light use in net CO_2 uptake, results in increased growth and therefore an increased rate of production of leaf area providing a feed-forward enhancement. This is reinforced by decreased water use, which further accelerates leaf development. As the plant develops to form a closed canopy, i.e., cover all available ground area, increased leaf area growth will have diminishing significance, but increased efficiency of light use will continue to result in increased production even after canopy closure. These changes, which both increase the efficiency of CO_2 uptake and water use, produce a wide range of secondary responses, most notably large increases in leaf nonstructural carbohydrates, improved plant water status including increased leaf water potential, and in many cases increases in plant carbon to nitrogen ratio (C/N), and decreases in leaf Rubisco activity, stomatal density, and root/shoot mass (reviewed in 40). Factors that appear unchanged with long-term growth at elevated [CO_2] are the ratio of intercellular to external [CO_2] (c_i/c_a) and the leaf area index (LAI) (40).

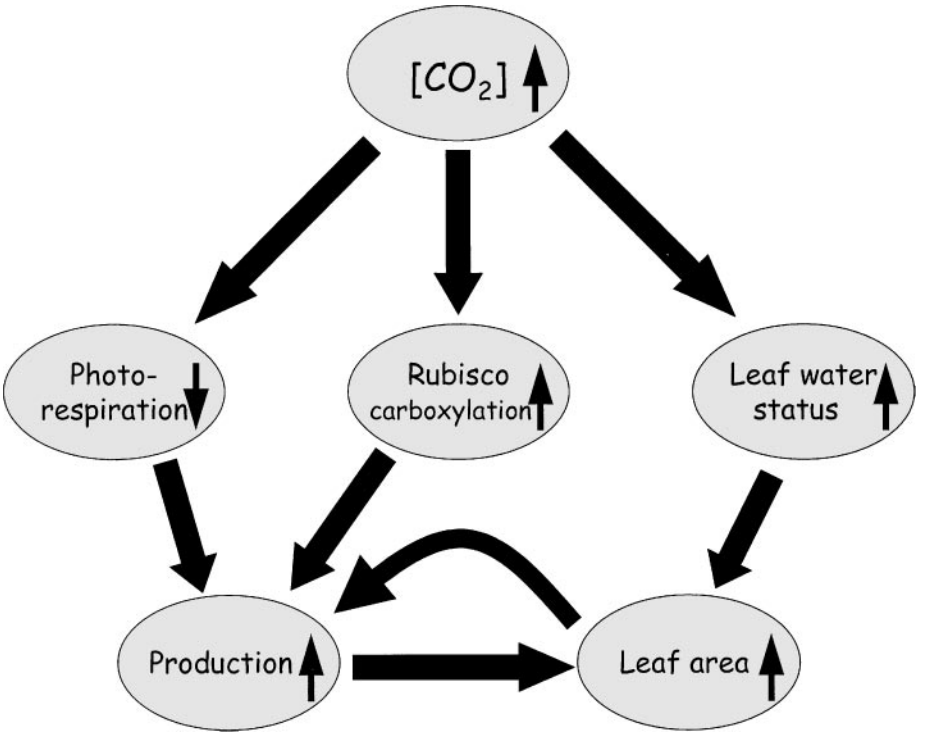


Figure 2 Schematic of the direct initial effects of rising [CO_2] on C_3 plant production. Increased [CO_2] increases the rate of carboxylation at Rubisco while inhibiting the oxygenation reaction and thus decreasing photorespiratory loss of carbon. Increased production allows increased leaf area development, providing positive feedback on the plant photosynthetic rate. This is further reinforced by decreased transpiration and improved leaf water status, which also favor increased leaf area growth.

What Increases in Photosynthesis and Production Might Be Expected under Elevated [CO_2]?

Here we explain, by reference to the Farquhar et al. (46) biochemical model of leaf photosynthesis, that the increase in photosynthesis with increase in [CO_2] will be greater when Rubisco is limiting, at high temperature, and when capacity for RubP regeneration is increased relative to Rubisco activity. Because the competing reactions of RuBP carboxylation and RuBP oxygenation dominate the response of C_3 photosynthesis to variation in [CO_2], this can be mechanistically modeled. Farquhar et al. (46) developed the steady-state leaf biochemical model by combining the kinetic properties of Rubisco with a model of the light response of electron transport to allow effective prediction of the light, temperature, CO_2, and O_2 responses of C_3 leaves. This model has proved remarkably robust, presumably

because of the conserved properties of photosynthesis and Rubisco across different vegetation types and forms. It has been combined with well-defined canopy properties to allow scaling from the leaf to canopies and landscapes (6, 22, 37, 210, 211). It is commonly incorporated into both simple (48, 49, 115) and complex canopy (37, 210) models, and into models of atmosphere-biosphere bidirectional interaction (48). The effective prediction of measured fluxes of CO_2 into large areas of forest is further evidence of the robustness of the model (116). The model successfully captures the effect of leaf temperature on the response of A to $[CO_2]$ (11, 12, 117). The relative increase in A with increase in $[CO_2]$ is greater at a high than at a low leaf temperature. Both the solubility of CO_2 in water relative to that of O_2 declines with increases in temperature and the activation energy requirement of the oxygenation reaction is greater than that of carboxylation. As a result rising temperature increasingly favors oxygenation (117, 193). Because CO_2 competitively inhibits oxygenation, the net increase in CO_2 uptake resulting from suppression of photorespiration rises with temperatures. Figure 3 uses the model of Farquhar et al. (46) and recent temperature parameterizations (11, 12) to predict the increase in leaf CO_2 uptake that would result from an increase in $[CO_2]$ by

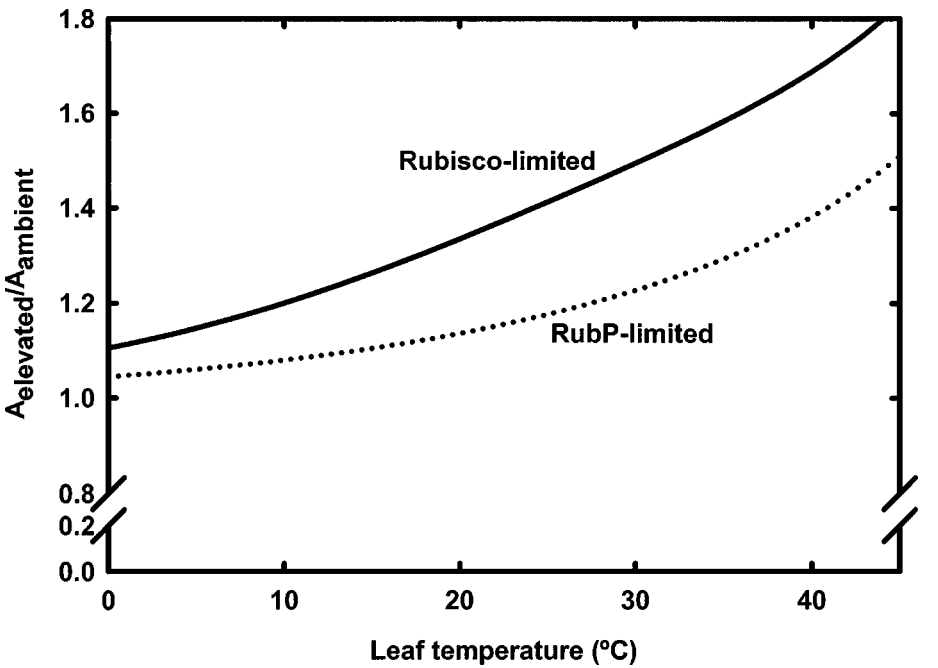


Figure 3 The theoretical increase in leaf CO_2 uptake (A) predicted from the properties of Rubisco from terrestrial C_3 plants at different temperatures, depending on whether *(a)* activity of Rubisco or *(b)* rate of RuBP regeneration limits photosynthesis (11, 12, 46, 118).

200 μmol mol^{-1} above present ambient concentration at a range of leaf temperatures. Two lines are illustrated: the lower shows the increase that would result if the rate of regeneration of RuBP were limiting and the upper shows the rate if activity of Rubisco were limiting. The increase is greater when Rubisco is limiting because elevated [CO_2] increases the velocity of carboxylation and competitively inhibits oxygenation. When RuBP is limiting only the latter factor increases the net rate of CO_2 uptake. A third possibility is that CO_2 uptake is limited by the rate of triose-phosphate utilization. In this situation the rate is independent of [CO_2] at all temperatures (66, 118).

Is it realistic to expect these short-term responses of leaf photosynthesis to elevated [CO_2] to be relevant to the long term and to affect the future production and ecology of C_3 plants in an elevated [CO_2] environment? Several factors can interfere with or nullify predictions based on these simple leaf photosynthetic responses (104, 133, 134, 158). In many early studies, the initial stimulation of photosynthesis and growth on transfer of plants to elevated [CO_2] diminished or disappeared in the longer term. It has been argued in the ecological literature that N availability in particular will limit the response to elevated [CO_2] (104, 133). However, at the photosynthesis and respiration level, there is no reason why N deficiency should diminish the response of carbon gain to elevation of [CO_2] (115). Loss of the initial stimulation may partially or fully be a result of growth conditions, in particular the restriction of rooting volume (9, 183). However, one of the first studies of growth of natural vegetation in situ under elevated [CO_2], and in the apparent absence of rooting volume limitation, also reported a loss of stimulation after just three years (158). The most prominent change in the leaf photosynthetic apparatus is a decline in the amount and activity of Rubisco. This has been suggested as the basis of the decrease in response of production to elevated [CO_2] and one that would inherently preclude a response in the long term (158). An alternative perspective is that the decline reflects a decreased requirement for Rubisco at elevated [CO_2] (120). The response of A to c_i is biphasic, such that as c_i increases from zero, A increases steeply where Rubico activity is limiting to a transition point beyond which RuBP is limiting and $\delta A/\delta c_i$ is small and approaches zero. At light saturation this transition is commonly at the c_i that occurs in the present atmospheric [CO_2]; typically c_i is about 0.7 of atmospheric [CO_2] (40). This implies that the amount of active Rubisco and capacity for RuBP regeneration are balanced (as Figure 1 illustrates). If atmospheric [CO_2] increases, c_i is expected to rise proportionately (40). As Figure 1 shows, if c_i increased by 50%, then photosynthesis would be limited solely by RuBP regeneration, and a substantial (30%) loss of Rubisco activity could occur without affecting photosynthesis. If plants could be engineered for a future atmosphere by decreasing their Rubisco content and activity by 15% and increasing their capacity for RuBP regeneration by 15%, the increase in A on elevation of [CO_2] would be 40% greater in the example in Figure 1. Because total quantities of protein invested in Rubisco and in the apparatus for regeneration of RuBP are similar, this greater increase could be achieved without any demand for extra resources (120). A key issue in CO_2

research is whether loss of the response to elevated [CO_2] is seen in long-term open-air experiments. We evaluate this issue below. First, what is the possible mechanism by which plants may acclimate to growth in elevated [CO_2]?

WHAT ARE THE MECHANISMS BY WHICH ELEVATED CO_2 MAY DOWNREGULATE PHOTOSYNTHETIC CAPACITY?

In this section we show that loss of photosynthetic capacity through acclimation, particularly Rubisco amount and activity, is most likely explained by decreased expression of specific photosynthetic genes or gene products in response to increased sucrose cycling within mesophyll cells. This results when photosynthesis exceeds the capacity for carbohydrate export and utilization, a response exacerbated by genetic limitations, such as determinate growth patterns, and environmental limitations, such as N deficiency or low temperature (5).

A common feature of plants exposed to elevated [CO_2] in chamber experiments is that they fail to sustain the initial, maximal stimulation of net CO_2 uptake rate under optimal microclimate conditions (40). Rogers & Humphries (179) demonstrated that this phenomenon could be attributed almost entirely to the decrease in Rubisco activity. Reduced carboxylation capacity with growth in elevated [CO_2] is well documented (40) and often associated with a reduction in the amount of Rubisco protein and the levels of transcripts for *rbc*S along with other photosynthetic genes (140).

Increased carbohydrate content is the most pronounced and universal change observed in the leaves of C_3 plants grown at elevated [CO_2] (40). Sucrose is a major product of photosynthetic cells, the main form of translocated carbon in most plants, and the main substrate for sink metabolism. Therefore, sucrose content in source leaves will reflect the balance of supply (photosynthesis) and demand (growth, storage, nutrient assimilation), and changes in the sucrose pool can communicate whole-plant carbon flux (47). Because sugars are not just substrates but play a regulatory role in controlling the expression of many plant genes (103), the hypothesis that excess carbohydrate at elevated [CO_2] feeds back on photosynthetic gene expression and leads to acclimation is attractive (108, 188, 194). Although carbohydrate accumulation at elevated [CO_2] is phenomenologically linked with acclimation, it is often poorly correlated with a loss of photosynthetic capacity (139). This is partially a result of the heterogeneous distribution of sugars within source tissue that may confound the meaningful correlation of bulk carbohydrate content with acclimation (106, 107). In addition, following the mass-flow hypothesis of translocation, even if sink metabolism can fully utilize the products of increased photosynthesis, an increase in sucrose concentration at the source is necessary to generate the additional flux through the phloem, assuming there is no increase in total phloem tube cross section. At the whole-plant level a restricted capacity to utilize photosynthate drives a loss of photosynthetic capacity at elevated [CO_2]. Ainsworth et al. (5) illustrated this by showing similar photosynthetic rates at

ambient [CO_2] in an indeterminate soybean cultivar and an isogenic determinate line of this cultivar derived by a single gene mutation. When the cultivar and its mutant grew at elevated [CO_2], a marked decrease in carboxylation capacity occurred in the determinate mutant, which was genetically limited in its capacity to add "sinks" for photosynthate, while no acclimation occurred in the wild type. So, if the correlation between carbohydrate accumulation and acclimation is often poor, how else might the photosynthetic cell sense inadequate capacity in the plant for utilization of additional photosynthate?

Sucrose is the form of carbohydrate exported from the leaf in most terrestrial plants, and also the major form in which it is stored within the vacuole. When photosynthesis exceeds the capacity for utilization and export of carbohydrate, sucrose will accumulate in the leaf phloem and mesophyll vacuoles. How can the imbalance between the supply and demand of carbohydrates be sensed and rectified through downregulation? Although there are some inconsistencies (61, 196), the model proposed by Moore et al. (140) makes a convincing case for the molecular control of Rubisco content at elevated [CO_2] via sucrose cycling (Figure 4). Excess sucrose from photosynthesis is hydrolyzed by vacuolar and apoplastic invertases and the resulting hexoses are phosphorylated by hexokinase and then used to resynthesize sucrose. The flux of hexose through hexokinase signals the source-sink imbalance, as demonstrated by Jang et al. (92), who showed that transgenic plants expressing antisense hexokinase have a reduced sensitivity to sugars (92). Nocturnal starch hydrolysis liberates maltose and glucose (185, 186). Glucose, including that resulting from the amylomaltase catalyzed conversion of maltose to sucrose, provides substrates for hexokinase, possibly involving a hexokinase bound to the outer envelope of the chloroplast membrane (217), thereby providing 24-hour signal production for a hexokinase sensing system (Figure 4).

Elucidation of the signal transduction system that links hexokinase to decreased photosynthetic capacity (reviewed recently in 180, 192) is incomplete, but there is evidence that a number of factors are involved including protein kinases, protein phosphatases, and Ca^{2+} as a second messenger. Control mechanisms initiated by the carbohydrate signal vary among species but target the small subunit of Rubisco through transcriptional or translational control or by interfering with the assembly of the holoenzyme (140).

There is evidence for cross talk between sugar signaling and several other possible regulatory compounds, in particular nitrate and ABA (94, 181, 195). However, it is not clear how relevant these interactions are in the downregulation of photosynthetic genes in plant source leaves, given that both N deficiency and water stress lower the capacity of the plant to utilize photosynthate and would exacerbate sucrose cycling in the source leaf.

An alternate concept is that acclimation is the result of a nonselective decrease in leaf N content (29, 89, 123, 189). In this model, the decrease observed in Rubisco reflects a general decrease of leaf protein due to relocation of N within the plant (123, 146) or earlier leaf senescence in N-limited plants (149, 150, 196). Under conditions of N limitation, acclimation may accelerate in elevated [CO_2] because

the plants are larger and therefore may experience acute N limitation sooner, or to a greater extent. Farage et al. (45) demonstrated that even when growth is restricted by low –N, photosynthetic acclimation in elevated [CO_2] could be ameliorated if N was added in direct proportion to plant growth, supporting the concept that N dilution, rather than N supply, causes Rubisco acclimation. In open-field conditions where plants can expand the volume of soil exploited in response to nutrient demand, such N dilution is less likely. Evidence of a greater decrease in Rubisco than N or protein content under open-field conditions such as in FACE will be key to separating these two competing, but not necessarily exclusive, hypotheses (i.e., selective decrease in proteins versus a general N dilution).

FACE: WHY AND WHAT?

Why FACE?

There have been thousands of experimental studies evaluating the response of vegetation to the increases in atmospheric [CO_2] expected to occur over this century; these have been summarized in several reviews (2, 28, 30, 40, 88, 100, 129, 131, 165, 207). With so much data already, what more could be needed? Most information about plant responses to elevated [CO_2] has been derived from experimental studies that used greenhouses, artificially illuminated controlled environment chambers, and in the field, transparent enclosures or open-top chambers (OTCs). With size limitations on these systems it is not surprising that most of these studies have focused on the early stages of plant growth. Many of these studies, including some of the field studies, have used plants grown in pots. Arp (9) showed that rooting volume suppressed the response of plants to elevated [CO_2], essentially demonstrating that loss of a response to increased [CO_2] through acclimation was an artifact of pot size. Although it has been suggested that this "pot effect" is a result of nutrient exhaustion (105), experiments have shown that independent of nutrient supply there is a strong feedback when roots encounter a barrier. This was shown by using different pot sizes with high fertilizer levels (200), using soils with different physical resistances (125), and using hydroponics where nutrients could be manipulated without restricting rooting volume (45). Even large pots restrict the response of plants to elevated [CO_2]. Ainsworth et al. (2) surveyed all studies of soybeans grown at elevated [CO_2] and found that plants grown in the field without restriction on rooting volume showed four times the yield increase of those grown in large pots (>10 liters volume). This emphasizes the importance of calibrating findings made with plants grown in containers with those grown in the field.

Most field studies have been based on the use of OTCs. Despite the fact that the top, or the larger portion of the top, of an OTC is open to the atmosphere, there are still important differences between the environment within the best-engineered OTCs and the environment surrounding the chamber. As a result the effect of enclosure in the OTC without elevation of [CO_2] may exceed that of any additional effect due to elevation of [CO_2] to twice the current ambient levels (34,

41). Whitehead et al. (216) evaluated the performance of the widely used large OTCs described by Heagle et al. (68) and compared microclimatic conditions with those outside. When the outside photon flux was 1600 μmol m^{-2} s^{-1}, air temperature was 4.3°C higher and water vapor humidity deficit 0.8 Pa higher. In cloudy conditions the mean transmittance of solar irradiance into the chambers was 81%; on clear days this decreased to 74% with increasing solar zenith angle. The ratio of diffuse to total solar irradiance in the chambers was 13% and 21% greater than that outside for cloudy and clear conditions, respectively. Transmittance of visible solar irradiance (400–700 nm) through the plastic wall material decreased by 7% after one year of exposure at the site. Other obvious effects of an OTC are that wind is removed, preventing wind damage and dispersal of pathogens and pests, rainfall interception is dramatically decreased, and plant-atmosphere coupling is altered. Most materials used in the construction of OTC walls do not transmit UV-B wavelengths (280–315 nm). These factors may contribute to a varying extent to changes reported in the growth form of vegetation inside versus outside an OTC (143, 202). In summary, OTC studies have examined the effects of elevated [CO_2] on plants in an aerial microclimate that may be duller, warmer, and drier than the norm. Because control OTCs in which [CO_2] is maintained at the current atmospheric level replicate these chamber effects, do they matter? Temperature, humidity, and light modify the response of plants to elevated [CO_2] (2, 28, 30). Although these chamber effects may not cause a change in the direction of a response, they will almost certainly alter its magnitude.

Additionally, small isolated plots in agronomic trials and ecological experiments are well known to overestimate biomass, production, and yields (173). Increased radiation interception at the edges of small plots in particular can exaggerate the effect of a treatment. Open-top experiments and some closed-chamber experiments have suggested very large increases in the yields of C_3 crops under elevated [CO_2] (100). The maximum practical size of OTCs, typically ca.2-m diameter, limits each plot to a ground surface area of <3.1 m^2. In a 2-m diameter chamber more than 50% of the vegetation is less than 30 cm from the chamber wall, and 75% is within 50 cm of the wall. The plot sizes used in agricultural trials usually include a border or buffer area that is twice the vegetation height (173). Therefore, even a 50-cm high semidwarf wheat crop would require a buffer zone of 1 m, and thus no area within the OTC would be free of edge effects. Consequently, knowledge of crop responses to elevated [CO_2] is currently derived from experiments that are considered unacceptable in standard agronomic trials (128).

For forests, which contain more than 90% of the carbon of earth's living organisms (52), the situation is worse. OTCs can only accommodate one or two moderately sized trees, and therefore edge effects are likely extreme and natural canopy closure is prevented. As Lee & Jarvis (112) noted, "trees... do not fit into simple experimental enclosures. Furthermore, trees and forests are very well coupled to the atmosphere, and this coupling is often greatly reduced when tress are enclosed in chambers, introducing an additional artifact." With a few exceptions, experiments with trees have been limited to seedlings and juvenile

trees (112). Given that juvenile trees respond very differently to light when compared to mature individuals, it is likely that their responses to elevated [CO_2] are also misleading as to the effects on mature trees (112). For tall trees in individual chambers, root systems often spread beyond the area covered by the chamber unless restricted by an artificial physical barrier. Such a barrier prevents roots from exploiting soil outside the chamber and vice-versa, but may artificially induce a feedback inhibition of photosynthesis and production (9).

The greater size of FACE plots (8–30-m diameter) by comparison to OTCs not only reduces edge effects but also allows simultaneous study of many plant processes. Within chamber systems such holistic approaches are precluded by the damaging effect that would result from destructive sampling of soil and leaves. For example, if we assume that no more than 2% of a leaf canopy should be removed by destructive analyses within a year if it is to remain representative of undisturbed canopy, this would provide just 160 cm^2 per unit canopy from a 1-m diameter OTC, compared to 100,000 cm^2 per canopy in a 25-m diameter FACE plot (128). This greater quantity of available tissue would allow the sampling of the quantities needed for the genomic, proteomic, and metabolomic approaches needed to fully understand plant response to rising [CO_2]. FACE systems have already allowed simultaneous study of leaf and canopy gas exchange, biochemical and molecular analysis of photosynthesis, secondary metabolism, leaf area and canopy development, above- and belowground biomass accumulation, shoot and root development, canopy energy balance, stem water flow, soil moisture, nutrient extraction, and final reproductive yield all within single treatment plots (128).

In summary, although chamber studies have been critical to developing understanding of plant responses to elevated [CO_2], a range of technical limitations have necessitated the development of open-air field treatment systems to re-evaluate our hypotheses on plant responses to elevated [CO_2].

What is FACE?

Although miniFACE systems as small as 1-m diameter have been developed (135), they do not escape all of the problems of enclosures outlined above. This review is therefore limited to full-size FACE systems of >8-m diameter plots. A single FACE plot of this type is approximately circular and surrounded by a ring of pipes that release CO_2, or air enriched with CO_2, at vertical intervals from just above the ground surface to just above the top of the plant canopy (Figure 5). Wind direction, wind velocity, and [CO_2] are measured at the center of each plot and this information is used by a computer-controlled system to adjust CO_2 flow rate, controlled by a mass-flow control valve, to maintain the target elevated [CO_2], typically either 550 μmol mol^{-1} or 600 μmol mol^{-1}. Only pipes on the upwind side of the plots release CO_2, unless wind velocity is less than 0.4 m s^{-1} when it is released alternately from adjacent release points (128). For vegetation of low stature, e.g., a wheat crop, only one or two vertical release points are necessary, whereas for tall vegetation, e.g., 12 m pine forest, several vertical release points

are needed to enrich the whole canopy (73, 114, 128, 136, 138). Quantities of released CO_2 decrease with depth into the canopy to reflect the profile of wind speed. The fast feedback Proportional Integral Differential (PID) algorithms avoid large overshoots in response to fluctuations in $[CO_2]$ and provide a stable elevation of $[CO_2]$. This basic FACE system has been utilized with some variations and technical developments in several experiments including studies of cotton, wheat, grassland and desert ecosystems, and forest and plantation trees (Table 1).

FACE is not without limitations (128). Long-term continuous records of $[CO_2]$ within FACE rings show that 1-minute averages of actual $[CO_2]$ are typically within $\pm 10\%$ of the target concentration for about 90% of the time in low stature vegetation such as most arable crops, and within $\pm 20\%$ for 90% of the time in forests (128). On shorter timescales (i.e., less than 1 minute), as in OTCs, there are larger fluctations around the target elevated $[CO_2]$ (73, 145). An important issue is whether these fluctuations are perceived by the plant, and in particular whether they affect net CO_2 exchange. Because the response of photosynthesis to $[CO_2]$ is nonlinear (see Figure 1), if $[CO_2]$ fluctuates, A at a given mean $[CO_2]$ will decrease as the amplitude of variation around that mean increases, providing that the chloroplasts are exposed to this fluctuation. Diffusion and solubilization dampen fluctuations, so at what frequency is there an effect on photosynthesis? Measuring net CO_2 uptake when the background $[CO_2]$ is fluctuating rapidly is fraught with technical difficulties and potential errors. Hendrey et al. (74) used modulated chlorophyll fluorescence to monitor whole-chain electron transport through photosystem II (J_{PSII}) in wheat leaves during controlled oscillations in $[CO_2]$ of 225 μmol mol^{-1} amplitude around a mean of 575 μmol mol^{-1}. Under nonphotorespiratory conditions, i.e., in 1% $[O_2]$, J_{PSII} is directly proportional to A. Oscillations of 1 minute or less in frequency had no effect on J_{PSII}, but lower frequency oscillations resulted in progressively greater decreases of J_{PSII}. Given that 1-minute averages are usually within 10% of the target $[CO_2]$ in FACE, these results suggest that the lower frequency oscillations necessary to decrease the response of photosynthesis to elevated $[CO_2]$ are uncommon (74).

The advantage of using the wind as the carrier gas, as in FACE, is that the perturbation of the natural microclimate is minimal in contrast to enclosure methods. The disadvantage is that a dilution gradient is generated across the treatment plot. So although the center is maintained close to the target, the upwind side may be 100 μmol mol^{-1} above and the downwind 100 μmol mol^{-1} below the target. With a strong prevailing wind a gradient effect would occur across each plot. At some FACE facilities the CO_2 used has a significantly different ^{13}C or ^{14}C content than that of the CO_2 in the bulk atmosphere. This is reflected in the vegetation formed within the treatment plots. Analysis of isotopic composition across these plots shows a remarkable uniformity, suggesting that although transient gradients occur, averaged over growing seasons these gradients are not detectable (111). Figure 5 shows that even on a shorter timescale a surprising spatial uniformity in the response of transpiration to elevated $[CO_2]$ may be seen via elevation of leaf temperature within the FACE ring.

TABLE 1 Location, vegetation, years, treatment, replication, and plot sizes of the FACE experiments covered in this review

Location	Vegetation	Year	$[CO_2]$ μmol mol^{-1}	Other treatments	n	Diam.
1. Experiment Station, University of Arizona, Maricopa, AZ, USA	Cotton (126)[a]	1989–91	550*s	2 water (split-plot)	4	25
	Wheat (101)[a]	1993/4	550*s	2 water (split-plot)	4	25
	Wheat (1)[a]	1996/7	Amb+200*s	2 N (split-plot)	4	25
	Sorghum (23, 161)[a]	1999/0	Amb+200*s	2 water (split-plot)	4	25
2. Rapolano Terme, Italy	Potato (137)[a]	1998/9	560s		3	8
3. Experiment Station, University of Illinois, Urbana, IL, USA	Soybean (175)[a]	2001	550s		4	20
	Soybean (5)[a]	2002	550s	2 ozone		20
	Corn (110)[a]	2002	550s			20
	Soybean[a]	2003	550s	2 ozone (factorial); 2 water (split-plot)	4	20
4. Shizukuishi, Iwate, Japan	Rice (98, 99)[a]	1998–present	589s	3 N (split-plot)	3	10
5. Experiment Station, ETHZ, Eschikon, Switzerland	Perennial ryegrass and white clover (3, 70)[a]	1993–2002	600s	2 N × 2 cutting freqs. (split-plot)	3	18
6. Bulls, Manawatu, New Zealand	Grazed pasture of 25 species, including C_3 and C_4 grasses (42)	1997–present	475	None	3	12
7. Mojave Desert, NV, USA	Desert scrub and C_4/C_3 grasses (84, 93)	1997–present	550*	None	3	23
8. Cedar Creek, Bethel, MN, USA	Tall grass prairie (172)[a]	1997–2001	550s	4 levels of species × 2 N in 4 m^2 subplots	3	24
9. Duke Forest, NC, USA	Loblolly pine & understory species (73)	1996–present	Amb+200 (1996/7*)s	None	3	30
10. Rhinelander, WI, USA	Aspen, Red maple, Paper birch (95)[a]	1997–present	Amb+200s	2 ozone (factorial)	3	30
11. Tuscania, Italy	White, black, and hybrid poplars (138)[a]	1999–present	Amb+200s	2 N (split-plot)	3	30
12. Oak Ridge, TN, USA	Sweetgum (154)	1998–present	550s	4 levels of species × 2 N in 4 m^2 subplots	2	25

[a]treatment from planting; * = day and night fumigation (otherwise daytime only); s = growing season fumigation only; Amb = site ambient $[CO_2]$.

A further potential disadvantage of FACE is that it depends on continuous air movement. During daylight hours the continual flux of solar radiation and resulting convective currents ensure that still periods are rare, except around dawn. However, at night, still conditions commonly occur. All but three of the FACE systems in Table 1 predilute CO_2 into air that is pumped into the plots at the release points. This flow of CO_2-enriched air moves air into the plot under still conditions. These systems can therefore enrich the atmosphere under still conditions. However, still conditions also result in a climatic inversion, i.e., cold air forms at the surface overlain by warm air. Pumping air into the plot brings the warm air to the surface thus disrupting the inversion (168). Enrichment can be achieved under still conditions, but only by significantly altering the microclimate as, for example, in OTCs operated under still conditions. The system described by Miglietta et al. (138) does not predilute CO_2 but releases pure CO_2 at supersonic velocity through minute nozzles into the wind. The energy of these turbulent jets generates a predilution of the CO_2 before the wind carries it back over the treatment plot. This system depends completely on some air movement and cannot operate under perfectly still conditions (128).

WHAT HAVE WE LEARNED FROM FACE? A META-ANALYSIS

In contrast to many chamber experiments, the FACE experiments have either treated plants for their entire life cycle, with annual and short-lived perennials, or used multiyear treatment, as with long-lived perennial systems. The database is therefore limited to plants or tissues that have developed entirely under elevated [CO_2]. Although chamber studies have used a wide range of elevated [CO_2], averaging 700 μmol mol^{-1}, FACE studies (Table 1) have used an elevation of 550–600 μmol mol^{-1}. This aids comparison across FACE experiments and provides experiments with more immediate relevance, given that these atmospheric concentrations will likely occur mid-century (169). The liability of these lower elevations is that important changes that are small in magnitude might not be resolved with this lower treatment concentration, particularly considering the relatively low replication level in FACE experiments ($n = 2$–4; Table 1). In the preceding sections we identified questions that might be answered by FACE. The following section quantitatively reviews the results of the FACE experiments using meta-analysis to answer these questions.

FACE Meta-Analysis: The Procedure

The experiments compiled in Table 1 cover diverse plants and systems, different durations of experiment, and different levels of replication and statistical power. Answering the questions ideally requires a quantitative measure of response derived from these studies. Meta-analytic techniques have been developed for quantitatively integrating research results from independent experiments (72), and have

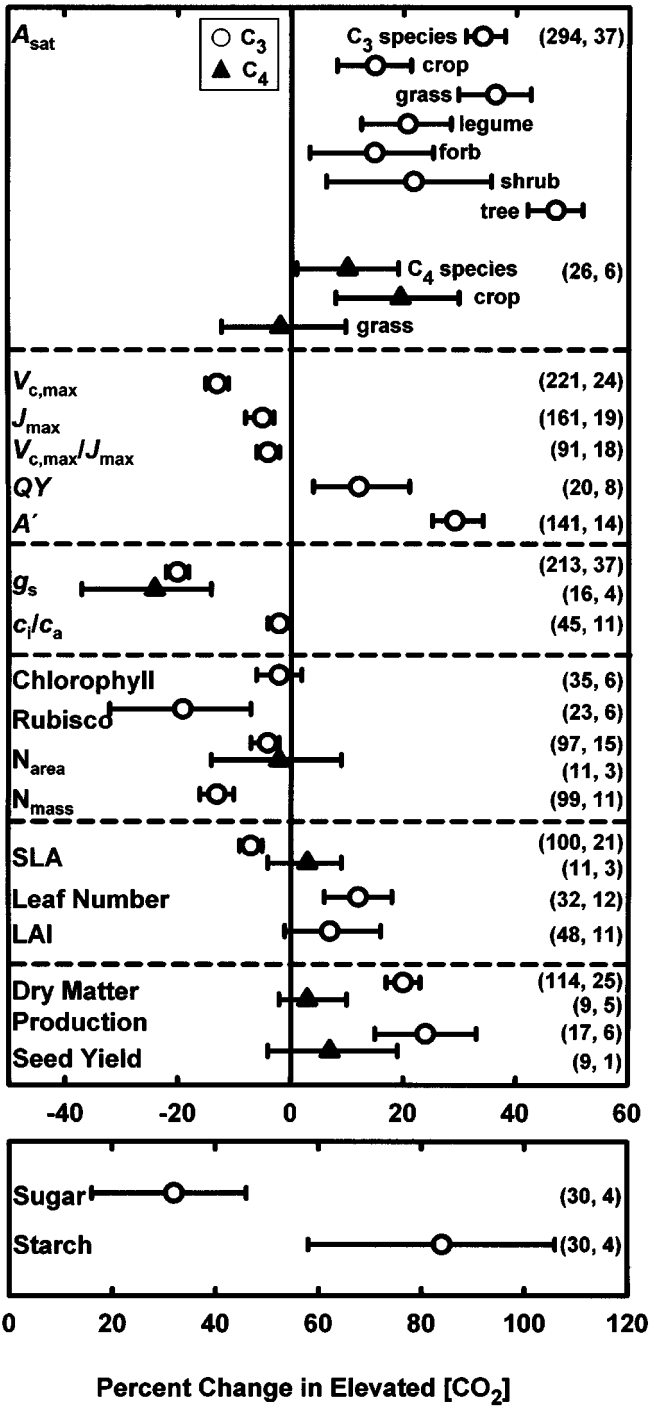
been widely adapted to summarize the effects of elevated [CO_2] on vegetation (2, 3, 28, 30, 97, 129, 131). The method provides a means to quantitatively review the literature. Particularly valuable in this context is the use of the response ratio, i.e., the ratio of values for a measure, such as photosynthetic rate, at elevated [CO_2] compared to ambient [CO_2]. Comparing the response ratio eliminates the problem that results from different absolute rates, e.g., when slow- and fast-growing species are compared. For this review, responses of different species, cultivars, stress treatments, and responses from different years of the FACE experiments are considered independent and suited to meta analysis. Thus, one FACE experiment examining a number of species in a multifactorial design could contribute multiple observations to a given response variable (30).

Literature searches of primary FACE research in published peer-reviewed journal sources were conducted with the *Current Contents* citation index and the *Web of Science* citation database (ISI, Philadelphia, PA). Data from 93 manuscripts that analyzed more than 40 species from 12 FACE sites (Table 1) were extracted for the analysis of gas exchange, leaf chemistry, leaf area, and yield variables (Figure 6). Response means of variables, standard deviations, and sample sizes from elevated and ambient [CO_2] treatments were either taken from tables, digitized from figures using digitizing software, or obtained directly from the authors of the primary studies, as described previously (2).

The natural log of the response ratio (r = response in elevated [CO_2]/response in ambient [CO_2]) was used as the metric for analyses (71, 182), and is reported as the mean percent change ($[r - 1] \times 100$) at elevated [CO_2]. The meta-analysis procedure followed the techniques described previously (30), using the statistical software MetaWin (182). A mixed-model analysis was used based on the assumption of random variation in effect sizes among FACE studies. A weighted parametric analysis was used and each individual observation of response was weighted by the reciprocal of the mixed-model variance, which is the sum of the natural log of the response ratio and the pooled within-class variance (71). If a 95% confidence interval did not overlap with zero, the response to elevated [CO_2] was considered significant.

What Are the Average Responses of Plants in FACE?

Figure 6 summarizes the percent change of growth at elevated [CO_2] of a range of physiological and biochemical plant variables averaged across all published FACE studies, as of August 2003, in ISI listed journals or in preprints of articles accepted for publication in these journals to which we were given access. Where the mean and lower confidence limits exceed zero, the variable is overall significantly larger in plants grown at elevated [CO_2], e.g., seed production in C_3 plants. Where the mean and upper confidence limits are less than zero, there is an overall significant decrease in plants grown at elevated [CO_2], e.g., stomatal conductance (g_s). Because sugars and starch show such large increases overall, they are plotted on a separate scale at the base of Figure 6. This summary shows highly significant



sustained increases in photosynthesis (A') integrated over the day of over 30%, with similar increases in light-saturated photosynthetic rate (A_{sat}). Compared to the 53% average increase across 50 greenhouse and OTC studies summarized by Curtis & Wang (30), this may in part be explained by the lower mean elevation of $[CO_2]$ used in the FACE studies, ca. 700 vs 570 μmol mol^{-1}. Nevertheless, because the response of A to $[CO_2]$ is nonlinear (Figure 1) the comparison suggests that the increase is less than may have been expected from chamber studies. As noted previously (128), this could also reflect the fact that alteration of microclimate by OTCs could exaggerate the effect of elevated $[CO_2]$ on photosynthesis. Overall production is increased by ca. 20% in C_3 plants with a similar increase in seed production, compared to 32% in a meta-analysis of greenhouse and OTC studies (30). LAI does not significantly increase, suggesting that increased production results from increased photosynthesis per unit leaf area, rather than increased assimilatory area (cf. Figure 1). These increases in photosynthesis and production occur despite a near doubling of leaf starch content, suggesting an imbalance in source versus sink activity and corresponding highly significant decreases in Rubisco content and stomatal conductance. This increase in starch content exceeds that observed in a summary of mainly chamber studies (30) even though the mean elevation of $[CO_2]$ in the FACE studies was about 50% of the mean level used in the chamber studies. One explanation might be that FACE has no effect on the light reaching the vegetation; plants in chamber studies, even in the field (128), generally receive less light and therefore there is less probability that photosynthesis will exceed capacity to remove carbohydrate from the leaf. Summarizing the FACE experiments as a whole, the results provide the best evidence yet that the elevation of $[CO_2]$ predicted for mid-century will result in a substantial increase in vegetative and reproductive production, decreased transpiration, and decreased tissue quality, with respect to protein and N content of leaves (Figure 6). There are significant differences

Figure 6 Meta-analysis. The percent change with growth at elevated $[CO_2]$ of light-saturated CO_2 assimilation (A_{sat}), maximum carboxylation rate ($V_{c,max}$), maximum rate of electron transport (J_{max}), the ratio of $V_{c,max}$: J_{max}, maximum apparent quantum yield of CO_2 uptake (QY), daily integral of leaf CO_2 uptake (A'), stomatal conductance (g_s), ratio of intercellular (c_i) to atmospheric $[CO_2]$ (c_a), leaf chlorophyll content per unit leaf area (chlorophyll), Rubisco content per unit leaf area (Rubisco), leaf N content per unit leaf area (N_{area}), and per unit leaf dry mass (N_{mass}), specific leaf area (SLA), leaf number, leaf area index (LAI), dry matter production, seed yield, leaf sugar content expressed on an area basis (sugar), and leaf starch content expressed on an area basis (starch). Symbols represent the percent change at elevated $[CO_2]$ and their 95% confidence intervals. Sample size (n), followed by the number of species included for each variable appear in parentheses after the symbol. (1, 3, 4, 10, 13–18, 23–26, 32, 35, 36, 38, 43, 44, 50, 51, 56, 59, 60, 62–64, 70, 75–87, 95, 96, 98, 99, 102, 110, 113, 122, 126, 127, 135, 137, 144, 147, 148, 150–153, 156, 157, 160–163, 166, 167, 171, 172, 174–178, 187, 190, 191, 197–199, 201, 203–205, 212, 215, 218–221).

in the response of C_4 and C_3 species, and among C_3 functional types (Figure 6), which suggests that the elevation of $[CO_2]$ will, and likely already is, altering competitive balance within plant communities. Overall increases in production and photosynthesis in FACE are broadly similar to projections from chamber studies. However, there are significant differences in the apparent response of leaf chemistry (cf. 30). Most importantly, only a small increase in LAI is indicated, and this is not statistically significant. Many current models of global vegetation response to rising $[CO_2]$ assume an increase LAI (reviewed in 27). This is not supported by the FACE studies (Figure 6) and consequently suggests these models may overestimate future evapotranspiration and photosynthetic carbon uptake at the landscape level.

Downregulation: Does It Occur?

As noted above, analysis of studies prior to FACE show that downregulation of photosynthesis at elevated $[CO_2]$ is often attributable to rooting volume limitation or nutrient limitations, thus leaving open the question of whether it would occur under field conditions. Downregulation typically involves a decrease in the amount and/or activity of Rubisco (40, 183, 194). Rubisco content expressed on an area basis decreased on average by a highly significant 19%, which could account for the decrease in N content in leaves grown under FACE (4% when expressed on an area basis; Figure 6). $V_{c,max}$, which provides an in vivo measure of Rubisco activity,was also reduced by 13% with growth in FACE, whereas J_{max}, which provides an in vivo measure of RuBP-regeneration capacity, was reduced only slightly by 5%. The smaller decrease in $V_{c,max}$ compared to Rubisco content suggests that Rubisco activation levels may be higher on average in the plants grown at elevated $[CO_2]$. This contrasts with prior summaries of chamber studies that found that decreased $V_{c,max}$ was a result of decreases in both the amount and activity of Rubisco (40). The results provide clear evidence that Rubisco loss is not just a feature of plants grown in chambers or pots.

However, does this loss of Rubisco mean that A is lower in plants grown and measured at elevated $[CO_2]$? Figure 1 explains why a loss of Rubisco may have no effect on A_{sat} when measured at an elevated growth $[CO_2]$. Figure 6 shows that on average A_{sat} increased by 34% in C_3 species; the average temperature at which these measurements were made was 26.6°C. Figure 3 shows that according to the theoretical increase at this temperature based on Rubisco catalyzed reaction kinetics should be within the range of 14% to 44%, depending on whether RuBP regeneration or Rubisco activity limit A_{sat}, and assuming that c_i/c_a remains constant with $[CO_2]$ elevation, i.e., no stomatal acclimation. If we assume some colimitation between these two capacities, then the 34% increase observed across FACE studies is strong evidence that no or very little loss of stimulation of photosynthesis has occurred despite significant loss of Rubisco.

In field crops and natural vegetation, total carbon uptake is not simply a function of light-saturated photosynthesis, but also of light-limited photosynthesis, which

may account for up to 50% of canopy carbon uptake. The initial slope of the response of A to photon flux (Q) is the maximum quantum yield of CO_2 uptake (QY) and the phase of photosynthesis that is exclusively light limited. Light-limited photosynthesis is determined by the rate of regeneration of RuBP, and will increase as $[CO_2]$ increases because less ATP and NADPH is diverted into photorespiratory metabolism, and therefore more is available for CO_2 uptake. The theoretical enhancement of QY by elevation of $[CO_2]$ from 372–550 μmol mol^{-1} is given by the lower line in Figure 3, which predicts a 14% increase at the mean temperature of measurements in the FACE experiments. Some previous chamber experiments have shown that increases in QY with growth at elevated $[CO_2]$ coincide almost exactly with theoretical expectation (119, 159); others have revealed a significant decrease in some species, implying significant downregulation of QY (20, 208). Across the FACE experiments, the observed increase is 13% and very close to theoretical expectation in the absence of downregulation. In contrast to findings in controlled environments and glasshouses (20), there is no evidence of downregulation of QY in the FACE studies.

As noted above, chamber studies have suggested that a decline in Rubisco reflects an overall decline in leaf N and protein content, implying that downregulation is part of a general decrease in investment in proteins under elevated $[CO_2]$. This is consistent with a summary of chamber studies that found an average decrease in leaf N, total protein, and Rubisco of 17%, 14%, and 15%, respectively (40). The summary of the FACE studies (Figure 6) provides a different picture: a 20% decrease in Rubisco, but just a 4% decrease in N per unit leaf area. The large decrease in Rubisco is substantiated by a parallel decrease in $V_{c,max}$ based on 221 independent sets of measurements. If we assume that Rubisco constitutes about 20% to 25% of leaf N, then decreased Rubisco could account for nearly all of the decrease in leaf N per unit area. However, there are many more observations of N than Rubisco, and not all of the studies of Rubisco report N content. The overall findings of FACE therefore suggest that Rubisco loss is a selective change. As observed in previous meta-analyses (2, 30, 209), growth at elevated $[CO_2]$ leads to large and significant increases in foliar carbohydrate content (Figure 6), indicative of a source-sink imbalance and consistent with feedback control of Rubisco content (Figure 4). No loss of stimulation of A_{sat} or QY at elevated $[CO_2]$ suggests that the changes that do occur should be regarded as acclimation [in the sense that they appear to fit the plant to the elevated $[CO_2]$ growth conditions (see Figure 1)] rather than downregulation. Lower Rubisco levels without loss of stimulation of photosynthesis by elevated $[CO_2]$ may be explained if the levels found in plants grown at current ambient $[CO_2]$ are in excess at elevated $[CO_2]$. The mean decrease in Rubisco may simply be eliminating part of this excess. Further evidence of a selective loss of Rubisco is provided by the statistically significant decrease in $V_{c,max}/J_{max}$. This is also reflected in changes in amounts of proteins within specific experiments. In ryegrass, a significant decrease in Rubisco occurred when levels of chloroplastic fructose-1:6-bisphosphatase (FBPase) and sedoheptulose-1:7-bisphosphatase (SBPase) (178) remained unchanged.

Both enzymes may control RuBP regeneration and therefore J_{max} (67, 170). In wheat, the ratio of the amount of Rubisco to chlorophyll proteins declined more rapidly at elevated than ambient [CO_2] (150). Similar changes were observed in some chamber experiments (53). If decrease in Rubisco simply reflected a general loss of leaf N, this ratio would not be affected. Specific evidence that decline in Rubisco within FACE is an acclimatory response, in the sense that it better fits the plant to the changed environment, comes from the ETH FACE experiment with ryegrass in Switzerland. There was little loss of Rubisco at elevated [CO_2] in ryegrass grown with a high N supply, but there was a significant loss at low N supply (178). However, the enhancement of A by elevated [CO_2] was the same in both N treatments (3). The findings imply that N is not sequestered into Rubisco that would otherwise be in excess at elevated [CO_2] when growth is strongly N limited, but that the decrease in Rubisco is insufficient to remove any enhancement of A by elevated [CO_2].

Saxe et al. (184) concluded that downregulation of photosynthetic capacity was mostly associated with stressed plants, at least for trees. Our analysis revealed a significant difference in the way stressed plants or plants grown under low N fertilization conditions respond to FACE ($Q_B = 23.41$, $P < 0.005$). Plants grown under "unstressed conditions" showed a 9% reduction in $V_{c,max}$, whereas those grown under low N showed a 22% reduction in $V_{c,max}$. Is this a direct response to N supply or an indirect response through low N, limiting sink capacity for additional photosynthate and leading to the feedbacks illustrated in Figure 4. The FACE experiment with ryegrass (Table 1) provides one answer. In the low N treatment there was a highly significant decrease in Rubisco and $V_{c,max}$. However, when the crop was partially defoliated, levels returned close to those seen at ambient [CO_2]. This implies that the response to low N is indirect, via sink/source balance, and when partial defoliation decreases source activity, Rubisco levels are restored (178). In summary, and by reference to Figure 4, the treatise of FACE studies suggests that feedback of sucrose cycling on Rubisco content does not cause a decrease in photosynthesis, but acts to increase the efficiency of N use by the plant.

Acclimation of $V_{c,max}$ in wheat grown under FACE conditions in Maricopa, Arizona, depended primarily on leaf position, leaf age, and crop development (1, 149, 150, 160), and secondarily on N fertilization level (1). Acclimation did not occur in the flag leaf of wheat, but in older shaded leaves (160), and was exacerbated by low N fertilization (1). The meta-analysis of all FACE studies also supports the claim that the leaf environment affects acclimation ($Q_B = 34.63$, $P < 0.01$). Sun leaves or upper canopy leaves did not show any change in $V_{c,max}$ with growth under FACE conditions whereas $V_{c,max}$ was reduced by 10% in plants growing in lower levels of the canopy.

Körner (105) suggested that the most important criterion by which data should be grouped in meta-analysis of elevated [CO_2] is plant age. Meta-analysis revealed a trend toward acclimation in old leaves but not in young leaves; however, the result was not statistically significant (confidence intervals overlapped with 1). Evidence from *Pinus taeda* grown at the FACE experiment in Duke Forest showed that age

of needles is an important factor in predicting acclimation (176). Rubisco activity and levels of the large subunit of Rubisco were 25% to 35% lower in FACE-grown needles over one year old, but unaffected in young needles (176). Similarly inferred loss of Rubisco from gas exchange measurements increased with age of both a perennial ryegrass and spring wheat crop (3, 160). N fertilization level, developmental stage, leaf age, and canopy position all affect the sink activity of plants, and therefore will likely affect the long-term response of Rubisco content to growth at elevated [CO_2].

SUSTAINED INCREASE IN PHOTOSYNTHESIS? Models have often projected that the initial stimulation of photosynthesis observed on elevation of [CO_2] would be lost in the longer term not only because of feedback within the plant, but also because of feedback within the ecosystem where increased plant production would cause sequestration of nutrients in litter and soil organic matter (121). This system-level limitation is consistent with the apparent loss of stimulation of arctic sedge tundra after three years of treatment in chamber studies (158). Luo & Reynolds (121) projected that these feedbacks would result in a loss of stimulation within about six years in grassland systems and considerably longer in forest systems. The FACE experiment with ryegrass in Switzerland ran for 10 years, and provides perhaps the best dataset to test these model projections. There was no evidence of a decline in the stimulation of *A* with duration of the experiment, either in the high or low N treatment; stimulation in 2002 was almost identical to that observed when the swards were first established in 1993 (3). As noted above, in the FACE experiments in general, the average increase in A_{sat} is close to theoretical expectation, implying that over their present duration there is no evidence that stimulation is transitory. However, the surprising lack of any significant increase in LAI across the FACE experiments might suggest that system feedbacks are at the level of the amount of leaf area produced, rather than the assimilatory capacity of that leaf area.

Is C_4 Photosynthesis Increased?

On average, C_4 photosynthesis increased by 10% and stomatal conductance decreased by 24% with growth in FACE (Figure 6). The increase reported in this analysis is much lower than the 25% increase in C_4 photosynthesis reported for wild C_4 Poaceae species (207); this is partially explained by the smaller elevation of [CO_2] in the FACE studies. Our results further differ from those of previous studies (207, 222) in that the stimulation in photosynthesis was only significant for C_4 crop species [*Sorghum bicolor* (L.) Moench and *Zea mays* (L.)] and not for the wild C_4 grassland species [*Andropogon gerardii* Vit., *Schizachyrium scoparium* (Mich.) Nash, *Sorghastrum nutans* (L.) Nash] (Figure 6). All wild C_4 grassland species in this meta-analysis were grown on the nutrient-poor, sandy soils at the Cedar Creek Natural History Area in east-central Minnesota, conditions that might be expected to favor a response to increased [CO_2] were PEP carboxylase levels lowered by nutrient deficiency to a level that it became limiting at present ambient

[CO_2]. This finding is consistent with the theoretical analysis of Ghannoum et al. (55), which concluded that bundle sheath leakiness, direct CO_2 fixation in the bundle sheath, or the presence of C_3-like photosynthesis in young C_4 leaves are unlikely explanations for the high CO_2 responsiveness of C_4 photosynthesis. Stimulation of C_4 photosynthesis in maize was associated with greater intercellular [CO_2], lower stomatal conductance and transpiration, and corresponded to transient drought events, but was absent following periods of heavy rainfall (110).

Previous chamber studies of elevated [CO_2] effects show a 49% decrease in phospho-enol pyruvate carboxylase (PEPC) in sorghum (213) and a 23% decrease in carboxylation efficiency in maize (124), implying a loss of Rubisco activity in vivo and a decrease in in vivo PEPc activity. Watling et al. (213) also found a significant decrease of about 12% in CO_2 saturated photosynthesis. By contrast, there was no significant change in PEPc or Rubisco in mature, FACE-grown sorghum leaves (206). However, photosynthesis and production were strongly enhanced by drought in this study. The variation in response to elevated [CO_2] is probably attributed to differences in chamber versus field-growth conditions, which may include the lower increase in [CO_2] and much higher photon flux of the field studies. To date, the FACE studies provide no evidence of the photosynthetic acclimation observed in chamber studies of C_4 species, but are consistent with the hypothesis that increased photosynthesis and production result from conservation of soil moisture due to decreased stomatal conductance by a highly significant 24% in the FACE studies (Figure 6).

Is There an Independent Acclimation of Stomatal Function?

Stomatal conductance decreased on average by 20% for C_3 plants grown in elevated [CO_2] in FACE, encompassing more than 200 independent measurements (Figure 6). This decrease is consistent with the reduction in g_s reported for 28 species with growth at elevated [CO_2] (40). Stomatal sensitivity to elevated [CO_2] is not lost over time with growth in FACE. In experiments with perennial plants, the decrease in g_s was the same in the first year of the experiment as in subsequent years (3, 59, 113, 153, 219). The theoretical stimulation of photosynthesis in the absence of any change in the activity of Rubisco and capacity for regeneration of RuBP (Figure 3) against which we have compared actual stimulations assume that c_i/c_a is unchanged. This only occurs if the decrease in stomatal conductance exactly balances the increase in external [CO_2] and increase in A. Remarkably, across 45 independent measurements in FACE, c_i/c_a remained unchanged. Although a small (3%) decrease was indicated, this was not statistically significant (Figure 6). Actual mean c_i/c_a was 0.72 in control and 0.70 at elevated [CO_2] in these FACE studies. This agrees closely with the conclusions of an earlier summary of chamber studies (40), which found no effect of growth at elevated [CO_2] on c_i/c_a. As noted above, there was no change in LAI with growth under FACE conditions (Figure 6). Thus, reduced g_s should lead to reduced stand evapotranspiration and increased soil water content.

Does Increased Photosynthesis Translate into Increased Production?

The increase in light-saturated photosynthesis was 34% for C_3 plants grown under FACE conditions. Daily carbon uptake was stimulated slightly less (by 29%), whereas dry matter production and seed yield were increased to an even lesser extent (20% and 24%, respectively). This trend, that the yield response is less than the photosynthetic response, is consistent with a meta-analysis of more than 100 chamber studies of soybeans grown at elevated [CO_2] (2), and modeled for a number of cereals and legumes (58). Sorghum was the only C_4 species for which seed yield information was available for our analysis. The average increase in *S. bicolor* photosynthesis was 21% (24, 25, 218), but the average 7% increase in seed yield was not significant (23, 38, 161).

CONCLUSION

FACE was developed as a means to grow plants in the field at a controlled elevation of [CO_2] under fully open-air conditions. FACE studies now provide our most realistic estimates of how plants in their native environments will respond to the atmospheric [CO_2] predicted for the middle of this century, and our best validation data for models predicting the responses of natural vegetation and crops to this ongoing change. Predictions, from earlier enclosure studies, that stimulation of photosynthesis and production would be transient have not been borne out in FACE. Given that the longest running FACE experiment was 10 years, that prediction cannot be ruled out, particularly for long-lived species. A quantitative meta-analytic summary of the 93 peer-reviewed publications reporting plant responses in FACE show trends that agree with parallel summaries of enclosure studies; however, important quantitative differences emerge. Averaged across these studies, light-saturated C_3 photosynthesis increased by 34% and production by 20%, somewhat less than forecast by enclosure studies. Also in contrast, LAI is not significantly increased, with very important implications for projecting the response of future vegetation to predicted increase in [CO_2]. In common with many enclosure studies, Rubisco content was decreased by about 20%, but in contrast there was little change in capacity for Ribulose-1,5-bisphosphate regeneration and little or no effect on photosynthetic rate at elevated [CO_2]. Also in contrast to enclosure studies, the loss of Rubisco cannot be explained as the result of an overall decline in leaf N, but instead appears specific and accounts for most of the decrease in N per unit of leaf area. These results suggest that loss of Rubisco in FACE is more appropriately described as an acclimatory change benefiting N use efficiency rather than as downregulation. Both genetic and experimental modifications of source-sink balance in FACE provide results consistent with current models of carbohydrate feedback on Rubisco expression. Unlike in chamber studies, there is no evidence of acclimation in C_4 species, and increases in photosynthesis and production are

consistent with the hypothesis that this results from improved water use, because stomatal conductance is decreased on average by 20%. The findings have important implications both for predicting the future terrestrial biosphere and understanding how crops may need to be adapted to the changed and changing atmosphere.

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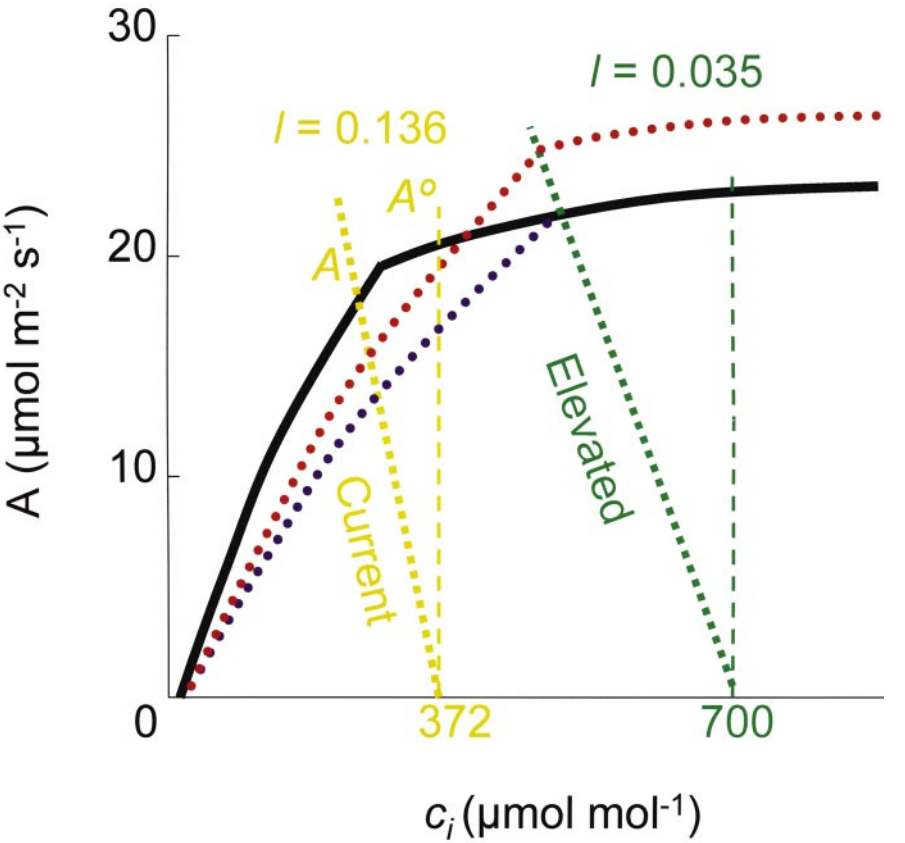

Figure 1 The response of leaf CO_2 uptake (A) to intercellular [CO_2] (c_i), i.e., demand function, as predicted from the leaf biochemical model of photosynthesis of Farquhar et al. (46). The dotted black lines illustrate the decline in c_i that occurs with increasing A at a constant g_s, i.e., the supply function. This is illustrated both for the current ambient and a future elevated CO_2 concentration. The vertical dashed lines show the supply function if stomatal conductance is assumed infinite. The dotted blue line illustrates how the demand function would alter if a 30% decrease in Rubisco activity occurred, and the dotted red line shows the demand function if a 15% decrease in Rubisco activity and 15% increase in RuBP regeneration capacity occur. l represents stomatal limit at the two CO_2 concentrations (see text for details).

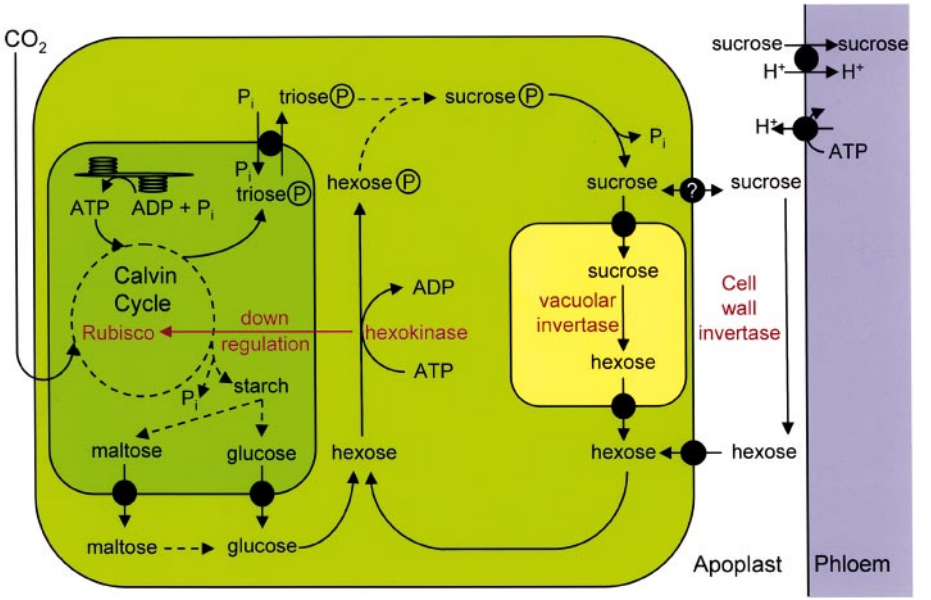

Figure 4 A diagrammatic representation of the hypotheses that seek to describe the mechanism underlying loss of photosynthetic capacity when sucrose accumulates in the mesophyll. Increased levels of sucrose accumulating in source leaves can potentially reduce photosynthetic capacity in both the long term, through downregulation of Rubisco and other photosynthetic genes (140), and in the short term, by reducing the capacity for ATP production within the chloroplast (66). Increased levels of sucrose are sensed via increased sucrose cycling through invertase (139) and perceived by a hexokinase sensing system (92). Nocturnal degradation of starch will also supply substrates for hexokinase. Depending on the species, the hexokinase-generated signal reduces Rubisco content by downregulating transcription of the*rbcs* family, translation of the mRNA, and/or affecting assembly of the holoenzyme. Components of the signal transduction pathway and the molecular control of Rubisco content were recently reviewed (140, 180, 192). Broken lines indicate multiple-step processes that have been simplified for clarity. The diagram was constructed by combining the models of Moore et al. (140), Bush (21), and Weise et al. (217).

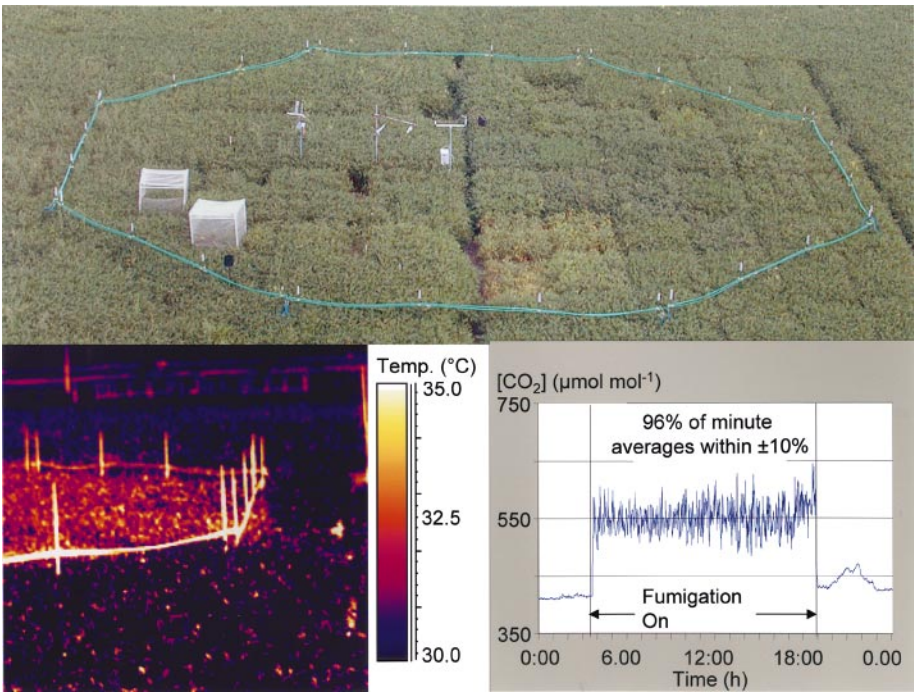

Figure 5 One of 16 FACE rings within a soybean crop at the University of Illinois SoyFACE facility (5, 175). CO_2 is released into the wind under high pressure from nozzles in the green pipe. Release is always on the upwind side of the ring and the release rate governed by the wind speed and $[CO_2]$ is measured at the center of the ring. The lower right panel shows an example from a test ring of controlled elevation over a 16-h period. The lower left panel shows a thermal image of part of the ring shown above. Elevation of $[CO_2]$ decreases stomatal conductance and transpiration; consequently, the vegetation within the ring is significantly warmer. This image also provides graphical illustration that the elevation is relatively uniform (pictures couresy of Andrew Leakey, Tim Mies & Hans Bohnert).

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