Project title:	Increasing crop yield and resource use efficiency via root-zone CO_2 enrichment
Project number:	CP 143
Project leader:	Ian Dodd
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Key staff:	Estibaliz Leibar-Porcel, PhD student Martin McAinsh, co-supervisor
Location of project:	Lancaster University
Industry Representative:	Philip Morley, British Tomatoes Growers' Association
Date project commenced:	1/10/2015
Date project completed (or expected completion date):	30/12/2018

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with 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.

A**U**THENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Professor Ian Dodd

Project leader

Lancaster University

Signature 

Date……24/12 /17.....

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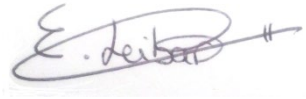
Signature 

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CONTENTS

GROWER SUMMARY

Headline

Gaseous CO_2 enrichment (1500 ppm) of the root-zone of aeroponically-grown plants increased lettuce biomass by 20%. Bicarbonate application (1-5 mM) to hydroponic solutions (which releases CO_2 to the solution) increased shoot growth of lettuce and pepper by 10-20%.

Background

Photosynthesis uses light energy to convert CO_2 and water into sugars, which are required for growth and respiration. Biomass accumulation is the difference between the photosynthesis rate and respiration rate. Greenhouse operators often inject extra CO_2 into the aerial environment to increase photosynthesis and biomass accumulation. However, when the humidity or the temperature is very high, the greenhouse is vented and CO_2 is released into the atmosphere (Figure 1), which is economically wasteful and releases a greenhouse gas to the atmosphere.

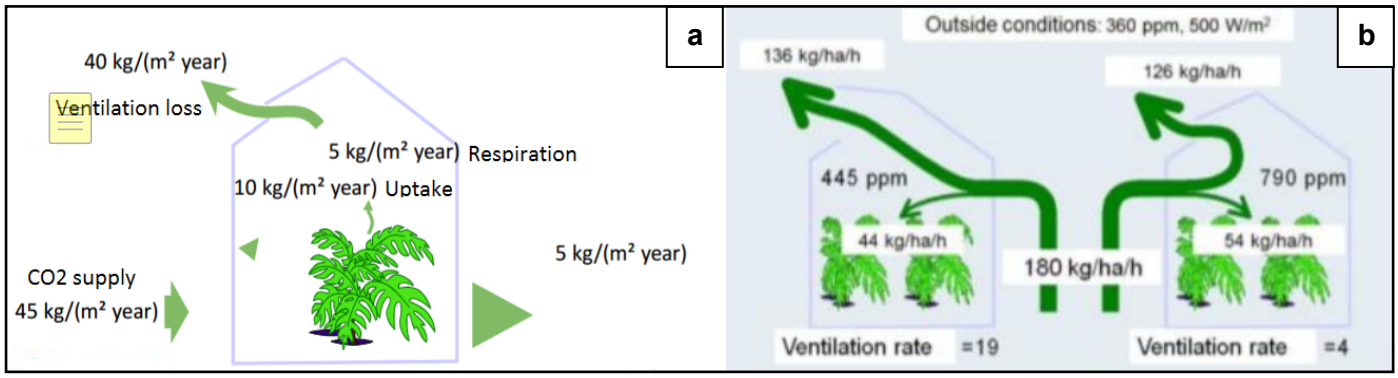

Figure 1. CO_2 balance model. a) General balance model when supplying 45 kg/ (m^2 year). b) CO_2 balance model when supplying 180Kg/ha/h CO_2 and different ventilation rates are applied with same outside conditions. *Wageningen University & Research, Business Unit Greenhouse Horticulture.*

Sources of CO_2 for enrichment include boiler, combined heat and power (CHP) and burner exhaust gases and liquefied pure gas. Flue gases from natural gas boilers are widely used in the UK as a source of CO_2 for enrichment. This practice has high energy costs of £200.000 per annum for a 5 Ha glasshouse (HDC 2011;

http://www.hdc.org.uk/sites/default/files/research_papers/PE%20003%20Final%202011_0.pdf). CO_2 gas is a “greenhouse gas” that contributes to global warming and climate change. Despite the efforts of growers to minimize spending and maximize production through technical improvements, it is necessary to consider other systems such as localized root-zone CO_2 enrichment, to improve crop production while minimising environmental emissions.

This project focused on improving resource use efficiency, the cost-effectiveness and the environmental performance of tomato, lettuce and pepper production, by testing whether root-zone CO_2 enrichment with soilless culture systems provided a viable alternative to aerial CO_2 enrichment.

Summary

Previous studies have shown that applying either bicarbonate hydroponically at low concentrations (5 mM HCO_3^-) or gaseous CO_2 at high concentrations (2000-50,000 ppm) to the roots increased growth of some crops such as tomatoes or lettuce. Also, initial studies carried out at Lancaster University by a previous AHDB-funded PhD student indicated that applying 700 ppm CO_2 to the root-zone of semi-aeroponically grown lettuce (without altering the aerial CO_2 concentration) increased biomass by 10%. Therefore, root-zone CO_2 enrichment in greenhouses may provide an alternative technique to increase yield.

In Year 1 of this project, initial studies identified that applying low concentrations of bicarbonate (1-5 mM) to the nutrient solution of hydroponically grown pepper and lettuce increased shoot biomass by 10%. In addition,, hydroponically grown tomato plants enriched with 1500 ppm root-zone CO_2 increased dry biomass by 11%. Although gaseous root-zone CO_2 enrichment is still undergoing additional research, some experiments showed greater biomass (7-10%) in aeroponically grown lettuce. However, these experiments need to be repeated to reach a final conclusion.

Financial Benefits

Developing techniques to more effectively apply CO_2 will decrease the cost of supplying liquefied CO_2 or energy consumption (natural gas boilers) in commercial scale greenhouses.

Action Points

1. Understand that there are potential alternatives to the current practice of aerial CO_2 enrichment in greenhouses that decrease CO_2 usage and reduce pollution, while maintaining crop yields.

Introduction

Carbon acquisition by terrestrial plants occurs predominantly through fixation of atmospheric carbon dioxide, yet plants can also gain inorganic carbon through their roots (Bialczyk, *et al*. 1992, 1994,1995, 2004, 2005; Yang, *et al.* 1994 ; Siddiqi, *et al.* 2002; Gao, *et al.* 1997; Cramer & Richard, *et al*. 1999 ; Cramer, *et al.* 1999; Van der Merwe, *et al.* 2000; Viktor, *et al.* 2003; Viktor, *et al*. 2005; He, *et al.* 2007, 2010, 2016).

Carbon isotopes have been very useful to study carbon flow processes in photosynthesis and in the translocation of different photosynthates to various parts of the plant. There are three naturally occurring isotopes with molecular weights of 12, 13 and 14. ^{12}C and ^{13}C are stable isotopes and their relative abundances are 98.9% and 1.1% respectively. ^{14}C is an unstable radioactive isotope present at <0.01%.

The ^{13}C content is usually determined with a mass spectrometer which measure the ratio (*R*) between ^{13}C and ^{12}C. *R* is approximately 0.0112 and just the last digit in the ratio varies. *R* values are commonly converted to values of $\delta^{13}C$ and the units are called “per mil” or ‰.

$$\delta^{13}C = [\ (R \text{ sample} / R \text{ standard}) - 1] \times 1000$$

The standard in determination of carbon isotopic ratios is carbon in carbon dioxide obtained from limestone, called PDB, from the pee dee formation in South Carolina (Craig 1957). Most natural materials such as plant biomass have a negative $\delta^{13}C$ value compared to the standard.

It has long been known that isotope fractionation of carbon is different depending on the photosynthesis type (C3 *versus* C4) of the plant. The value of atmospheric CO_2 is -8‰. Plants contain less ^{13}C than the atmosphere because ^{13}C is heavier than ^{12}C and forms stronger chemical bonds. Therefore, during photosynthesis plants discriminate against ^{13}C and they fix mostly ^{12}C. However, C3 plants are isotopically distinct from C4 plants. C3 plants incorporate CO_2 through the enzyme ribulose bisphosphate carboxylase (Rubisco) and they have $\delta^{13}C$ values between -33 to -24 ‰. Within the leaves of C4 plants, a two-step CO_2 incorporation process occurs, such that CO_2 is first taken up by phosphoenolpyruvate carboxylase and then the carboxylation product is transported from the outer mesophyll cells to the inner bundle sheath layer where decarboxylation and refixation occurs by Rubisco

(Figure 2). Values in C4 plants are in the range of -16 to -10 ‰ (Farquhar et al 1989, O'Leary 1981, 1988).

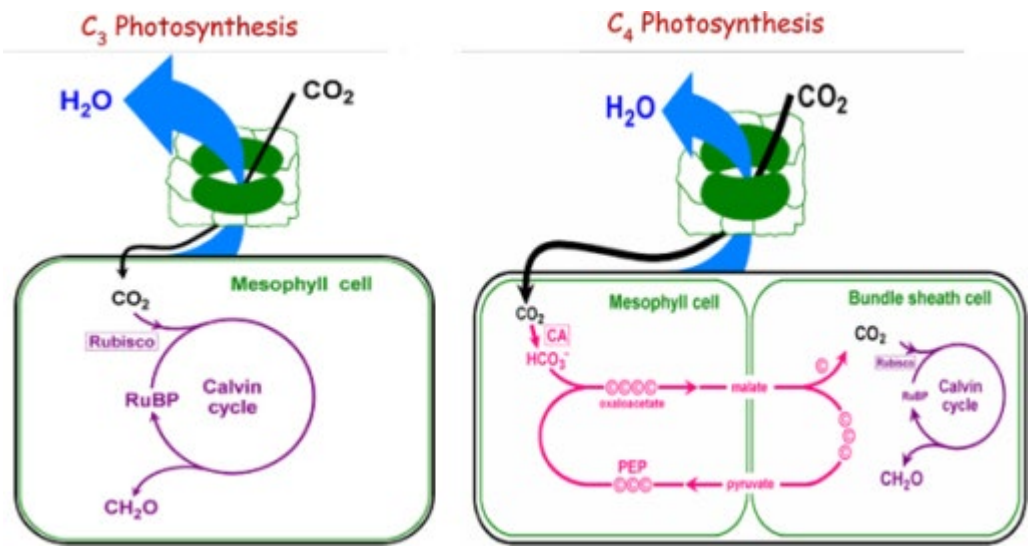

Figure 2: Diagram of C3 and C4 plants photosynthesis pathways (Wang, *et al.* 2012).

Previous studies have shown the uptake of dissolved inorganic carbon through the roots using both labelled ^{14}C or ^{13}C in a variety of crops and trees (Viktor & Cramer 2003, Vuorinen, *et al.* 1992; Kimerer, *et al.* 1993), with incorporation of dissolved inorganic carbon into organic products in the roots occurring through the activity of phosphoenolpyruvate carboxylase (PEPc). Labelled organic acids are transported by the xylem to the shoots to provide a ready source of CO_2 by being partly decarboxylated in the shoot, and the released CO_2 re-fixed (via Rubisco) through photosynthesis. However, studies concluded that the small contribution of the root-derived carbon cannot explain the observed increase in growth (Viktor & Cramer, 2003).

1.4 Objectives

The experiments conducted this year aimed to determine the effect of root-zone CO_2 enrichment on lettuce biomass in the aeroponic system and the mechanism(s) by which high dissolved inorganic carbon (DIC) root-zone concentrations promote growth.

Materials and methods

Experiment 1: Root-zone CO_2 enrichment of aeroponics

Aim: Determine the effects of 1500 ppm root-zone CO_2 on biomass of lettuce plants.

Experimental procedures:

Four experiments were carried out between January and August 2017, two in a naturally lit (with supplementary lighting when PPFD was < 400 μmol m^{-2} s^{-1}) glasshouse and two in an artificially lit controlled environment room (300 μmol m^{-2} s^{-1}).

Crisphead and butterhead lettuce types grown in Grodan rockwool were transferred to two aeroponic systems (Platinum aero pro-8) at the 4-leaf stage. The hypocotyls of the plants were inserted through a collar made with impermeable CO_2 sealant (Qubitac) in the lids of 11 L pots with one plant per pot and 8 plants per system. Nebulisers (flow rate: 12-14 L h^{-1}) misted roots with recirculated half-strength Hoagland's solution coming from a 60 L reservoir (Figure 3). The pH was monitored every day to have a near-constant pH between 6 – 6.3 by manually adjusting each day with dropwise HCl or NaOH addition.

After transplanting, two different CO_2 treatments (400 and 1500 ppm), were applied into each bin. The system consisted of an enriched channel supplemented with CO_2 and a non-enriched channel supplied only with compressed air. The air from the enriched channel was completely mixed in a mixing box before entering the aeroponic bins. The CO_2 in the mixing box was monitored continuously using a CO_2 gas analyser (PP Systems, WMA-4).

To prevent leakages, the lid was sealed with self-adhesive rubber foam around the rim. The air above the lid and at the shoot base was routinely sampled with a LI-COR 6400 with no significant difference compared to the ambient air.

Figure 3: Lettuce aeroponic system

Experiment 2: Carbon uptake of hydroponically grown lettuce plants.

Aim: Determine whether plants absorb dissolve inorganic carbon (DIC) through the roots and the form of carbon taken up.

Experimental procedures:

Two time-course experiments were developed. Butterhead lettuce type seedlings (*Lactuca sativa* cv. Sunstar) grown in vermiculite were transferred to two different hydroponic systems (recirculating and non-recirculating system) at the 4-leaf stage. The hypocotyls of the plants were inserted through a closed cell foam collar and the nutrient solution in each pot was constantly aerated through a 6 mm pipe connected to an air pump.

In the non-recirculating system, 10 lettuce plants (*Lactuca sativa* cv. Sunstar) were each placed in a 300 mL jar with nutrient solution (Figure 4). After 3 days, 1 mM ^{13}C labelled sodium bicarbonate was added to 4 jars at 08.30. Two plants were harvested at 08.00 (non-enriched controls) and another two plants (control and enriched) were harvested 4, 8, 12 and 24 hours after the $NaH^{13}CO_3$ was added. At harvest, plants were divided into leaves and roots, which were rinsed 3 times in dH_2O to remove any nutrient solution.

Figure 4: Lettuce in hydroponics system for $NaH^{13}CO_3$-labelling experiments.

Three recirculating hydroponic systems were used, each containing 5 lettuce plants with each one placed in a 300 mL jar (Figure 5):

- Control system with half-strength Hoagland solution with the pH 5.8 manually adjusted at the beginning of the treatment.
- Labelled ($NaH^{13}CO_3$ addition) system with naturally fluctuating pH (Figure 6)
- Labelled ($NaH^{13}CO_3$ addition) system with pH constantly controlled at pH 5.8 using a pH automatic controller (pH Kontrol 01, Prosystem Aqua).

$NaH^{13}CO_3$ addition occurred at 08.30, three days after plants were introduced to the systems. Prior to addition of the label at 8am, three plants, one from each system, were harvested and divided into leaves and roots, which were rinsed 3 times in dH_2O. The same procedure was performed 4, 8, 12 and 24 hours after the $NaH^{13}CO_3$ was added.

Figure 5: Lettuce in a recirculating hydroponic system

All the plant material was freeze-dried and ground to a fine powder using standard precautions to avoid cross-contamination. For all biomass fractions, a 2 mg subsample was analysed on an isotope ratio mass spectrometer.

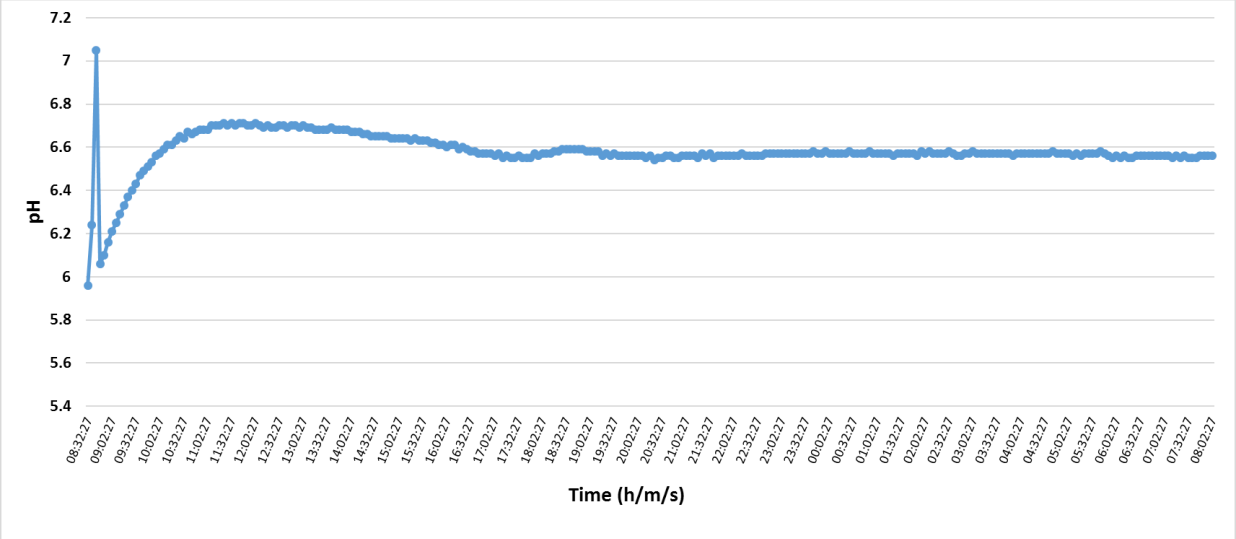

Figure 6. pH values *versus* time in the labelled ($NaH^{13}CO_3$ addition) system. Maximum pH after adding 1mM $NaH^{13}CO_3$ was 6.7. The prevalent form of carbonates at pH ≤6.36 is CO_2 and H_2CO_3, at pH between 6.36 and 10.33 is HCO_3^-, and CO_3^{2-} is predominant at pH >10.33 (Lindsay, 1979).

Results

Experiment 1:

Root-zone CO_2 enrichment significantly increased dry shoot biomass in lettuce by about 20% compared to those grown with 400 ppm root-zone CO_2 regardless of the variety and location of the experiment (Table 1).

Lettuce variety	Location	Increase
Butterhead (Sunstar)	Glasshouse	22% (NS)
Crisphead (Antartika)	Glasshouse	19% ($p<0.05$)
Crisphead (Consul)	CE room	25% ($p<0.05$)
Crisphead (Consul)	CE room	27% ($p<0.01$)

Table 1. Shoot biomass increase (%) of aeroponically grown lettuce in the glasshouse and CE room.

Experiment 2:

The $\delta^{13}C$ values of leaves increased significantly over time in bicarbonate-enriched plants, therefore confirming root DIC uptake. The $\delta^{13}C$ values of roots increased significantly over time however, it seems that the uptake is higher at the beginning, suggesting DIC transport from the root to the shoot (Figure 7).

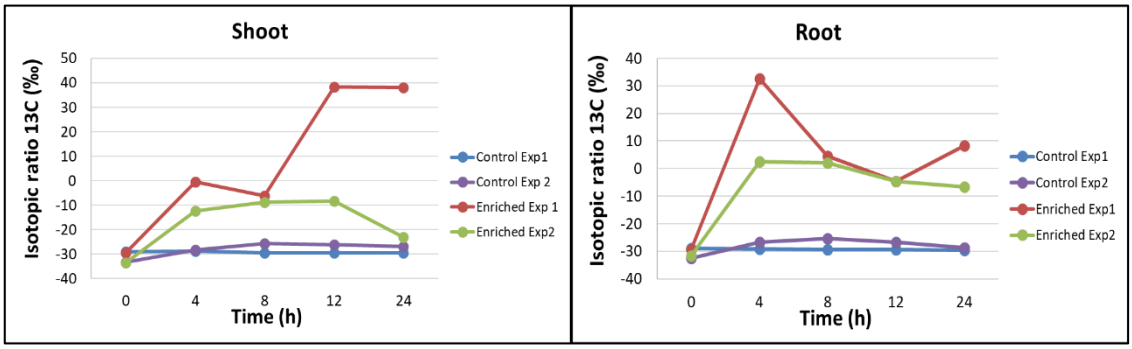

Figure 7. δ13C (‰) for leaves and roots containing 0 or 1 mM $NaH^{13}CO_3$ *versus* time for DIC uptake by lettuce. Points are from individual plants grown in two replicate experiments.

Root $\delta^{13}C$ values in plants exposed to different solution pHs were similar, indicating DIC incorporation is independent of the form of carbon taken up: at pH 5.8 the ^{13}C will be in the form of CO_2, while when solution pH naturally fluctuates (between 6.3 and 6.7) the ^{13}C will be in the form of HCO_3^-. However, greater ^{13}C translocation from the roots to the shoot occurred when nutrient solution pH was allowed to naturally fluctuate (Figure .8).

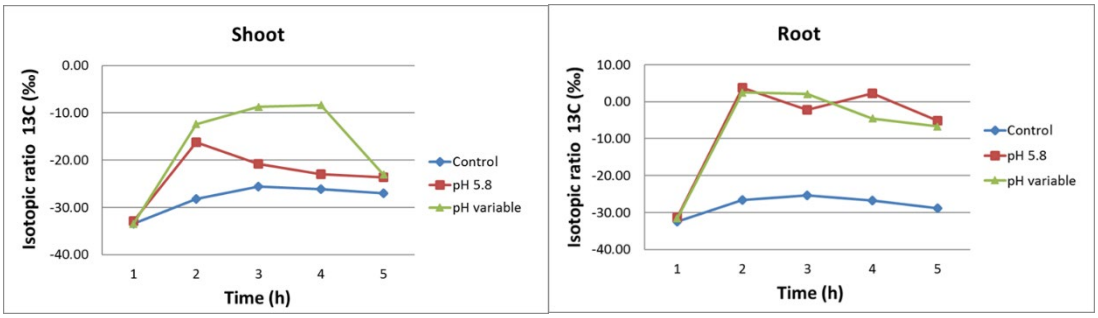

Figure 8. δ13C (‰) for leaves and roots containing 0 or 1 mM $NaH^{13}CO_3$ *versus* time for DIC uptake by lettuce. Each point is from an individual plant.

Discussion

Comparable previous studies showed that twelve days of applying elevated root-zone CO_2 (2000 ppm) to aeroponically grown lettuce increased shoot growth (~18%) compared to plants aerated with ambient CO_2 (360 ppm) (He, *et al*. 2010). Although ambient temperatures and PPFD were higher in that study, in our study, we found that similar shoot growth enhancement occurred in plants grown under elevated root-zone CO_2 (1500 ppm) at irradiance for 10 days (Table 1). In contrast, previous measurements of root dry weight showed a significant increase at elevated root-zone CO_2 (He, *et al.* 2010), whilst no effect (data not shown) was detected in our study, likely because the small pot size limited root growth. The effects of high root-zone CO_2 concentrations occurred after few days of treatment: decreased g_s, less water loss, higher midday leaf RWC, higher sink capacity (larger root systems enhanced NO_3^- uptake and increased the capacity for utilizing photoassimilate) and higher levels of reduced NO_3^- (He, *et al.* 2010). Further studies are needed in our aeroponics system to accurately measure leaf gas exchange and nutrient concentrations to compare with the conclusions of previous studies.

The uptake of DIC through the roots has been repeatedly demonstrated (Vuorinen, *et al.* 1992, Hibberd, *et al.* 2002, Cramer, *et al.* 1995,1999, Bialczyk, *et al.* 1992), although its effects on plant responses are not well known. Inorganic carbon absorbed through the roots is converted to organic and amino acids which are exported to the shoots, where they are decarboxylated to augment photosynthesis (Bialczyk, *et al.* 1992, 1995, Cramer, *et al.* 1995, 1999, Viktor & Cramer, 2005). However, since this small contribution to the total carbon budget of the plant cannot explain the stimulation of growth (Viktor & Cramer, 2003), it is necessary to explore other routes that can promote the plant growth.

The pH of the xylem sap plays an important role in growth regulation, with drought-induced alkalisation of xylem sap decreasing leaf elongation (Bacon, *et al.* 1998). When plants of *Vigna unguiculata* were perfused with different pH solutions, xylem exudate pH was adjusted due to the high buffering action of the xylem wall apoplast, and protonation of a specific cell wall layer was an essential prerequisite for cell wall extension independently of the auxin-induced acidification (Mizuno and Katou 1991), which does not always occur. The pH of the xylem sap also has a role in drought signalling (Willkinson and Davies, 1997; Wilkinson, 1999; Zwieniecki, *et al.* 2001; MacRobbie, 1998) and plant nutrition (Mengel, 1994; Bialczyk, 1995), but the effects of bicarbonate addition to hydroponic solutions on xylem sap pH are inconsistent. Adding bicarbonate to the nutrient solution of hydroponically grown maize alkalised the xylem sap, from 5.52 to 6.02 upon adding 5 mM bicarbonate and from 5.23 to

6.71 upon addition of 20 mM bicarbonate (Wegner & Zimmermann, 2004). On the other hand, several studies did not detect any significant change of the xylem sap pH when more than 10 mM bicarbonate was added to the rhizosphere (Alhendawi, *et al.* 1997; Nikolic & Römheld, 1999). Since the isotopic ratio of ^{13}C in the shoot differed when the nutrient solution was at pH 5.8 or as high as 6.7 (Figure 6), and previous studies linked the pH with cell wall extension, measuring xylem sap pH is necessary to determine whether xylem pH variation affects plant growth. Although changes in rhizosphere pH in response to bicarbonate addition may not always change xylem sap pH (due to the buffering capacity of the xylem sap – Gollan, *et al.* 1992), the direction of change (xylem sap alkalisation) means it is difficult to reconcile bicarbonate-induced growth promotion (see Year 1 report) with known xylem pH impacts on leaf elongation (Bacon, *et al.* 1998).

For this reason, it will be necessary to measure a range of growth promoting phytohormones e.g. (auxins, cytokinins, abscisic acid, gibberellins and ethylene - Davies, 2004) in plants exposed to a different root-zone CO_2 concentrations, to investigate additional mechanisms of growth regulation.

Conclusions

- Applying 1500 ppm root-zone CO_2 to aeroponically grown lettuce plants stimulated growth by 20%.
- The uptake of DIC through the roots of lettuce plants was demonstrated using $NaH^{13}CO_3$.
- Further analysis is needed to explain the mechanism(s) by which root-zone CO_2 enrichment enhances plant growth.

Knowledge and Technology Transfer

Conferences:

Leibar-Porcel, E. Increasing crop yield and resource efficiency via root-zone CO_2 enrichment. The Great British Tomato Conference. Chesford Grange Hotel. 28-29th September 2016.

Leibar-Porcel, E. Increasing crop yield and resource efficiency via root-zone CO_2 enrichment. Plant & Crop Science Postgraduate Conference, Lancaster University, 4th October 2016.

Leibar-Porcel, E. Increasing crop yield and resource efficiency via root-zone CO_2 enrichment. AHDB Studentship Conference. Stratford Manor. 6-7th November 2017.

Posters:

Leibar-Porcel, E. Increasing crop yield and resource efficiency via root-zone CO_2 enrichment. LEC PGR Conference, Lancaster University, 21-22th April 2016.

Leibar-Porcel, E. Increasing crop yield and resource efficiency via root-zone CO_2 enrichment. *2nd* Agriculture and Climate Change Conference, Sitges, 26-28th March 2017.

Glossary

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Appendices

