Project title**:**	Cucumbers: The role of environmental and agronomic factors in carbon dioxide toxicity.
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The results and conclusions in this report are based on a single series of experiments. The conditions under which the experiment was carried out and the results have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

CONTENTS

PRACTICAL SECTION FOR GROWERS

Objectives and background

It has been demonstrated in commercial conditions and in small scale experiments that cucumber plants respond to carbon dioxide enrichment by showing increased productivity. Many growers though, have reported the development of phytotoxic symptoms on cucumbers evident as severe leaf bleaching, which has prevented wide scale uptake of CO_2 enrichment to levels used by UK glasshouse tomato growers.

This project will use the whole plant approach to determine the physiological and biochemical processes leading to damage. Through a combination of growth cabinet and glasshouse studies, it aims to provide cucumber growers with guidelines that will enable them to maximise the benefit obtained from CO_2 enrichment without risk of crop damage.

Summary of results

1. Symptom development

Studies both in the controlled environment cabinets (CEC) at Lancaster University and in the glasshouses at HRI Stockbridge House have confirmed the symptoms of carbon dioxide damage. The symptoms are expressed as inter-veinal leaf bleaching which is first seen around the edge of the leaves approximately 3 weeks after the introduction of a high (2000 ppm) CO_2 regime. If left exposed to high CO_2 enrichment levels the symptoms progress until the entire leaf area becomes bleached.

In the glasshouse studies the symptoms occurred initially on expanded leaves at the top of the plant canopy and along south facing walls indicating a possible interaction between leaf age and light levels.

2. Investigations of photosynthetic performance

Investigations into the photosynthetic performance of a crop grown at 350 ppm and 2000 ppm CO_2 were measured on whole plants grown in the CEC at Lancaster University. After 4 weeks in elevated (2000 ppm) CO_2 there was a change in the slope of the relationship between photosynthetic rate (A) and the CO_2 concentration in the intercellular spaces (C*i*). That is, the slope of the A/C*i* curve is reduced compared with that produced with plants grown in control (350 ppm) chambers.

Both carboxylation efficiency (the efficiency with which CO_2 is captured) and regeneration capacity of Ribulose bisphosphate (RuBP), the primary carbon acceptor, showed a decline in the 2000 ppm regime suggesting that there are significant direct effects of elevated CO_2 on photosynthetic metabolism. This photosynthetic down regulation at high CO_2 concentrations will be expected to limit predicted increases in CO_2 uptake.

Coincident with these physiological changes, total protein levels in the leaf, when expressed on a dry weight basis (mg/g dry wt.), showed a significant decline in the 2000 ppm CO_2 treatments. Taken together with direct assessment of RUBISCO levels (data not shown here) and other physiological data, this indicates a possible down regulation of the RUBISCO protein (Ribulose-1, 5-bisphoshphate carboxylase oxygenase) which is responsible for catalysing the reaction between CO_2 from the atmosphere with RuBP.

No significant starch accumulation in the leaves of plants grown in 2000 ppm CO_2 was found compared to those grown in 350 ppm CO_2. This suggests that starch accumulation may not be as critical component in the onset of symptom expression as is observed with some plant species.

Measurements of stomatal density taken on a 4 week old crop have revealed that the abaxial (lower) leaf surface has significantly less stomata per cm^2 in the upper and mid canopy layers when plants are enriched with 2000 ppm CO_2 compared to plants enriched with 350 ppm CO_2. This change in morphology will contribute to the limitation in stomatal conductance observed at high CO_2. These changes may result in significant stomatal limitation of photosynthesis when the CO_2 concentration falls as the glasshouse vents are opened in the April / May growing period. In addition a decreased stomatal conductance may contribute to photoinhibition injury on bright days. This can occur due to a decrease in the plants ability to

dissipate excess energy when photosynthesis is restricted. Also lower stomatal conductances could cause an increase in leaf temperature resulting in an increase in photorespiration. Changed stomatal differentiation may be accompanied by changes in xylem development and both of these changes will impact on plant water relations.

Action points for growers/Information points for growers

Please note that this report is based on the results of the first year of a three year project. The results must be regarded as preliminary at this stage and more robust guidance will become available as the project progresses.

- The results indicate a clear damaging effect of high (2000 ppm) concentrations of CO_2 on cucumber leaf structure and functioning.

Glasshouse studies

- Glasshouse studies indicate that damage can be induced using pure CO_2. Symptoms are expressed as inter-veinal leaf bleaching which is first seen around the edge of the leaves approximately 3 weeks after the introduction of a high (2000 ppm) CO_2 regime. If left exposed to high CO_2 enrichment levels the symptoms progress until the entire leaf area becomes bleached.

- The use of pure CO_2 in these experiments rules out the involvement of air pollutants (e.g. oxides of nitrogen) in symptom development

- The symptoms occur initially on expanded leaves at the top of the plant canopy and along south facing walls indicating a possible interaction between leaf age and light levels.

- Physiological measurements of plant functioning in the glasshouse are continuing and it is too early to suggest a clear hypothesis for symptom development but physiology of leaves is certainly affected by high CO_2 and we predict that yields will also be reduced as a result.

- Future investigations will focus on these issues.

Growth cabinet studies

- Leaf damage is not a result of excessive starch accumulation as reported for other plant species.

- Initial results suggest a damaging effect on CO_2 photosynthetic metabolism.

- The current hypothesis to explain reductions in photosynthesis promoted by high (2000 ppm) CO_2 is that photosynthetic down regulation is combined with damage to the light harvesting and reaction centres of the leaf, a combination of responses that will certainly affect carbon accumulation and yield.

Potential and financial benefits

Optimal control of CO_2 enrichment of tomato crops is estimated to increase grower net income by £5 m^2. The value of cucumber crops could be expected to rise from £35 m^2 to 41 m^2 and a industry benefit of £11 million per annum.

SCIENCE SECTION

Introduction

In the UK there are approximately 190 ha of glasshouses devoted to cucumber production with an estimated value of £45 million per annum. Crop production is intensive and technically advanced. Growers have the capability to control and manipulate the aerial environment in terms of temperature, humidity and CO_2 enrichment.

CO_2 enrichment with set points of 800 to 1000 ppm has become standard practice for the winter period when glasshouse ventilation is restricted. In the past computer set points for the summer period have been reduced due to the economics of CO_2 generation. However with the installation of Combined Heat and Power (CHP) Units growers are now able to time shift the heat burn to produce plentiful quantities of CO_2 during the summer months. The heat generated during this process is stored in hot water tanks for use in the glasshouse at night.

It is well established that CO_2 enrichment will provide benefits in terms of increased photosynthesis and greater allocation of biomass to fruits (Nederhoff, 1994) but no relationship between enrichment level and potential yield at high summer CO_2 levels has been defined (tomatoes or cucumbers). In addition, at high CO_2 concentrations a phytotoxic reaction (severe leaf bleaching) has been observed. It is therefore necessary to devise optimal distribution and control systems for cucumber production that will enable growers to use the gas to its full potential without risk of crop damage. Before this can be achieved the conditions under which phytotoxicity occurs must be identified.

The overall aim of this project is to identify those environmental and agronomic factors that are associated with carbon dioxide toxicity and to provide initial guidelines that will enable cucumber growers to maximise the benefit obtained from enrichment without risk of crop damage and yield loss.

A scientific and detailed literature review of the likely physiological and biochemical processes leading to crop damage is provided in Appendix 1.

MATERIALS AND METHODS

Investigation protocol

The work is reported in two parts. Part I, which was conducted during the first 3 months of the project was aimed at establishing CO_2 damage in a glasshouse crop grown at HRI Stockbridge House by enriching to 2000 ppm CO_2 once a crop canopy had been established. Gas exchange and chlorophyll fluorescence measurements were carried out during this period to assess the changes in photosynthetic performance during the onset of leaf damage.

Part II consists of the work carried out in the controlled environment cabinets (CEC) at Lancaster University. In these studies CO_2 concentrations of 350 ppm and 2000 ppm were used to ensure that the damage symptoms induced were comparable to those seen within the glasshouse trials detailed in Part I. Using gas exchange analysis, photosynthetic rate was measured and A/C*i* analysis was performed to characterise any acclimatory changes by the plants over a 4-week growth period. Analysis of starch and total leaf protein was undertaken to gain an impression of the plants ability to accumulate starch under high CO_2 conditions and to measure the possible effects of down regulation, since RUBISCO is the most abundant protein in plants. Histological work and growth analysis was used to confirm the occurrence of morphological changes as a result of growth in 2000 ppm CO_2.

Part I: Establishment of CO_2 damage in glasshouse cucumber crops.

HRI Stockbridge House

Crop details

A cucumber crop cv Sabrina was sown on 10 December 1998 and planted on 5 January 1999 into a modern, well sealed 200 m^2 4.2 m high Venlo glasshouse. During propagation the plants were exposed to 1000 ppm CO_2 and supplementary lighting was provided using 400 W high-pressure sodium lamps. The crop was grown on the V-system at 1.4 plants m^{-2} according to good commercial practice.

CO_2 regimes

Pure CO_2 was used for all enrichment strategies such that damage could be attributed to CO_2 toxicity and not NO*x*. Immediately following planting a CO_2 regime of 600 ppm rising 200 ppm on light was set in the environmental computer. CO_2 enrichment strategies were changed with crop growth and once a full canopy had developed high levels were targeted to induce damage symptoms (Table 1).

Table 1. CO_2 set points and crop growth stage.

Regime	Date	CO_2 set points (ppm)	Crop growth stage
1	5 January	600 + 200 on light	(trans)planting
2	2 March	2000 + 400 on light	Full canopy
3	9 April	800 + 200 on light	Completion of study by Lancaster University

Crop measurements

During the growth of the crop 4 visits were made by Lancaster University, namely 12 January to establish base line data. Subsequent visits were then made after 1 day, 10 days and 30 days growth post 2000 ppm set point (Table 1). During each visit gas exchange studies (see page 8) were carried out and A/C*i* curves for the crop were constructed. On each visit a different leaf was monitored. A Plant Efficiency Analyser (PEA, Hansatech Instruments, UK) was also used to measure fv/fm values. This measurement provides the ratio of variable to maximum fluorescence and enables the health of the light harvesting centres within the plants to be monitored (see page 10). The data was used to assess the changes in photosynthetic performance during the onset of leaf damage, which is visualised as leaf bleaching.

HRI Stockbridge House collected data relating to the aerial environmental within the glasshouse and crop yield data.

Part II: Investigation of CO_2 damage in cucumber plants grown under ambient and elevated CO_2 regimes in controlled environment cabinets.

Lancaster University

Crop details

Seeds of the cv Sabrina were supplied by HRI Stockbridge House. The crop was grown in rockwool fed with a hydroponic nutrient solution using a standard commercial feed recipe supplied by HRI Stockbridge House. Plants were propagated under ambient (350 ppm) CO_2 conditions prior to transfer at the 3-leaf stage to the CEC cabinets where they were grown for a period of 5 weeks. Following transfer from the propagation unit the plants were allowed to establish for 1 week prior to introduction of the experimental treatments. Two CO_2 enrichment strategies were adopted, namely 350 ppm and 2000 ppm. To maintain a constant light intensity throughout the growth of the crop artificial lights were used (Table 2).

Table 2. Incident PAR (μmol m^{-2} s^{-1}) measured at various heights within the CEC cabinets.

Cabinet height	Incident PAR (μmol m-2 s-1)
0 cm	650
25 cm	750
50 cm	1020

Controlled environment cabinets

The experimental facility consisted of four 105 cm x 180 cm CEC cabinets. A central cooling unit was used to maintain the temperature within the desired set points to each cabinet. The humidity was controlled using Vapac humidifiers, controlled via a Quick Basic computer programme. The same computer programme was also used to control the lights and to provide graphical output of the actual environmental conditions achieved. For CO_2 enrichment pure CO_2 was used for all experiments.

Plant Assays

Within the CEC studies various physiological plant assays were conducted namely;

1. Gas analysis to determine plant assimilation rates under low and high CO_2 regimes.
2. Leaf starch and protein assays to determine the accumulation potential of leaf starch and levels of the RUBISCO enzyme respectively under low and high CO_2 regimes.

Also various histological studies were conducted namely;

3. Growth analyses to determine plant dry weights under low and high CO_2 regimes.
4. Measurements of stomatal density under low and high CO_2 regimes.
5. Fluorescence microscopy to determine quantity of starch grains in the leaf under low and high CO_2 regimes.
6. Chlorophyll fluorescence to provide indication of plant health under low and high CO_2 regimes.

1. Gas exchange studies

Gas exchange measurements were taken at weekly intervals during the first 3 weeks following plant transfer to the CEC using a portable gas exchange unit (Ciras-1, PP Systems, Hitchin UK). Using this equipment the assimilation rate of the plant was established by measuring the difference in CO_2 concentration in the air before and after it had passed through the leaf. The difference in CO_2 concentration in the air-flow is determined using infra-red gas analysers. Ciras-1 then calculates the intercellular CO_2 concentration (C*i*). Using this equipment the effect of the stomata is removed and the metabolic activity of the plant can be investigated using an A/C*i* curve (Figure 1) where A = assimilation rate ($\mu mol\ m^{-2}\ s^{-1}$). The initial slope of this curve describes the carboxylation efficiency of the plant that is how rapidly RUBISCO catalyses the reaction between the 5-carbon sugar RuBP and CO_2 at the start of the Calvin cycle and how rapidly RuBP is saturated. Thereafter the rate becomes dependent on the regeneration of RuBP and is an indication of the maximum rate of assimilation (Figures 1a and 1b).

Figures 1a and 1b

2. Starch and protein assays

For both starch and protein assays 1 cm leaf discs were taken from 3 randomly selected plants within the ambient (350 ppm) and high (2000 ppm) CO_2 CEC at 9 a.m. when the lights had reached maximum intensity. Since symptoms of CO_2 damage are evident on older leaves a comparison of upper and middle canopy leaves was made.

2.1 Starch assay

The discs were immediately frozen in liquid nitrogen and stored at –18ºC prior to analysis. For determination of the starch content each sample of 2 discs were subsequently ground in 4 cm^3 of ice-cold citrate buffer (pH 5) using a chilled pestle and mortar. A further 6 cm^3 of citrate buffer was then added and the extracts were centrifuged at 3000 rpm for 10 minutes. 3 ml of solution was then taken and boiled for 5 minutes. After which time 0.2 cm^3 of iodine solution (1g iodine, 2 g potassium iodide, in 300 ml distilled water) was added to each tube and mixed thoroughly. A further 4.7 cm^3 distilled water was then added and the absorbance was read using a linear spectrophotometer (Cecil Instruments, Series 2, Cambridge UK) at 600 nm and compared to a 0.2 gl^{-1} soluble starch stock solution.

2.2. Protein assay

Samples were extracted in 1 cm^3 of 0.1 M potassium phosphate / 1 mM EDTA buffer (pH 7.5) and spun for 5 minutes at 13,000 rpm in a microfuge. The samples were then stored at –18ºC. 3 cm^3 of stock solution (100 mg Coomassie blue G250, 50 cm^3 ethanol, 100 cm^3 Phosphoric acid-made up to 1 litre and filtered before use) was then added to a plastic disposable cuvette. 20 µl of plant sample was added and mixed by inversion. The subsequent solution was left for 2 minutes to allow development of a blue colour and the absorbance at 595 nm was measured using a spectrophotometer. The data was compared to a standard curve of Bovine Serum Albumin (Bradford, 1976).

3. Growth analysis

After 5 weeks growth in the CEC the total dry weight and leaf area of a selected crop was measured from the 350 ppm CO_2 and 2000 ppm CO_2 treatments. This allowed biomass in each treatment to be expressed on a specific weight (g cm^{-2} leaf) basis.

4. Stomatal density

Leaf samples were taken from selected plants growing in the 350 ppm CO_2 and 2000 ppm CO_2 treatments. Small sections of leaf were painted with clear nail varnish. Once dry the varnish was pealed away from the leaf surface using forceps and stomatal density determined by counting the pores using a light microscope.

5. Fluorescence microscopy

Leaf cross sections were taken and mounted in a solution containing calcofluor and iodine. The sections were viewed using an inverted microscope (Olympus IMT-2) and photographic records of starch grains were taken.

6. Chlorophyll fluorescence

A Plant Efficiency Analyser (Hansatech Instruments, UK) was used to measure *fv/fm* values for intact cucumber plants grown in the glasshouse at HRI Stockbridge House. This measurement (*fv/fm*) gives the ratio of variable to maximum fluorescence and provides an indication of the health of the plants light harvesting centres. A value of 0.8 indicates that the plant is healthy, any value below this being indicative of photo-damage, which is apparent as leaf bleaching.

RESULTS

Part I: Establishment of CO_2 damage in glasshouse cucumber crops.

Glasshouse conditions

The achieved daily CO_2 levels within the glasshouse for the imposed CO_2 regimes detailed in Table 1 are illustrated in Figure 2. The objectives of the CO_2 strategy was to produce a full plant canopy and then induce crop damage by setting supra-optimal CO_2 targets within the glasshouse. The arrow (Figure 2) indicates the time that classic symptoms of CO_2 damage were first expressed.

Figure 2

Gas exchange studies

The A/ci curve for cucumber plants 7 days after (trans)planting is shown in Figure 3. When this curve is compared to that of plants after 1 day of exposure to 2000 ppm CO_2 (Figure 4) there is little difference in the slope of the curve or net assimilation rates. However following 10 and 30 days exposure to 2000 ppm CO_2 the assimilation rates of the plants in 2000 ppm are higher (Figure 4) than those grown at 600 ppm, compare Figures 3 and 4. However it should be noted that on each sampling occasion a different leaf was used so there also may be an effect of leaf age here.

Crop damage

Crop damage was first noted on 26 March 1999, 24 days after the imposition of 2000 ppm CO_2. The classic CO_2 damage symptoms are illustrated in Plate 1. There was also a decline in the fv/fm value to about 0.5. However until damage was apparent to the naked eye the chlorophyll fluorescence data showed no changes to that of healthy leaves (0.8) indicating that this measurement would not help ascertain the onset of CO_2 damage (data not shown).

Figures 3 and 4

Plate 1: Visual leaf damage caused by high CO_2 levels.

Plant yield

Total weight of cucumbers harvested during weeks 7 to 20 is shown in Figure 5. As expected yields increase rapidly from the start of harvesting in week 7. The CO_2 target levels were increased to 2000 ppm in week 9, illustrated by the arrow (Figure 5) and crop damage was first noted during week 12 (Figure 5). Shortly after damage was noted crop yield reached a plateau and then fell dramatically during week 16 before rising again in weeks 17-20 (Figure 5). It should be noted that although these yield figures were obtained from 12 rows of cucumbers, these plants were all contained within a single glasshouse and there were no replicated plots and no control treatment. This experiment should be repeated with replication and controls.

Figure 5

Part II: Investigation of CO_2 damage in cucumber plants grown under ambient and elevated CO_2 regimes in controlled environment cabinets.

Gas exchange studies

A/C*i* curves for whole intact cucumber plants grown under ambient (350 ppm) and enriched (2000 ppm) CO_2 regimes after 1 and 4 weeks are shown in Figures 6a and 6b respectively. The initial slope of the line in Figure 6b is lower than that in Figure 6a suggesting that after 4 weeks exposure to 2000 ppm the plants were expressing a decrease in the carboxylation efficiency, that is down-regulation of RUBISCO activity. These results contradict those obtained from the glasshouse studies (Figures 3 and 4), however the latter could be attributed to sampling different individual leaves at each crop visit.

Starch assay

No significant differences were found in the starch levels within the leaves of plants grown at 2000 ppm compared to 350 ppm CO_2 (data not shown). However Fluorescence microscopy work illustrated that starch does accumulate in the pallisade layer of leaves grown at 2000 ppm (Plate 2).

Protein assay

The result of the protein assay (Bradford, 1976) shows a significant loss of protein in the leaves of plants grown at 2000 ppm, in both upper and mid canopy layers (Figure 7). These results complement those obtained from the A/c*i* curves (Figures 6a and 6b) and are consistent with data obtained from a RUBISCO assay performed subsequently (data not shown). Lower total protein probably suggests reduced investment in RUBISCO which will result in photosynthetic down regulation.

Growth analysis

There were no differences in leaf area between plants grown under 350 ppm CO_2 compared to those grown under 2000 ppm CO_2 (data not shown). However specific leaf weight was increased at higher CO_2 implying an increased leaf dry weight at high CO_2 levels.

Stomatal density

There was a decrease in stomatal density on the abaxial (lower) leaf surfaces in the upper and mid canopy layers of plants grown in 2000 ppm CO_2 compared to those plants grown under 350 ppm CO_2 (Figure 8). However no significant differences were found between adaxial (upper) leaf surfaces (Figure 8). Figure 9 also shows that stomatal conductances are lower for plants grown under the elevated CO_2 regime.

Figures 6a & 6b

Figures 7 8 and 9

Plate 2: Starch grains in pallisade layer of leaves grown at 350 ppm CO_2 (Plate 2a) and 2000 ppm CO_2 (Plate 2b).

Plate 2a:

Plate 2b:

DISCUSSION

The results shown in this report indicate a clear damaging effect of high concentrations of CO_2 on cucumber leaf structure and functioning. A review of the literature (Appendix I) suggests that this is not a response that is restricted to cucumber. Our results do suggest that lesions in leaf functioning in cucumber are not related to accumulation of starch in leaves, an association which is reported for other species.

Experiments in controlled environments suggest a directly damaging effect of high CO_2 on photosynthetic metabolism. Part of this may be a down regulation of photosynthesis rather than damage per se, but we must also investigate further effects of high CO_2 on the light reactions of photosynthesis and other effects on carbon metabolism. Preliminary glasshouse experiments show clear visual symptoms of high CO_2 but physiological measurements made in glasshouses are inconclusive. This is not surprising in that these measurements were made during the first few weeks of the project while techniques were still being developed.

Our current hypothesis to explain reductions in photosynthesis promoted by high CO_2 is that photosynthetic down-regulation is combined with damage to the light harvesting and reaction centres of the leaf. This may be caused by a restricted supply of CO_2 through a reduced number of stomatal pores which seem to be tightly closed. This closure is a reaction to high CO_2 but may also be a reaction to mild shoot water deficit arising from limitations in xylem development at high CO_2. We plan to further investigate this hypothesis.

Experiments are underway at Stockbridge House to determine whether the reported photosynthetic lesions result in a limitation to yield. Investment in extra CO_2 supplementation may be wasteful unless yield benefits can be shown.

APPENDIX I

Literature review of the physiological and biochemical processes associated with crop damage at high levels CO_2 enrichment.

Leaf injury caused by high levels of CO_2 enrichment has been observed and reported in tomato, cucumber, chysanthenum, gerbera (Van Berkel, 1984), bean (Ehret & Jolliffe, 1985) and spinach (Holbrook *et al*., 1993). In all these cases the authors describe symptoms of injury as regions of inter-veinal chlorosis which eventually result in complete necrosis, with leaf roll also occurring in tomato. The most often-cited reason for this has been the accumulation of starch in the choroplasts, which cause physical disruption and distortion of the thylakoid lamellae. Cucumber plants are known as starch storers (Goldsmidt & Huber, 1992) and it can be postulated that such plants are less susceptible to damage at high CO_2 compared with non-starch storing plants, such as tomato.

It is interesting to note that very similar injury symptoms have been observed on cucumber varieties where no CO_2 enrichment takes place. Injury is apparent during or after periods of very bright sunshine and when vegetative growth has stopped because of the fruit load hanging on the plants. When the fruits are harvested, the plants reserve of water is removed and the plants capacity to sustain a positive water balance in times of high transpirational demand is reduced. The ensuing increase in leaf temperature (from stomatal closure could then induce chlorophyll breakdown and hence cause a decline in photosynthesis (Van Berkel, 1984). Another plausible, and perhaps more likely explanation is stomatal closure due to resulting drought stress which results in photoinhibitory effects discussed previously.

The enriched CO_2 environment may cause developmental changes to take place in the plant architecture, such changes in stomatal density and xylem development, which would exacerbate the problem of leaf heating / photoinhibition.

The decline in rates of net photosynthesis seen in CO_2 enriched atmospheres have been studied extensively by climate change physiologists. They have found that photosynthesis can acclimate to elevated CO_2. Though the precise mechanisms have yet to be fully defined, the main framework for regulatory changes appears to have been elucidated. However, it is not apparent whether symptoms of damage and acclimatory responses operate independently of each other or are inextricably linked.

One explanation for acclimation is a decrease in the activity of RUBISCO. Pivotal to this may be a photosynthetic end product inhibition, caused by the fact that the stimulation of primary carbon assimilation at elevated CO_2 cannot be fully exploited by plants due to the lack of an increase in carbon export or consumption (Grimmer *et al*., 1999). This repression of photosynthetic activity genes by an increased soluble carbohydrate concentration was tested by Nie *et al* (1995) in a field crop of spring wheat grown at ambient and elevated (550 μmol mol^{-1}) CO_2. Their results showed that elevated CO_2 induced an increase in leaf non-structural carbohydrates and a decrease in the concentration of gene transcripts coding for specific proteins of the photosynthetic apparatus. However they did conclude that the effects on the transcripts varied greatly with stage of crop and leaf development and time of day, which suggests that subtle changes in conditions could be invoked which would result in an avoidance of an acclimatory response to elevated CO_2.

The 'sink' regulation of photosynthesis was explored further by Krapp *et al* (1991) who found that feeding glucose to the detached leaves of spinach caused a gradual decrease in photosynthesis rates over a 7 day period with a concomitant decrease in RUBISCO protein and loss of chlorophyll.

Recent work by Cheng *et al* (1998), whereby *Arabidopsis thaliana* was exposed to 1000 μmol mol^{-1} CO2 for 40 days supported this result. They observed a two-fold increase in non-structural carbohydrate, large reductions in rbcL, the gene encoding the large sub-unit of RUBISCO and rbcS, the gene encoding the small sub-unit; this result being attributed to a prolonged night time hexose metabolism which deleteriously affected transcript accumulation.

Similar work was carried out by Socias et al (1993), who exposed *Phaseolus vulgaris* to two CO_2 regimes (350 and 650 μmol mol^{-1}). They found that the maximum rate of ribulose-1, 5-bisphosphate (RuBP) consumption was lower in plants grown at 650 μmol mol^{-1}. This was due to reduced carbamylation, not a loss of protein, caused by feedback inhibition as judged by a lack of response to removing oxygen from the air stream.

It has also been reported that decreased expression of RUBISCO could be due to faster growth in elevated CO_2 accelerating leaf senescence due to ontogenetic shift or because nutrients, principally nitrogen, become limiting (Ludewig *et al*., 1998). In a study where N was added either in direct proportion to plant growth, relative addition rate (RAR), or as a set

concentration where there was free access to N, Farage *et al* (1998) investigated the effect of a low nitrogen supply on acclimation to elevated (650 μmol mol^{-1}) CO_2 in wheat cultivars. They constructed A/Ci curves to gain an understanding of the biochemistry of photosynthesis and found that in high CO_2 there was a significant effect of both CO_2 and N in the treatment that allowed a dilution of N (Free access). However no significant differences were observed in the RAR treatment. The authors concluded that low rates of N supply need not cause acclimation of photosynthesis to elevated CO_2 and the strategy apparently adopted to avoid acclimation is an adjustment of plant growth rate to match N supply.

Using transgenic plants that are unable to accumulate starch, due to leaf mesophyll-specific antisense expression of AGP B (a gene which encodes ADP-glucose biosynthesis) Ludewig *et al* (1998) were able to to show that in elevated CO_2 (1000 ppm) photosynthesis was inhibited to a greater extent in the antisense transformants compared with the wild type plants and the antisense plants grown in ambient CO_2 conditions. They interestingly found there was no significant effect of elevated CO_2 on RUBISCO in the wild type plants or transformants but discovered an increased expression of AGP B that allowed an increased accumulation of carbohydrate. This led them to propose that acclimation was not caused by starch accumulation, instead the rate of photosynthesis can become limiting by the rate of end product inhibition.

Despite some of these conflicting reports as to the nature of the signal that is responsible for photosynthetic acclimation, the intercellular sucrose concentration must be tightly regulated since this compound is the main osmotic substance for the maintenance of cell turgor. The plants photoassimilatory characteristics may determine whether of not starch accumulates to high levels and how the sucrose is cycled within the cell, especially in the case of cucumbers, where stachyose is the main transport sugar.

Which ever mechanism holds true it should be noted that most of these experiments reported in the literature were conducted at twice ambient CO_2 concentration and it seems therefore that the higher CO2 levels which are often used by cucumber growers could cause an upset in the regulatory pathways discussed.

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