

**Carbon dioxide and oxidative techniques to improve growth and
yield of beetroot, Swiss chard and green beans**

by

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DECLARATION

I hereby certify that this dissertation is my own work, except where duly acknowledged. I also certify that no plagiarism was committed in writing this seminar.

Signed

A handwritten signature in black ink, appearing to read 'Bekal', written over a horizontal line.

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ABSTRACT

Carbon dioxide and oxidative techniques to improve growth and yield of beetroot, Swiss chard and green beans

by

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Degree: Master of Science (Agric) Horticulture

The current population of almost 8 billion is projected to reach 9.55 billion by 2050 and will cause a greater demand for food. Dietary changes will further add to the demand for food.. To meet the growing demand, food production will have to be increased between 60 -110%. Intensification and land clearing are two methods that have been developed to increase yield and to close the yield gap, however, there are negative problems associated with them. Genetic manipulation, management and production practices are mainly focused on to increase yield. The release of additional oxygen and carbon dioxide in close proximity to plants or the improvement of existing CO₂ and O₂ fertilization are techniques that can be explored further as a possible solution to the yield gap problem. Main purpose of this study was to investigate the application of additional carbon dioxide and oxygen on plant growth and yield.

In this study a quantitative research approach was taken to determine the effects of the CO₂ and O₂ treatments on plant growth and yield. In the preliminary pot trial additional CO₂ was given to the bean plants via a calcium carbonate and hydrochloric acid reaction as well as via a carbon dioxide tablet treatment on the soil surface. In the second trial, the same treatments were applied to the beans, beetroot and Swiss chard plants. The reactions took place within Boston round bottles planted into the pots next to the plants. Unfortunately, due to COVID-19 the CO₂ tablet treatments were terminated as no new stock could be imported. Weekly plant growth measurements, photosynthetic measurements, stomatal conductance and laboratory analyses of the leaf material (stomatal density, chlorophyll concentration) were measured. Other measurements such a root length, root mass, biomass and yield were taken

at the end of the trial or per harvest (bean pods, edible part of the beetroot). The data was analysed to determine whether the means were significantly ($P < 0.05$) affected by the treatments. Initially the treatments in the preliminary trial showed promise in increasing plant height, chlorophyll A and B concentrations and stomatal density; however, the soil treatment affected the plant roots and soil electrical conductivity (EC), which caused the plants to die. In the second pot trial both the beans and beetroot had grown under an elevated CO_2 concentration showed improved growth but varied between the crops for example, only beans showed increased height, beans and beetroot had improved root and biomass growth, it greatly affected the shoot to root ratio in Swiss chard, the number of pods per bean plant and yield in all crops except for Swiss chard. Calcium carbonate in the second pot trial improved bean and beetroot plant growth. The plant growth parameters, bean yield and beet measurements did not differ significantly between the control (ambient glasshouse) and the plants receiving the additional CO_2 through CaCO_3 .

The field study consisted of beans, beetroot and Swiss chard plants grown in an open field at plot scale. The plant leaves were sprayed with two concentrations (0.5 and 0.1 ml L^{-1}) of hydrogen peroxide at two application rates (once and twice a week) to determine whether additional O_2 would improve not only plant growth but also yield. The foliar applications of hydrogen peroxide were found to be effective in increasing plant height in all three crops. The biomass and yield of Swiss chard plants treated weekly with the low H_2O_2 concentration were positively affected whereas the bean biomass was improved with the weekly application of the high H_2O_2 concentration. Regardless of application rate the beetroot leaf area index and biomass improved with the low H_2O_2 concentration treatment whilst yield increased under the high H_2O_2 concentrations. The stomatal density in all three crops were unaffected by any of the treatments. The photosynthetic pigments (chl A, -B and total carotenoid concentration) increased overall in all the treatments of the bean plants. The beans treated weekly with the low H_2O_2 concentration resulted in greater photosynthetic pigment concentrations regardless of application rate. The beetroot plants had a similar reaction in its photosynthetic pigments than the beans except that it was with the higher H_2O_2 concentration applied twice a week. The Swiss chard plants treated weekly with the low H_2O_2 concentration resulted in the increase of its photosynthetic pigments.

Beetroot and Swiss chard plants were grown in a suspended, non-circulating hydroponic pot system. A pump system was built to aerate the nutrient solution with either pure oxygen, carbon dioxide or air (21% oxygen) to determine if plant growth and yield would improve. The beetroot and Swiss chard plants aerated with air resulted in the best plant and root growth among the treatments. The beetroot plants that were aerated with pure oxygen resulted in the greatest biomass weight followed by those aerated with carbon dioxide. In

conclusion, the application of additional CO₂ to plants grown in pots, the foliar application of hydrogen peroxide to plants grown in open fields and aerating the nutrient solution of a hydroponic system with carbon dioxide or oxygen showed promising results but should be studied further.

Keywords: *Carbon dioxide, oxygen, hydrogen peroxide, growth, yield*

INTRODUCTION

The current global population of approximately 7.96 billion is roughly three times more than in the mid-twentieth century. It has been forecast that the global population will reach and astonishing 9.55 billion by 2050 (UN 2019). The United Nations Department of Economic and Social Affairs has predicted that the population will increase to 8 billion in mid-November 2022 worldwide (UNDESA 2022) and 8.6 billion people by 2031 (OECD/FAO 2021). The growth rate of the global population is currently 0.9% p.a. Which is slower than the 1.1% annual growth rate that was experienced over the last ten years. However, the growth rate do differ between developed and developing countries where a fast growth rate of 2.5% p.a. is expected in Sub-Saharan Africa over the next 10 years (OECD/FAO 2021). Together with a growing global population, the change in peoples' diets worldwide further adds to the food demand. In the 1860's people consumed 2250 kilocalories (kcal) per capita daily, by 2015 it increased by 630 kcal to 2880 kcal and is expected to reach 3900 kcal by the year 2050 (Pardey et al. 2014, Valin et al. 2014). This will not only cause an increase in food demand by the human population but also in crops to feed livestock. This will require an increased output of food (60%-110%) to meet growing demand by 2050 (Cirera and Masset 2010, Tilman et al. 2011, Alexandratos and Bruinsma 2012, Valin et al. 2014).

There are two methods to increase crop yields. The first is to increase the area of land used for cultivation and the second to increase yield on the land already utilized for crop production, which is called intensification. Numerous countries have the potential to increase their arable land, even though the land available for agricultural use is limited (FAO 2020). Between the year 1980 and 2000 approximately 83% of agricultural land was made available from forests of which more than 55% at the cost of intact forests and 28% from disturbed forests (Gibbs et al. 2010). Urbanisation, other human activities, desertification, soil erosion, salinization, degradation and unsustainable land management over the last few decades have led to agricultural land being lost (Nellemann 2009, Fitzgerald et al. 2020). For example, agricultural land has decreased by 34% in developed countries between 1995 and 2007, whilst land in developing countries increased (7.1%) particularly in tropical forests (Gibbs et al. 2010). Approximately five billion hectares of the global land surface is currently utilized for agriculture (FAO 2020). Clearing more land for crop production is not a viable nor a cheap solution. The land will be competed for as it can also be utilized for other human activities. Preserving the natural ecosystem which is a source of carbon storage and biodiversity can further add to the cost of the land for food production (Balmford et al. 2005). In the process of converting land for crop production (from native habitats) enormous amounts of carbon dioxide are released into the atmosphere as soil and plant biomass are the largest biological stores of carbon (2.7 times more than in the atmosphere). Carbon dioxide is rapidly released when the

habitat is burned and when plant biomass (fine roots and leaves) are decomposed by microbes in the soil. Greenhouse gasses (GHG) are released over a longer period as the coarser roots, branches and other wood breaks down or burn after the initial clearing of land (Winjum et al 1998, Silver and Miya 2001, West et al. 2004, Van Loon et al. 2019).

FAO and DWFI (2015) estimates that an 80% increase of crop production can be achieved through intensification. Intensification can be defined as increasing agricultural production per unit of input (such as labour, time, seed, fertilizer, cash etc.). Many researchers believe that intensification is the better choice when taking into consideration greenhouse gas emissions, loss of biodiversity and ecosystem degradation (Godfray et al. 2010, Foley et al. 2011, Tilman et al. 2011, Suh et al. 2020). Both increasing land and intensification have problems that are associated with them. The problems associated with land clearing and intensification has shifted the focus to reducing the yield gap and increasing production without the need to clear more land (Cassman 1999, Cassman et al. 2003, Van Ittersum et al. 2013). The difference between the farm yield actually obtained and those from experiments is defined by De Datta (1981) as the concept of yield gap and was first introduced in 1981. Various authors have also suggested that this would be the most justifiable pathway to lowering food insecurity (Green et al. 2005, Matson and Vitousek 2006, Godfray et al. 2010, Foley et al. 2011, Phalan et al. 2011a, Phalan et al. 2011b, Tscharrntke et al. 2012, Hulme et al. 2013, Zeidler and Schubert 2014). Yield potential is the highest yield that can be obtained from a crop cultivar when it is grown in conditions where water and nutrients are not limited as well as where biotic stress is successfully controlled (De Datta 1981, Evans 1993, Van Ittersum and Rabbinge, 1997). From about 1951 onwards the yield in farmland was greatly increased due to the green revolution resulting from technology developed after the Second World War. Crop yield worldwide was tripled between 1960 and 2014 whilst arable land was only increased by 10% (Pellegrini and Fernández 2018). The 2021 outlook of the Organisation for Economic Co-operation and Development (OECD)/FAO expects that a crop production increase of 88% in 2030 will be due to crop yield growth through improved genetic material, greater inputs and investments in production technology. Approximately seven percent of the predicted increase could be attributed to crop intensity (more harvests per area) (OECD and FAO 2021).

When farm yield reaches the yield potential threshold it will become more difficult to sustain the increase in yield as everything will have to be perfected. This means that small problem areas with the integrated management of pests, water, nutrients, soil and the crop will have to be eradicated. Economically this is not a viable solution as the yield will typically

stagnate at 80% of the yield upper limit (Cassman 1999). Various researchers have studied the yield gap with the use of statistical analyses (Licker et al. 2010, Johnston et al. 2011) and crop models (Václavík et al. 2013). Solutions to close the yield gap have also been researched (Foley et al. 2011). In these studies, they have concentrated on management practices in terms of water and nutrition to close the yield gap.

The main focus to increase yield is usually on plant genetics, management and production practices; and less on methods such as the release of oxygen and additional CO₂ in close proximity to plants (other than Free Air CO₂ Enrichment, i.e. FACE) in order to improve plant growth and yield. While CO₂ is directly involved in the growth and yield of crops via photosynthesis, the addition of O₂ is more linked to improved root health and growth resulting in improved growth of the plant and thus yield. Introducing additional CO₂ and O₂ to a plant could therefore potentially increase the growth and yield of a crop. However, it is expected that different crop types will react differently to increased levels of CO₂ and O₂, and therefore a leafy, fruiting and rooting vegetable crop will be included in this study. This study could be a possible solution for the yield gap problem. The main purpose of this study is to investigate the effect of additional carbon dioxide and oxygen on plant growth and yield. This first chapter will consist of the literature review. Chapter two will focus on the effect of additional carbon dioxide on the growth and yield of crops (Swiss chard, beetroot and beans) grown in pots within greenhouses and CO₂ chambers. The effect of hydrogen peroxide on the growth and yield of beans, beetroot and Swiss chard in an open field will be discussed in detail in chapter three. In chapter four the focal point will be the effect of pure oxygen and carbon dioxide, bubbled through a hydroponic system, on the growth and yield of the abovementioned crops.

CHAPTER 1

1. LITERATURE REVIEW

1.1 Food production

In the world there is an urgency for an increase in crop production and yield. This has resulted in more land being made available for agriculture and improved crop management practices, which includes the utilization of copious amounts of inputs (inorganic fertilizers, synthetic pest and weed control) and irrigation (Oldfield et al. 2019). Such practices have led to the degradation of both water quality and soil characteristics; groundwater pollution; soil erosion to accelerate and food quality to reduce. This has also led to the encouragement of sustainable intensification initiatives. These initiatives are aimed at increasing yields on the land already used for crop production whilst at the same time reducing the impact on the environment (Godfray et al. 2010, Foley et al. 2011, Mueller et al. 2012, Campbell et al. 2014, Wang 2014). For example, organic crop production is an advocated agricultural practice as it lowers environmental pollution. It is for this very reason that it been introduced in numerous countries to replace chemical dependent farming (Oquist et al. 2007).

Eighty percent of the required increase in agricultural productivity can come from intensification (FAO and DWFI 2015). In order to reach the global food demand and the production target it will require higher crop intensification as well as an increase in crop productivity per annum. Increasing crop productivity will be difficult to achieve as it varies between regions and depends on the environment, practices and inputs. As there are also negative effects associated with intensification it is essential that it transpires in a profitable, maintainable and a socially acceptable manner. It should not degrade natural resources, cause eutrophication or unnecessary nutrient losses, negatively impact biodiversity nor enhance the emission of greenhouse gasses (Alcock et al. 2015, Liu et al. 2020a, Harrison et al. 2021).

1.2 Effective and sustainable management of agricultural crops

The effective as well as sustainable management of agricultural crops is crucial in order to continuously supply food and prevent the loss of agricultural crops within the food supply chain (Kiaya 2014, Adom 2018). Any method or practice that is green, environmentally friendly, technically sound and will reduce or prevent general problems that arise in agricultural production will be the best management practice to apply (UNEP 1998). As agroecosystems are interrelated as well as complex in nature (Menalled et al. 2008) it is important to carefully

consider any crop management practice in order to avoid affecting the interactions between crops and their environment. It is also particularly important that the management practices must not be connected with environmental costs or interrupt maintenance and conservation interests. Both the long- and short-term objectives of the practices should improve land resilience and biodiversity as well as take the benefits to local communities, in close proximity to the farmlands, into consideration (Adom 2018).

1.3 Current crop management practices that aids in sustaining and or increasing crop yields.

Best management practices defines the manner in which agricultural activities are managed and should be conducted in order to maintain agricultural production whilst alleviating the contamination of surface and groundwater (Prokopy et al. 2008). There are universally accepted management practices specifically for agricultural crop yield results. These practices include the utilization of cover crops, intercropping, agroforestry, crop rotation, suitable water and irrigation management, effective pesticide and/or fertilizer application, record keeping, conservation tillage, soil testing etcetera (Prokopy et al. 2008). If close attention is paid to these management practices it is possible to not only maximise crop yield but also guarantee the conservation of biodiversity and improve soil quality and reduce general environmental costs.

1.3.1 Cover crops

Plants that are cultivated in the off-season period, growing low to the ground are known as cover crops. The crops are planted in preparation of the land for cash crops (White 2014, Creamer and Baldwin 2019). On the other hand, researcher White (2014) found that cover crops could also be used as a living mulch year-round, planted after harvesting the cash crop or intercropping with the cash crop as it performs a significant role in sustaining agricultural produce and enhancing the quality and health of soil. Not only will this sustain microbial biodiversity in the soil (Lee et al. 2016, Sharma et al. 2018) but also improve water quality, ameliorate soil quality and fertility, inhibit soil erosion (Sharma et al. 2018) and inhibit leaching of nitrogen or runoff (Schonbeck 2017). Some cover crops are able to not only assist in sustaining soil pH levels by supplying nitrogen (legumes) but also in decreasing soil compaction (Rackham and McNeilan 1999). These crops can also function as a trap or a sacrificial crop. It can act as a decoy to attract pests and prevent it from damaging the agricultural crop. Therefore, many agriculturists recommend using cover crops as one of the

best and sustainable management practices that farmers can use (Shelton and Badenes-Pérez 2006, Badenes-Pérez 2018).

1.3.2 Crop rotation and intercropping

Both crop rotation as well as intercropping are considered to be some of the best management practices in crop production. These management practices have been utilized in organic farming for some time and more recently in conventional farming as well (PAN 2013). The agricultural practice of cultivating different crops or unrelated crops on the same land in different seasons, is known as crop rotation. Intercropping is when two or more crops are planted at the same time (in the same season) on the same land (Mousavi and Eskandari 2011). This practice is beneficial to the nutrient cycle and decrease the probability of soil erosion from occurring (Ball et al 2005, Edesi et al 2012). Ball et al. (2005) affirms that crop rotation will result in crop harvests of a high yield and quality, with a reduced environmental impact on agroecosystems. Not only does cover crops contribute to the structure, physical-, biological- and chemical characteristics of soil but also in decreasing pest, weed and other disease outbreaks (Mousavi and Eskandari 2011, Riedell et al 2022). Both intercropping and crop rotation will allow farmers to be less dependent on chemical fertilizers (Murphy-Bokern et al. 2017, Anon 2019), reduce numerous risks associated with crop production (Woodfine 2009, Renard and Tilman 2019, Huss et al. 2022) and increase crop yields (as a study that lasted five decades indicated) when compared to monoculture practices (Boincean 2014).

1.3.3 Pesticide management

The use of chemical pesticides to increase crop yield in agriculture is unrestrained. The use of these chemicals must be done in moderation. It is essential that these chemicals only be used as a last resort to protect the crops from pests. Agriculturalists pay careful attention to the selection of pesticides to ensure that it has minimal or no impact on the environment. The chemical solubility, degradation and volatility of the pesticide are considered to establish the possible effect of the chemical on the environment or whether it will leach through the soil.

All instructions and precautions on the label should be strictly followed in terms of application time, storage etc. (Appiah et al. 2018) as improper use of pesticides can result in secondary outbreaks of pests (Gross and Rosenheim 2011, Gill and Garg 2014); non-target

species to be killed (Ware 1980, Andrea et al. 2000); water, air and soil to become contaminated (Bedos et al. 2002, Arias-Estévez et al. 2008) and residues to remain within both primary and derived agricultural products (Osman et al. 2010). Incorrect use of pesticides can negatively affect crop production and yield.

1.3.4 Fertilizer management

It is typical for farmers to implement a management practice known as a “more fertilizer and much water” practice in order to attain higher yields. In this management practice farmers apply more nitrogen fertilizer during the early growth stages of the crop than in later development stages of the crop, which is more important (Zhou et al 2010, Deng et al. 2022). Applying more nitrogen fertilizer than what is most advantageous, in the early growth stages of the crop, will both impede the potential yield and the efficient use of nitrogen as well as increase the possibility of polluting the environment and wastewater (Adeyemi et al. 2020, Diatta et al. 2020). The use of compost and animal manure as organic fertilizer (Branca et al. 2011) should be favoured above the use of chemical fertilizers in order to improve soil structure, texture and fertility (Menalled et al. 2008, LeBlanc et al. 2011).

1.4 Improving yield through genetic manipulation

In utilizing available genetic variation, plant breeders have the ability to create enhanced and better adapted plant phenotypes that will meet human needs. This endeavour has been practiced nonstop for thousands of years. In the beginning farmers were the ones to practice plant breeding whereafter it became a science. Crop quality, diversity and efficiency is the main purpose in the breeding of both agricultural and horticultural crops (Bhargava and Srivastava 2019). The science of breeding crops is a fast-evolving science. This science has utilized current innovations in genetics and biotechnology in order to create improved crop varieties. After the first work on the genetics on the garden pea by Mendel there was a significant increase of interest in plant breeding, especially in breeders that aim at producing cultivars that are more robust and produce higher yields (Bhargava and Srivastava 2019). The Green Revolution that occurred between 1960 and 1980 was as a result of swift improvements through conventional breeding techniques and led to a noteworthy increase in rice and wheat production due to the development of varieties that produce higher yields (Evenson and Gollin 2003, Qaim 2017, Bhargava and Srivastava 2019, Eliazar Nelson et al. 2019).

1.5 Carbon dioxide levels in the atmosphere

The concern of climate change throughout the world has become more significant during recent decades due to the fact that it is hazardous to the environment (Asumadu-Sarkodie and Owusu 2016a, Owusu and Asumadu-Sarkodie 2016). Humans make use of fossil fuels for energy which contributes to the increase of carbon dioxide in the atmosphere. Carbon once stored in plants for eons are now released in a matter of a few centuries (IPPC 2014). Much attention has therefore been directed to the human activities that led to increases in greenhouse gas emissions (GHG). Methane, carbon dioxide, nitrous oxide, water vapour, etcetera are examples of greenhouse gases that intercepts infrared radiation from the sun, which therefore increases the greenhouse effect. The atmospheric carbon dioxide concentration has reached a peak at approximately 421 ppm but is still likely to increase (NOAA 2022). A dramatic increase (66.5%) in the atmospheric concentration of carbon dioxide has occurred since the Industrial Era (1750). The consequences of elevated carbon dioxide levels in the atmosphere on plants can either harm or benefit crops.

1.5.1 Effect of increased carbon dioxide levels in the atmosphere on crops and production

In the last 30 years scientists have found that the Earth has been greening (showed increased vegetation growth) and the primary cause is rising carbon dioxide (Zhu et al. 2016, Cortés et al. 2021) levels. These researchers also found, from their collected data, that our atmosphere has warmed more rapidly than was anticipated. The greenhouse effect is heightened with increased atmospheric levels of carbon dioxide and thus climate change. Climate change has an undeniable effect on agriculture (Noya et al. 2018) for example when a period of drought is followed by heavy rains, the probability of flooding increases thus creating favourable conditions for fungal infections in various plant parts. The frequency of extreme events such as flash floods and droughts is expected to occur more frequently (Thornton and Gerber 2010) and will therefore challenge the stability of agricultural productivity annually (Harrison et al. 2011, Harrison et al. 2012a, Harrison et al. 2012b, Phelan et al 2015). Climate change has also affected bees to such an extent that various local plant species have gone extinct (Ajetomobi 2016).

With the increase of temperature as a result of the heightened greenhouse effect, precipitation patterns will change, lead to drier climates, a decrease in rainfall whilst evaporation will increase (Vanuytrecht et al. 2012). This will affect not only plant water usage and efficiency but will also result in a greater demand for water in agriculture (Gosling and Arnell 2016). Temperature sensitivity in seedlings differs depending on growth stage and with

the increase of temperatures their sensitivity would increase as well as affect their biomass. It is predicted that by 2050 water stress due to climate change will affect twice the area of land than those that will be experiencing reduces water stress (Bates et al. 2010).

1.5.2 Effect of increased carbon dioxide levels in close proximity to plants in terms of crop production

Increased levels of carbon dioxide have a positive effect on plant growth. Elevated carbon dioxide both increase photosynthesis and promote plant water use efficiency (WUE), therefore plant productivity increases. This effect is known as carbon dioxide “fertilization” (Thompson et al. 2004). A summary of the results of FACE (Free Air CO₂ Enrichment) experiments over the last 30 years has been published in Global Change Biology (Ainsworth and Long 2020) and found that C₃ crops would be positively affected in terms of crop productivity. This effect would, however, be reduced in wet conditions as well as in soil with a nitrogen deficit. Crop response under elevated carbon dioxide levels indicated a strong association between crop yield and potential yield (Ainsworth and Long 2020). Initially the increase of carbon dioxide would positively affect plants in terms of photosynthesis, transpiration and plant yield (Long et al. 2005). Elevated carbon dioxide levels, therefore, have the potential to close the yield gap (difference between actual yield and potential yield) but will be dependent and vary between regions (Broberg et al. 2019, Ainsworth and Long 2020).

1.6 Hydrogen peroxide as a growth regulator

Phytohormones, commonly known as plant hormones, are chemicals which are produced by plants to regulate plant growth, reproductive processes, development, plant death etcetera (Dilworth et al. 2017). When plants grow under stressed and/or non-stressed conditions, these phytohormones regulate the growth of the plant and can release the genetic potential of plants when it comes to the yield output. Hydrogen peroxide has been identified as an important natural molecule amongst numerous other non-classical plant growth regulators (Li et al. 2012, Zelinová et al. 2013). Hydrogen peroxide is able to counteract biotic stress (Orozco-Cárdenas et al. 2001, Małolepsza and Różalska 2005) through the restoration of the cellular redox status of plants (Kumar et al. 2013) to achieve growth and development. It also interacts with other phytohormones for example abscisic acid (ABA), nitric acid, brassinosteroids, nitric oxide etcetera (Małolepsza and Różalska 2005, Kumar et al. 2010, Jiang et al. 2012, Liao et al. 2012). The promising roles that reactive oxygen species play in

both the growth and metabolism of plants in general, have led to reveal the notability of hydrogen peroxide in various plant development aspects (Hasan et al. 2016).

1.7 The role of hydrogen peroxide in plants

A signalling molecule such as hydrogen peroxide (a reactive oxygen species) plays an essential role during regular plant metabolism as well as in signal transduction (Karpinski et al. 1999, Dat et al. 2000, Niu and Liao 2016). Hydrogen peroxide plays an incredibly positive role in plants when they are exposed to countless abiotic factors. It has made us realise that it is not only a free radical, but also advantageous in maintaining cellular homeostasis in plants, specifically crop plants (Nazir et al. 2020). During the process of both photosynthesis and respiration, hydrogen peroxide is produced nonstop in plant cells, whilst unessential protective mechanisms strongly control the redox equilibrium (Arencibia et al. 2012). It is therefore essential in regulating photosynthesis, fruit and flower development, stomatal movement, the growth of pollen and leaf senescence, as research has shown over the last 200 years (Nazir et al. 2020). Hydrogen peroxide has a significant effect in both the growth and metabolism of plants in general. This has revealed the notability of hydrogen peroxide in various plant development aspects. It has been accepted that hydrogen peroxide has a dual effect on plants depending on the concentration (Xiong et al. 2015), therefore the species and the genotypic responses of hydrogen peroxide were tested in both abiotic stress as well as normal plant growth conditions (Hasan et al. 2016).

1.7.1 Effect of high and low hydrogen peroxide concentrations

Hydrogen peroxide in high concentrations is damaging to photosynthesis and sets a programmed cell death in motion. Research from Orabi et al. (2015) however, have indicated that treating plants with lower concentrations of H_2O_2 may have noteworthy beneficial effect on plant growth, antioxidant enzyme activity, growth regulators (ABA, Indole-3-acetic acid, Gibberellic acid-3) and fruit yield quality and quantity (antioxidant activity and total soluble solids). They discovered this when growing two tomato cultivars in a cultivation system that uses sand as the main medium under low temperature conditions. When hydrogen peroxide is applied at concentrations lower than 100 mg L^{-1} , improved plant growth (Ahmad et al. 2014, Hasan et al. 2016), improved root growth (Lin and Kao 2001, Foreman et al. 2003, Liao et al. 2009, Li and Jai 2013, Ma et al. 2014, Hernández-Barrera et al. 2015), boosting of the leaf chlorophyll content (Hasan et al. 2016), stomatal aperture controlled (Ge et al. 2015),

increased leaf growth is increased (Deng et al. 2012a , Guzel and Terzi 2013), general control plant metabolism (Gechev and Hille 2005, Carol and Dolan 2006) and many more improvements were observed. In a study conducted by Khandaker et al. in 2012 it was found that the exogenous application of hydrogen peroxide to wax apple trees led to increased physiological activities within the trees. The growth responses of numerous other plants has been proven to be enhanced with the external application of hydrogen peroxide through the regulation of both the physiological and biochemical responses (Ishibashi et al. 2011, Deng et al. 2012b, Ashfaq et al. 2014).

CHAPTER 2

Effect of additional carbon dioxide on the growth and yield of three vegetable crops grown in controlled environments

2.1 Introduction

The increased atmospheric concentration of carbon dioxide (CO₂) is a component of climate change that threatens food production worldwide (Wheeler and Von Braun 2013, IPCC 2014). Elevated temperatures, altered rainfall patterns and increased incidence of extreme temperatures and precipitation are other components of climate change (Wheeler and Von Braun 2013) that interacts with elevated CO₂ to affect vegetable production and food security (Schmidhuber and Tubiello 2007, Bisbis et al. 2018, Gruda et al. 2019b). It is for this reason that the optimization of environmental conditions, to improve vegetable production, became more important to not only farmers but researchers as well (Dong et al. 2018, Gruda et al. 2019a). For more than 100 years, elevated CO₂ levels had been used as an agricultural practice in the production of vegetables in greenhouses (Wittwer and Robb 1964, Mortensen 1994, Gruda et al. 2019b) as CO₂ is known to increase vegetable production in general. It alleviates, therefore the threat from a changing environment on vegetable growth (Long et al. 2004, Long et al. 2006a, Huang and Xu 2015, Parajuli et al. 2019). Vegetable production between 1997 and 2017 increased by approximately 1.8%, from 0.6 to 1.10 billion tonnes (FAO 2018, Zhou et al. 2019). It has been recommended that people incorporate vegetables in their everyday diet since it is a good source of vitamins, minerals, antioxidant and dietary fibre (Hounsborne et al. 2008, Slavin and Lloyd 2012).

A leguminous vegetable crop that is grown throughout South Africa is the green bean (*Phaseolus vulgaris* L.) a common bean variety. Beans are high in both carbohydrates and fibre but also contains vitamins and minerals (calcium, iron, vitamin A, -K, -C, potassium, magnesium, folate etc.) (USDA 2019). Swiss chard (*Beta vulgaris subsp. vulgaris*) is a highly nutritious leafy biennial vegetable, available throughout the year, is low in cost and can be used in multiple dishes (Gao et al. 2009). Both the leaf and stems contain elevated levels of vitamins (A, B and C) and minerals (calcium, iron, phosphorous) (Pyo et al. 2004). In South Africa, the consumption of Swiss chard was encouraged in order to mitigate the widespread nutritional deficiencies that are predominant in rural communities (Faber et al. 2007). A vegetable with increasing importance due to its numerous positive nutritional and physiological properties are red beetroots (*Beta vulgaris*). Apart from its considerable magnesium, potassium and low sodium content (Dóra and Mónika 2022) the vegetable is also a rich source of antioxidants (Chhikara et al. 2019) which are beneficial to human health. It is for this reason that beetroot is also classified as a superfood (Ben et al. 2014, Clifford et al. 2015, Guldiken

et al. 2016, Chhikara et al. 2019). Another benefit in beetroots are that they can be grown nearly everywhere as no additional care is required except thinning (Ceclu and Oana-Viorela 2020). This is due to these characteristics that Swiss chard, beans and beetroot were included as test crops in this and the following chapters.

In 1804, Saussure was the first to report the effect that CO₂ fertilization had on plant productivity (Farquhar et al. 1980). Numerous scientists in agriculture have since conducted in depth research on the utilization and development of carbon dioxide. It is now certain that increased CO₂ concentrations will contribute to increased crop yield (Zhu et al. 2010). A high photosynthetic rate ensures an increase in yield, when other factors are nonlimiting (Long et al. 2006b, Parry et al. 2010, Kant et al. 2012). Only between 5% and 10% of nutrients taken up from the soil are used by the plant for growth and development, whereas photosynthesis produces 90-95% of the plants' energy for yield production (Sharkey et al. 2000, Ainsworth and Rogers 2007). A process that has not only been well researched but also has models of the limitations to its pathway is photosynthesis (Zhu et al. 2010, Paul 2021). The main factor that limits the photosynthetic process is CO₂, due to its low concentrations in both the in vitro atmospheric environments and the carboxylation sites within leaves (Amthor 2001). Numerous scientists have proved that photosynthesis in plants can be increased by elevated CO₂ in the environment through genetic enhancement experiments (Jablonski et al. 2002, Hasegawa et al. 2013, Flexas 2016). In greenhouse grown vegetables such as tomatoes, lettuce and cucumbers, CO₂ was applied. Carbon dioxide dosing was applied to approximately 80% of greenhouses in 1995 (Esmeijer 1999). In the last few decades, a CO₂ gas fertilizer (AGROSOL) was developed and applied to crops grown in the field. This type of fertilization is based on the gas fertilizer entering the stomata on the leaves, reacting to the acidic environment within the stomata which then leads to the slow release of CO₂ in order to improve plant growth. There is room for more research regarding the mechanisms of CO₂ fertilization in photosynthesis.

The application of additional CO₂ in bursts as a form of CO₂ fertilization in close proximity to individual plants could therefore potentially increase not only plant growth but also yield. It is however expected that different crops will respond differently to the CO₂ fertilization therefore the inclusion of a leafy, fruiting and rooting vegetable crop in this study. The aim of this chapter was to investigate the effect of releasing CO₂ fertilization in small bursts in a glasshouse pot trial, will have on plant growth and yield. Swiss chard, beetroot and beans were used as test crops. It is expected that the beans will produce more bean pods than the plants grown at ambient CO₂. It is expected that the Swiss chard plants will have a greater biomass and photosynthetic rates when additional CO₂ is applied. The beetroot will have

greater stomatal densities and conductance than any of the other crops treated with the additional CO₂.

2.2. Methodology

2.2.1 Location and type of glasshouse

This study was conducted at Innovation Africa @UP (Previously known as the Hatfield Experimental farm, University of Pretoria) (25°45'06.4"S 28°15'33.5"E). One CO₂ chamber (CONVIRON) and two glasshouse structures were used. One glasshouse was equipped with a rotary table, an evaporative cooling pad system as well as a drip irrigation system. The other glasshouse was only equipped with a ventilation cooling system and required the plants to be watered by hand. Both glasshouses had a north-south orientation.

2.2.2 Experimental design

The preliminary trial consisted of a single vegetable crop having six treatments each, namely: A (700ppm, elevated CO₂ chamber), B (ambient CO₂, no treatment), C (1 whole CO₂ tablet), D (half a CO₂ tablet), E (15 ml of calcium carbonate and 30 ml HCl), F (7.5 ml of calcium carbonate and 15 ml of HCl) and five replicates within each treatment.

The second trial consisted of three vegetables crops having six treatments each, namely: A (700ppm, elevated CO₂ chamber), B (ambient CO₂, no treatment), D (8 g of calcium carbonate and 30 ml HCl), D (4 g of calcium carbonate and 15 ml of HCl) and E (ambient CO₂ chamber).

Treatment tests and application

The intend was to produce a slow CO₂ releasing fertilizer from scratch and use it in this trial to be applied as a foliar spray onto the leaves of the plants, however it was too expensive to make and COVID also did not make it easy. Therefore a different and cheaper method was developed for use in this study by which CO₂ was released close to the leaves of the plants using HCl and CaCO₃.

For the **preliminary trial**, only bush beans (STAR 2000) were used. Seedling cultivation began on 24 May 2021 by planting 105 seeds of bush beans in seedling trays filled with a sand and coir mixture (6:1 ratio). About 2 weeks after emergence, the seedlings were transplanted/replanted into 4 litre plastic pots filled with the same soil mixture and placed on a rotary table in the glasshouse. The seedling were given approximately 2 weeks to establish before the preliminary testing of the methodology commenced. As treatments, half a tablespoon (E) and a full tablespoon (F) of CaCO₃ were placed in crescent shaped

indentations (1 cm deep), made approximately 2 cm from the edge of the 4 L pot. Hydrochloric acid (HCl) was diluted with water (in different ratios) to test the rate at which CO₂ is released when mixed with calcium carbonate ($\text{CaCO}_3 + 2\text{HCl} \rightarrow \text{CaCl}_2 + \text{H}_2\text{O} + \text{CO}_2$). The ratio of one-part hydrochloric acid to six parts water allowed for more HCl to react to the calcium carbonate, which released more CO₂. The diluted HCl was slowly dripped onto the CaCO₃ with a pipette until no reaction was observed. The CO₂ tablet treatments (C and D) were applied in the same manner as stated above. In the second week of treatments, the stems of the beans that were treated with the CO₂ tablets and the CaCO₃ started to turn a brownish colour and no new growth from the apical meristem was observed. The soil EC was then measured and proofed to be too high to be read by the EC meter. Once it was established that the soil EC could be affecting plant growth, the soil was immediately irrigated with clear water to leach the substrate. This was done two times. The pots were then left to dry out normally. Two days later the EC was tested again and still too high to read. The leaching method was applied again before another EC measurement was taken. After the last EC measurement it was established that the CO₂ and CaCO₃ treatments acidified the soil which resulted in the high EC readings. Treatments C to F were applied for three weeks total and the plants in these treatments terminated at the end of the fourth week. The plants in the other two treatments (A and B) were terminated three weeks later.

The **second trial** consisted of same vegetable crops (beetroot – Crimson Globe, beans – Star 2000 and Swiss chard – Fordhook Giant) having five treatments each, namely: A 700ppm (elevated CO₂ chamber), B (ambient CO₂, no treatment), C (8 g of calcium carbonate and 16ml HCl), D (4 g of calcium carbonate and 8ml of HCl), E (ambient CO₂ chamber, 418 ppm) with five (C, D) and ten (A, B and E) replicates within the respective treatments. Treatments C and D were applied three times a week in the glasshouse with the rotary table. The plants that received no treatment (B) were grown in a separate glasshouse with stationary table, to prevent contamination with CO₂. Only the beans received a fifth treatment (E) as the availability and space was within the CO₂ growth chambers were limited.

On 21 June 2021, 105 seeds of each of the following vegetable crops, Swiss chard, beetroot and bush beans were sown into seedling trays and transplanted/replanted to 4 L plastic pots as described above. The treatment application also commenced 2 weeks after being transplanted/replanted into the pots. In the second trial the reactions took place in clear Boston round glass bottles. The CaCO₃ were weighed a week in advance and placed in small plastic pouches. Two clear bottles were planted into the soil next to the plants and received 4 g CaCO₃ treated with 8 ml of diluted HCl in each glass bottle for treatment C or 2 g CaCO₃

and 4 ml of HCl in each glass bottle for treatment D. For the CO₂ tablet treatments, the same method was used i.e., the whole tablet treatment had half a tablet in each of the glass bottles with 20 ml of water and the half tablet treatments had a quarter of the tablet in each glass bottle and 10 ml of water. For both trials the treatments were applied three times a week. Between treatments, the glass bottles were removed, cleaned and returned to the pots. For the duration of the trial, the plants were irrigated with water once a week with only tap water and twice a week with a Hydroponic fertilizer (1 g L⁻¹). The water was applied either through a drip irrigation system (treatments A, C, D and E) or by hand (B). Due to COVID 19, the demand for the CO₂ tablets could not be met by the supply. The CO₂ tablets were mainly imported from China with no local manufacturers. Therefore the CO₂ tablet trial had to be terminated within the first week of applying the treatments. The rest of the trial was not affected by supply issues and was terminated 10 weeks after the first treatments were applied.

Weather data

Certain weather measurements were taken over the duration of the trial as indicated in Table 2.1. In the glasshouse with the rotary table, the apparatus that measures the temp and humidity was broken and therefore no measurements were recorded.

Table 2.1: The conditions in which the plants grew during the study.

	Ambient CO ₂ glasshouse			Elevated and ambient CO ₂ chamber	Rotary table glasshouse
	Jul 2021	Aug 2021	Sep – 2 Oct 2021	Jul – Sep 2021	Jul – Sep 2021
Max temp (°C)	25.8	27.6	34.3	25	*
Min temp (°C)	10.3	12.2	15.0	16	*
Max Relative humidity (%)	65.0	70.7	64.3	50	*
Min Relative humidity (%)	24.0	28.3	24.3	50	*

2.2.3 Measurements

Various measurements were taken over the duration of the trial as indicated in Table 2.2. The bean plants produced bean pods over a period of time and those were harvested as they matured (***), while the beetroot and Swiss chard were only harvested once (*) at the end of the trial. Measurements associated with plant growth and photosynthesis were collected once a week (**).

In this dissertation, the term beet will refer to the edible root part of the beetroot plant, while biomass will refer to the leaf (Swiss chard and beetroot) or stem and leaf material (bean plants). The stem portion of the Swiss chard and beetroot were very small at harvest and ended up being part of the leaves and roots (Swiss chard) or beets (beetroot).

Table 2.2: All the measurements taken throughout the second trial.

	Beans	Beetroot	Swiss chard
Height	**	**	**
Photosynthesis	**	**	**
Stomatal conductance	**	**	**
Chlorophyll and carotenoid content	**	**	**
Stomatal density	**	**	**
Pod length and width	***	NA	NA
Number of seeds per pod	***	NA	NA
Beet length and width	NA	*	NA
Pod dry mass	*	NA	NA
Beet dry mass	NA	*	NA
Root length	*	*	*
Root dry mass	*	*	*
Biomass	*	*	*
General observations	**	**	**

* Measurements taken once trial was terminated, ** measurements taken weekly, *** measurements taken per harvest. NA not applicable

Plant height, root length, pod and beet length and width

The plant heights were measured weekly (Table 2.2, **). This variable was measured in centimetres (cm) using a ruler, placed vertically on the surface of the soil. The measurement was taken at the highest stem meristem for the beans, from the soil surface to the tip of the

longest leaf for Swiss chard and from the crown to the leaf tip of the longest leaf for the beetroot. The length and width of the bean pods and beets were measured per harvest and at the end of the trial, respectively, with a Vernier Caliper. The root length of the beetroot plants did not include the edible part of the plants.

Mass measurements

The fresh and dry mass of the bean pods, beets, biomass and root mass of the plants were weighed after each harvest and at the end of the trial. The plants were placed into labelled paper bags after the fresh weight and all other measurements of the pods and beets were taken and dried in an oven at 70°C for 2-3 days. After the samples have dried they were weighed again to measure the dry mass.

Leaf sample selection and analysis

Two samples of mature leaves were taken (from 2 different plants of the same crop) from each crop and treatment (12 samples per vegetable crop) in a randomised manner. Only mature leaves were taken for the samples for further analysis. The leaf samples were collected once a week between 10:30 and 11:30 am and placed in labelled plastic bags before immediately being taken to the laboratory.

Chlorophyll concentration: For the chlorophyll concentration measurements, four 1.5 cm discs were taken, mixed with acetone in a 15 ml centrifuge tube and crushed with a glass rod to release the chlorophyll. The acetone mixture was then left in a cooling chamber for one hour. It was then centrifuged at 2500 rpm for five minutes. The samples were then taken, analysed spectrophotometrically (a UV/Vis Spectrophotometer) and the absorbance determined at 470 nm, 647 nm, 663 nm wavelengths. At the end of every analyses the concentration of chlorophyll A, B and carotenoids were calculated using the following equations:

- $C_a (\mu\text{g ml}^{-1}) = 12.25(A_{663}) - 2.79(A_{647})$
- $C_b (\mu\text{g ml}^{-1}) = 21.50(A_{647}) - 5.10(A_{663})$
- $C_{x+c} (\mu\text{g ml}^{-1}) = ((1000 \times A_{470}) - (1.82 \times C_a) - (85.02 \times C_b))/198$

Where C_a represents chlorophyll A, C_b chlorophyll B, and C_{x+c} total carotenoids (i.e. carotenes plus xanthophylls).

Stomatal density: An area of approximately 2 cm^2 at the top of the same leaf samples were coated with clear nail polish (to get an impression of leaf epidermis), left to dry and peeled off using tweezers. The epidermal impression was then stuck to the microscope slide with a drop of glycerol (with an alcohol binding in it). The stomatal density was counted in three different 1 cm^2 squares by looking through a graticule eyepiece at 10x magnification of a light microscope.

Photosynthesis measurements

The LiCor 6800 was programmed beforehand to measure photosynthetic rate and stomatal conductance of the plants. Only a single LiCor 6800 was available for measurements between three students running their trials simultaneous. This led to the depletion of the CO_2 cylinders at a faster rate than expected and resulted in two weekly measurements to not be recorded. Using the Licor was a steep learning curve and unfortunately, as first time user, it led to some readings that were not usable, and thus resulting in some missing values in the data.

2.2.4 Statistical analysis

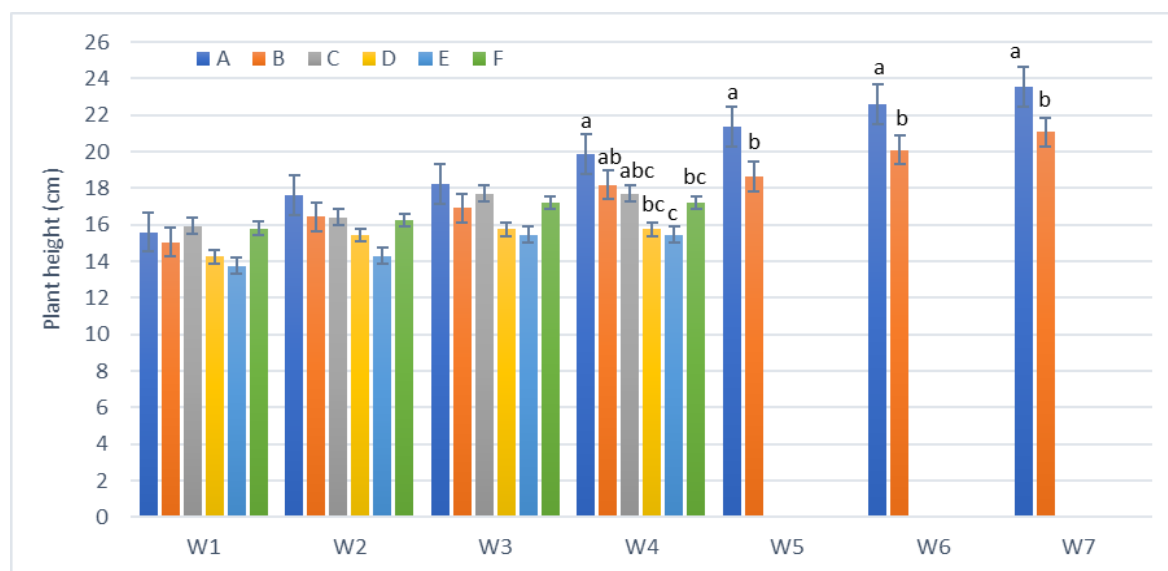
The data was analysed using the statistical package SAS (model 9.4) to test if the means were significantly ($P < 0.05$) affected by the treatments. The Tukey LSD test was used to determine if there was a significant difference at a 5% probability. The ANOVA tables are included in the Appendices A and B. The graphs in the results section were drawn using the mean yield per treatments.

2.3 Results

Preliminary pot trial

2.3.1 Bean plant height

Throughout the first three weeks of the study, no significant difference in terms of plant height occurred between the different treatments (Figure 2.1). Despite this, some trends emerged with the plants grown in elevated CO₂ (A) resulting in the tallest plants followed by those grown in the ambient CO₂ glasshouse (B). During the fourth week of the study, the first significant differences were observed. The bean plants that were grown in the elevated CO₂ chamber (A) resulted in plants that were only significantly taller than the plants that were treated with half a carbon dioxide tablet (D) as well as the plants treated with the high and low CaCO₃ treatments (E and F, respectively). The plants in treatment D, E and F did not differ significantly in terms of plant height. Due to toxic levels of salts (high EC) all plants treated with the CO₂ tablets (C and D) as well as the CaCO₃ (E and F) died and only the plants in the elevated and ambient CO₂ conditions survived. During the last three weeks of the trial the plants grown in the elevated CO₂ chamber (A) were significantly taller than the plants grown in the ambient CO₂ glasshouse (B).

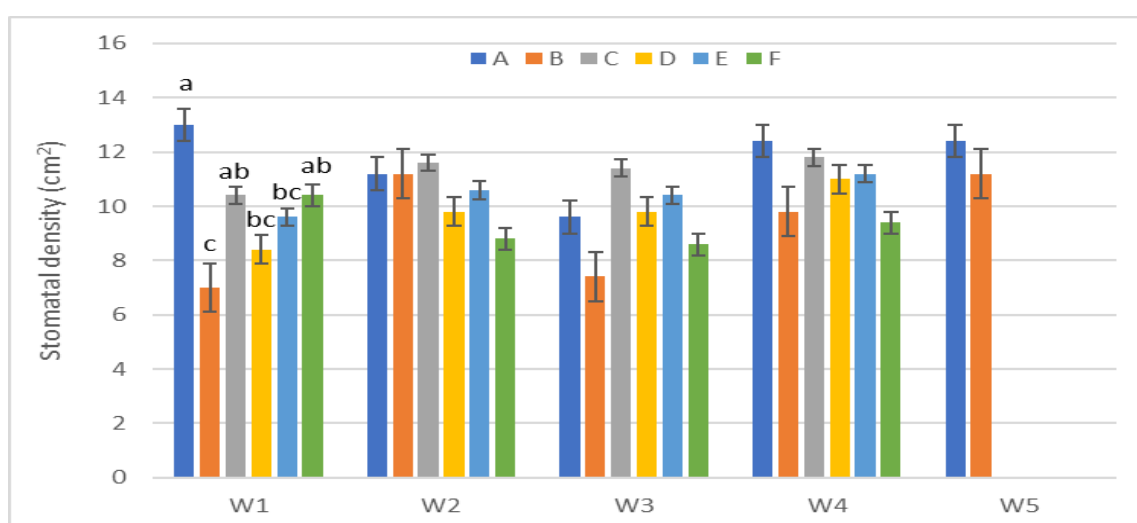


A to F represents the different treatments applied in the trial and W1-W7 represents weeks one to seven. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 1 whole CO₂ tablet, three times a week; D = ½ a CO₂ tablet, three times a week; E = 15 ml of calcium carbonate and 30 ml HCl, three times a week; F = 7.5 ml of calcium carbonate and 15 ml HCl, three times a week. Bars of the same week and no letter indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments.

Figure 2.1: Weekly changes in the plant height of beans exposed to different carbon dioxide treatments.

2.3.2 Stomatal density

The stomatal densities throughout the trial fluctuated in all the treatments (Figure 2.2). The plants that grew in the elevated CO₂ chamber (A) consistently had the highest stomatal density compared to the plants grown in the ambient CO₂ glasshouse (B). The plants treated with the whole CO₂ tablet (C) consistently had a stomatal density greater than the plants that received the half CO₂ tablet treatment (D). The plants treated with the high (15 ml) CaCO₃ (E) and low (7.5 ml) CaCO₃ had the same pattern as that of treatment C and D. Between the whole CO₂ tablet treatment (C) and the high CaCO₃ treatment (E), the former treatment consistently resulted in a higher stomatal density than treatment (E).

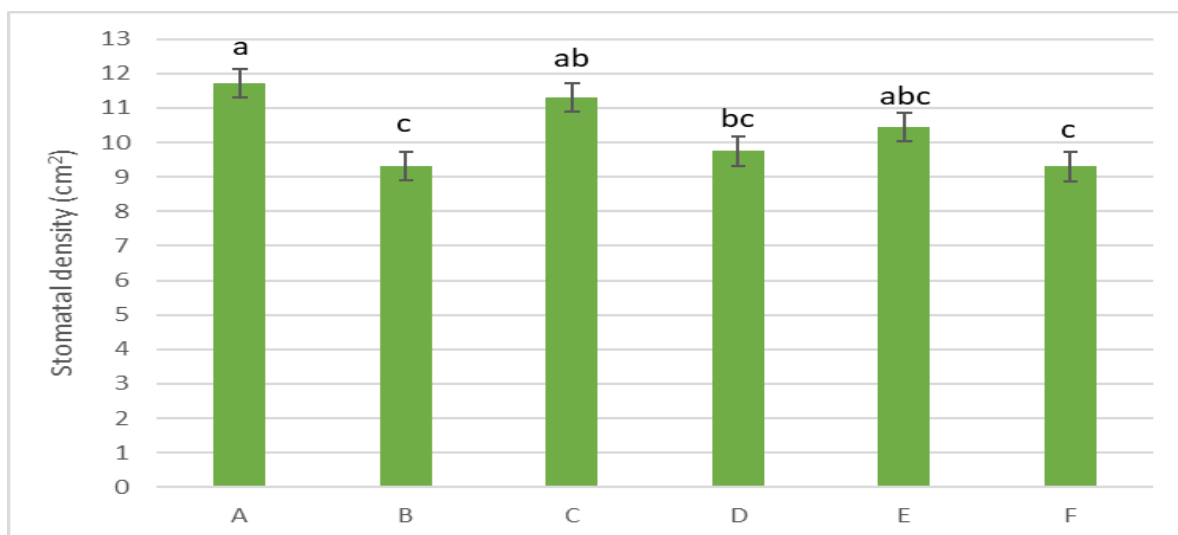


A to F represents the different treatments applied in the trial and W1-W5 represents weeks one to five. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 1 whole CO₂ tablet, three times a week; D = ½ a CO₂ tablet, three times a week; E = 15 ml of calcium carbonate and 30 ml HCl, three times a week; F = 7.5 ml of calcium carbonate and 15 ml HCl, three times a week. Bars of the same week and no letter indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments.

Figure 2.2: Effect of different carbon dioxide treatments on the weekly stomatal density of bean leaves.

Average stomatal density per treatment

The plants grown in the elevated CO₂ chamber (A) had an average stomatal density (Figure 2.3) which was significantly higher than all the other treatments, except those treated with the whole CO₂ tablet (C) and the high CaCO₃ treatment (E). The stomatal density of the plants treated with half a CO₂ tablet had an average stomatal density not significantly higher than the plants grown in the ambient CO₂ glasshouse or treated with the low CaCO₃ treatment.

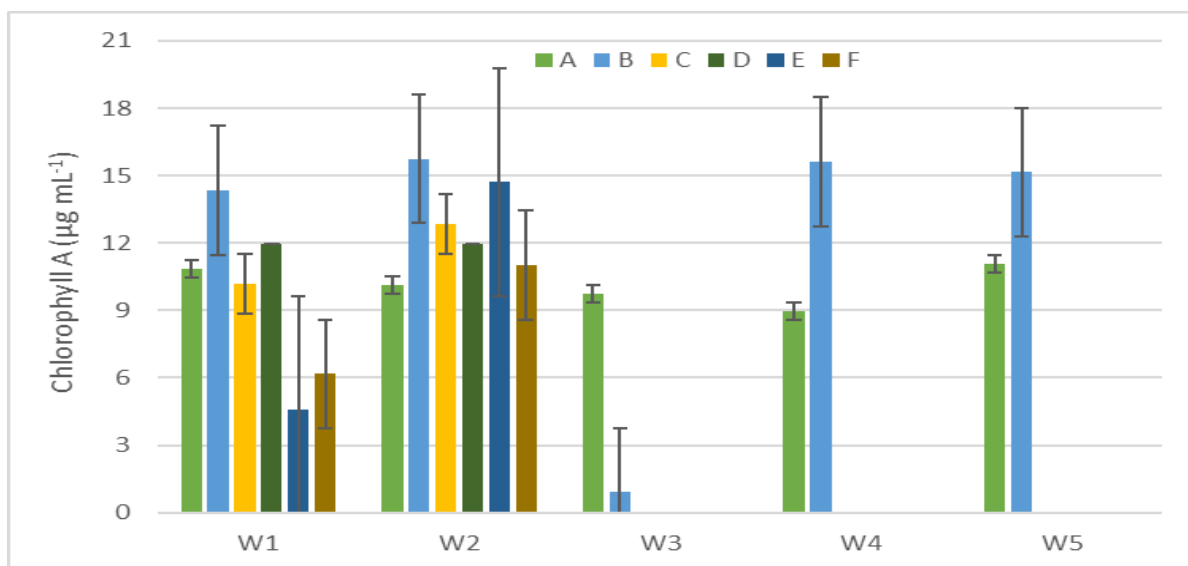


A to F represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 1 whole CO₂ tablet, three times a week; D = ½ a CO₂ tablet, three times a week; E = 15 ml of calcium carbonate and 30 ml HCl, three times a week; F = 7.5 ml of calcium carbonate and 15 ml HCl, three times a week. Bars with different letters indicates significant differences between treatments ($P < 0.05$).

Figure 2.3: Effect of different carbon dioxide treatments on the average stomatal density of bean leaves per treatment.

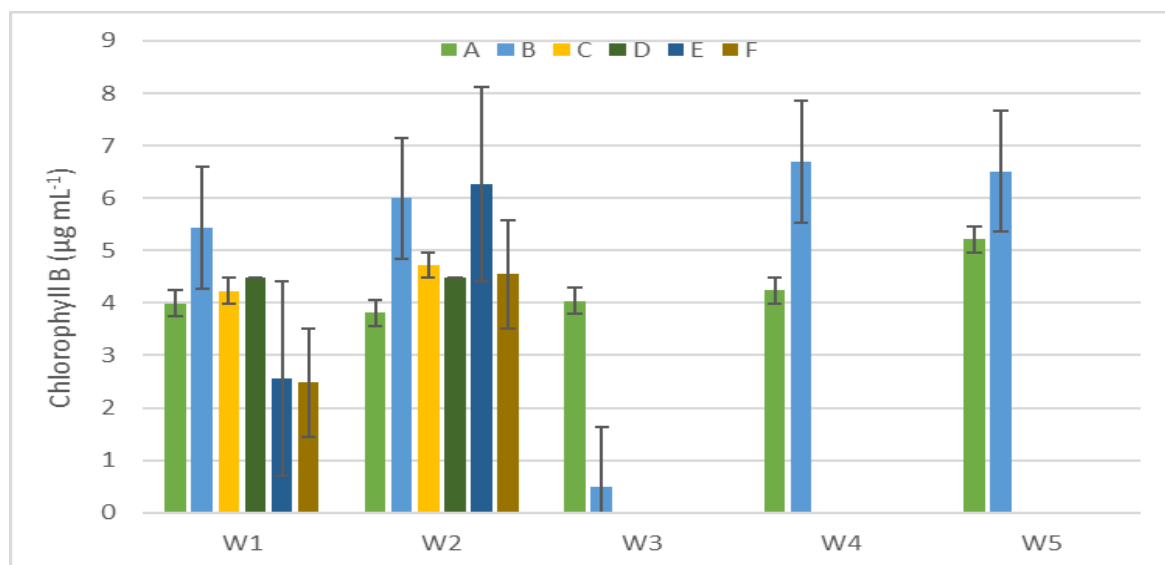
2.3.3 Chlorophyll and total carotenoid concentration

The plants grown in the ambient CO₂ glasshouse (B) consistently had the highest chlorophyll A and B concentration (Figure 2.4 and 2.5, respectively) except in week 3. The total carotenoid concentration of the plants in treatment B had a similar trends than both the chlorophyll A and B with the plants, except during week 2 and 3 where the plants in treatment B had the lowest total carotenoid concentration. The [chl A] of the plants that grew in the elevated CO₂ chamber (A) fluctuated throughout the trial whereas the [chl B] stayed relatively the same. The total carotenoid concentration (Figure 2.6) of the latter treatment increased throughout the trial. For the plants treated with the CO₂ tablets and the CaCO₃ treatments no definitive pattern can be discerned as the plants did not have any new leaf growth from which leaf samples could be taken for analysis after week two. In the first two weeks of the trial the plants treated with the whole CO₂ tablet (C), and high and low CaCO₃ concentrations (E and F, respectively) increased in both [chl A] and [chl B]. The total carotenoid concentration of the latter treatments stayed relatively the same. The plants treated with half a CO₂ tablet (D) showed a similar pattern in its [chl A], [chl B] and carotenoid concentration as compared to the carotenoid concentrations of treatments C, E and F which differed.



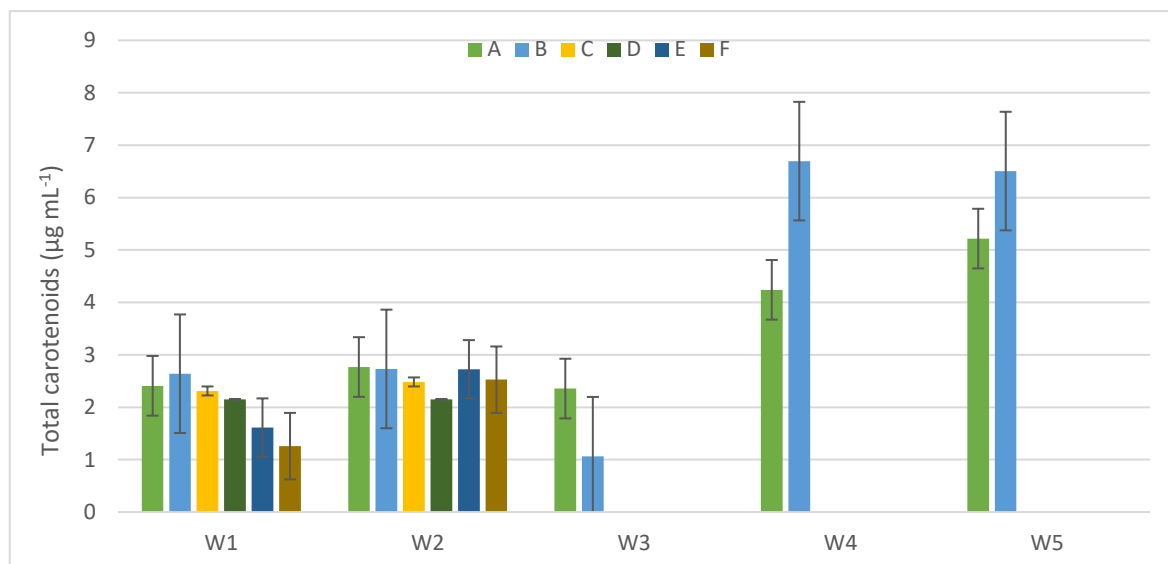
A to F represents the different treatments applied in the trial and W1-W5 represents weeks one to five. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 1 whole CO₂ tablet, three times a week; D = ½ a CO₂ tablet, three times a week; E = 15 ml of calcium carbonate and 30 ml HCl, three times a week; F = 7.5 ml of calcium carbonate and 15 ml HCl, three times a week.

Figure 2.4: Weekly changes in chlorophyll A in bean leaves treated with different carbon dioxide treatments.



A to F represents the different treatments applied in the trial and W1-W5 represents weeks one to five. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 1 whole CO₂ tablet, three times a week; D = ½ a CO₂ tablet, three times a week; E = 15 ml of calcium carbonate and 30 ml HCl, three times a week; F = 7.5 ml of calcium carbonate and 15 ml HCl, three times a week.

Figure 2.5: Weekly changes in chlorophyll B in bean leaves treated with different carbon dioxide treatments.



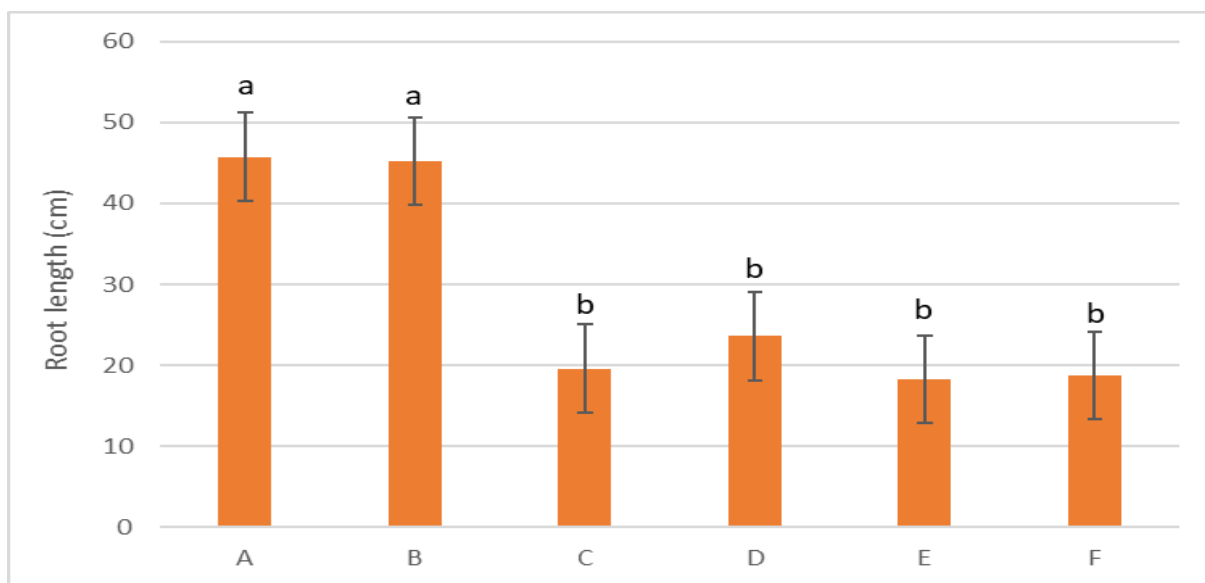
A to F represents the different treatments applied in the trial and W1-W5 represents weeks one to five. A = elevated CO_2 chamber (700 ppm); B = ambient CO_2 (in a glasshouse); C = 1 whole CO_2 tablet, three times a week; D = $\frac{1}{2}$ a CO_2 tablet, three times a week; E = 15 ml of calcium carbonate and 30 ml HCl, three times a week; F = 7.5 ml of calcium carbonate and 15 ml HCl, three times a week.

Figure 2.6: Weekly changes in the total carotenoid concentration in bean leaves treated with different carbon dioxide treatments.

2.3.4 Biomass and Root data

Root length

The plants that were treated with carbon dioxide in the elevated CO_2 chamber (A) and those grown in the ambient CO_2 glasshouse (B) resulted in the longest roots and did not differ significantly from one another (Figure 2.7). The latter treatments, however, had roots that were significantly longer than those that received carbon dioxide through a whole CO_2 tablet (C), half a CO_2 tablet (D), 15 ml of CaCO_3 (E) and 7.5 ml of CaCO_3 (F). The latter treatments (C to F) did not differ significantly from one another in terms of root length.

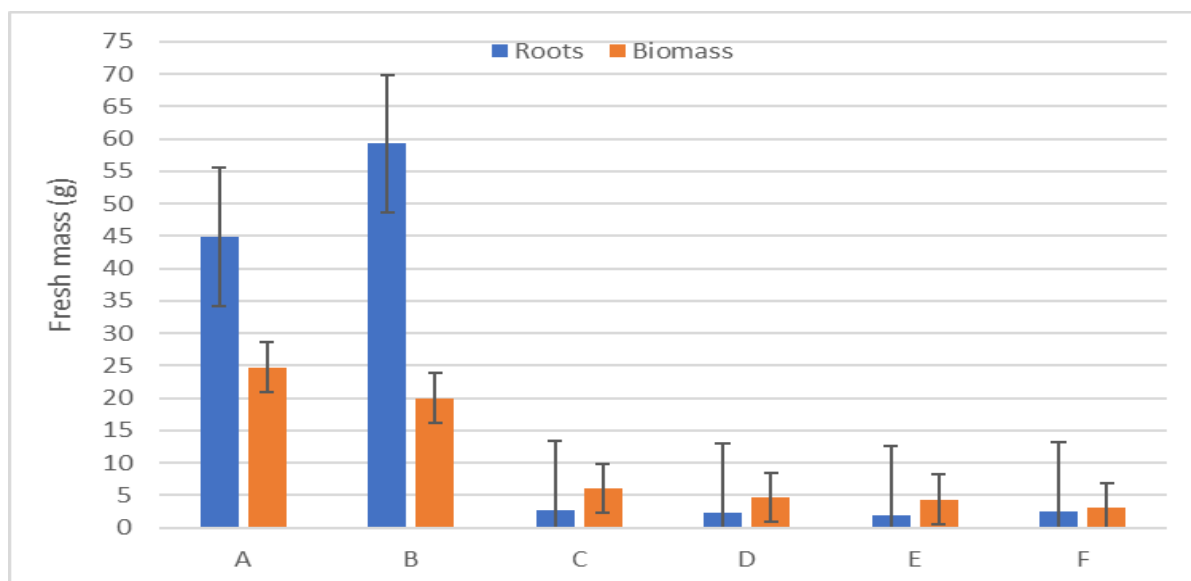


A to F represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 1 whole CO₂ tablet, three times a week; D = ½ a CO₂ tablet, three times a week; E = 15 ml of calcium carbonate and 30 ml HCl, three times a week; F = 7.5 ml of calcium carbonate and 15 ml HCl, three times a week. Bars with different letters indicates significant differences between treatments (P<0.05).

Figure 2.7: Effect of the different carbon dioxide treatments on the root length of beans.

Fresh root and biomass

The root fresh mass (Figure 2.8) of the plants that were grown in the glass house at ambient CO₂ (B) were significantly heavier than the roots in any of the other treatments. The plants grown in the elevated CO₂ chamber had the second heaviest roots followed by the roots of the plants treated with a whole and half of a CO₂ tablet (C and D, respectively) as well as those treated with the high and low CaCO₃ treatments (E and F, respectively). The biomass of the plants grown in the elevated CO₂ chamber (A) were the heaviest (25 g) among all the treatments, on average, followed closely by the plants grown in the ambient CO₂ glasshouse (B) at 20 g. Despite the fact that the plants in treatment B having heavier roots than those in treatment A, the biomass of the plant in treatment A were heavier than those in treatment B. The fresh biomass of the plants treated with the CO₂ tablets and CaCO₃ (C, D, E and F) did not differ a lot between the treatments but were twice to three times as heavy as the roots. The root mass of the plants grown in the ambient CO₂ glasshouse (B) were three times heavier than its biomass whilst the root mass of the plants in the elevated CO₂ chamber (A) were almost twice as heavy as the biomass.



A to F represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 1 whole CO₂ tablet, three times a week; D = ½ a CO₂ tablet, three times a week; E = 15 ml of calcium carbonate and 30 ml HCl, three times a week; F = 7.5 ml of calcium carbonate and 15 ml HCl, three times a week.

Figure 2.8: Effect of different carbon dioxide treatments on the average fresh root mass and biomass of bean plants per treatment.

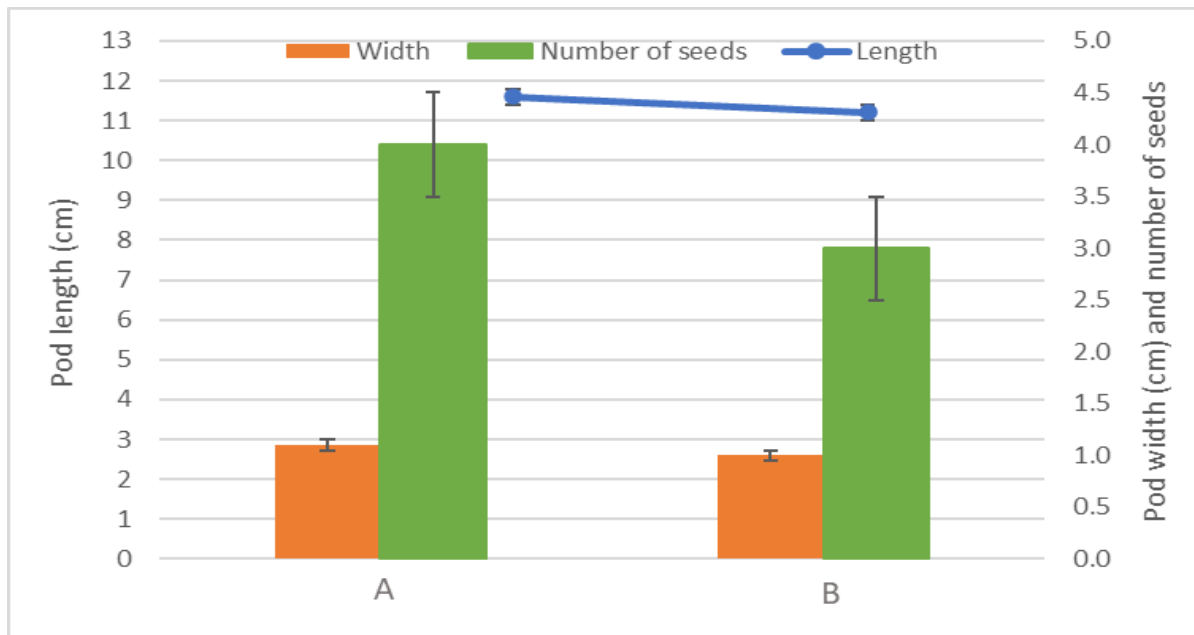
2.3.5 Yield

Bean pod length, width and number of seeds

In this first trial of the study, only the plants that were treated in the elevated CO₂ chamber (A) and those treated at ambient CO₂ glasshouse (B) produced bean pods (Figure 2.9). The longest pods were harvested from the plants that grew in the elevated CO₂ chamber (A) but they were not significantly longer than those harvested from the plants in the ambient CO₂ glasshouse (B). The pod widths did not differ significantly between treatments nor significantly in terms of the number of seeds (Figure 2.6). The pods of the plants that grew in the elevated CO₂ chamber (A) had one seed more (on average) than those in the ambient glasshouse (B) but did not differ significantly between treatments.

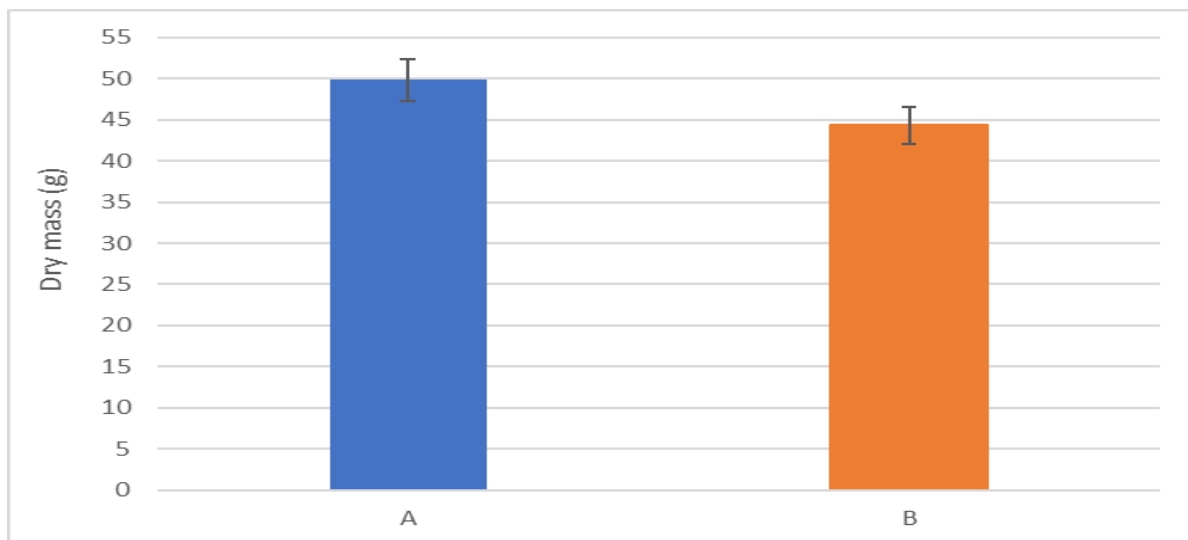
Dry mass

Throughout the trial (Figure 2.10), the plants that grew in the elevated CO₂ chamber (A) had a yield only slightly heavier than the yield obtained from the plants grown in the ambient CO₂ glasshouse (B) but did not differ significantly.



A to B represents some of the different treatments applied in the trial. A = CO₂ chamber (700ppm); B = ambient CO₂ (in a glasshouse). Bars and lines with no letters indicates no significant differences between treatments ($P < 0.05$).

Figure 2.9: Effect of different carbon dioxide treatments on the average length, width and number of seeds in the pods harvested in each treatment.



A and B represents some of the different treatments applied in the trial. A = CO₂ chamber (700ppm); B = ambient CO₂ (in a glasshouse).

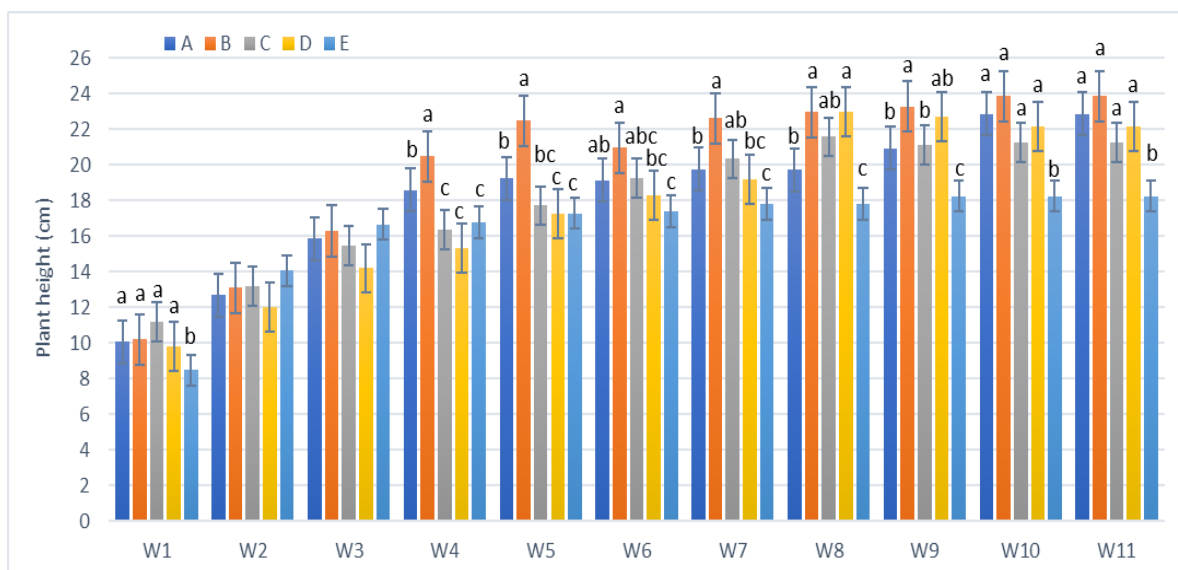
Figure 2.10: Effect of different carbon dioxide treatments on the average yield dry mass per treatment

Second pot trial

2.3.6 Plant height

Beans

During the first week of the trial (Figure 2.11) the plants that were grown in the elevated CO₂ chamber (A), in the glasshouse at ambient CO₂ (B), treated with 8 g CaCO₃ (C) and 4 g calcium carbonate (D) did not have a significant difference in plant height. The plants of these treatments were, however, significantly taller than the plants grown in the ambient CO₂ chamber (E). During week 2 and 3 of the trial, there was no meaningful difference in plant height between any of the treatments. In week 4, the plants grown in glasshouse at ambient CO₂ (B) were significantly taller than those treated with CaCO₃ (C and D) or grown in the elevated and ambient CO₂ chamber (A and E). Over time the difference in plant heights between the plants in the ambient CO₂ (A) treatment versus treatments C, D and E became smaller and at the end of the trial only the plant height of treatment E was significantly lower than that of all the other treatments. Initially (weeks 4 to 6) there was no significant difference in plant height between the CaCO₃ (C and D) treatments and those grown in the ambient CO₂ chamber (E), but over time this changed. From week 8 till the end of the trial, the CaCO₃ treated plants became and stayed significantly taller than those plants grown in the ambient CO₂ chamber (E). While the higher CaCO₃ (C) treatment initially (week 1 to 7) resulted in slightly taller plants than those of the lower CaCO₃ treatment (D), it changed from week 8 and stayed true till the end of the trial with the higher CaCO₃ (C) having slightly shorter plants than those that received the lower CaCO₃ (D) treatment. These differences were, however, never significant.



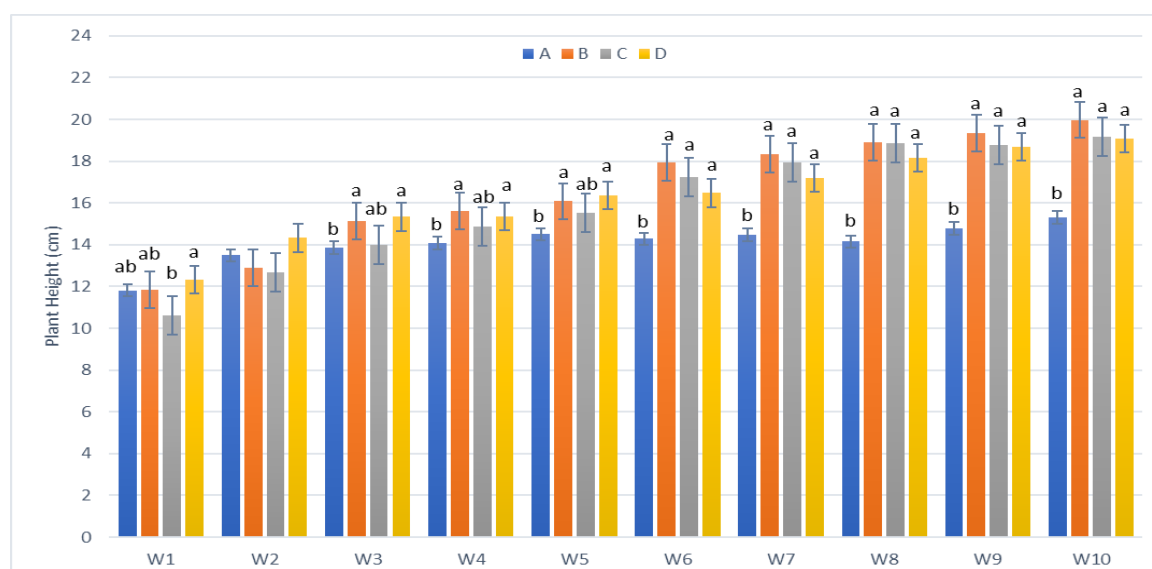
A to E represents the different treatments applied in the trial and W1-W11 represents weeks one to eleven. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars of the same week and no letter indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments.

Figure 2.11: Weekly changes in plant height of beans treated with carbon dioxide.

Beetroot

In the first week of the trial the beetroot plants (Figure 2.12) that received the 4 g CaCO₃ treatment (D) resulted in the tallest plants, however they were not significantly taller than the plants that grew in the elevated CO₂ chamber (A) nor the plants that grew in the glasshouse at ambient CO₂ (B). Over time the difference in plant heights between the plants that received the 4 g CaCO₃ (D) treatment versus those that received the 8 g of CaCO₃ (C) became smaller and at the end of the trial only the plant height of treatment A was significantly lower than that of all the other treatments. From week 3 to 11, the plants grown in glasshouse at ambient CO₂ (B) were significantly taller than those grown in the elevated CO₂ chamber (A) but not those treated with CaCO₃ (C and D). Initially the plants that received the high (8 g) CaCO₃ treatment (C) did not result in plants that were significantly taller than those grown in the elevated CO₂ chamber (A). From week 6 to 11 the plants in treatment C, however, was now significantly taller than those in treatment A. The beetroot plants reacted differently to the treatments as compared to the bean plants. The two main differences are that while the plants in the elevated CO₂ chamber (A) initially did not differ significantly, the plants did become significantly shorter as compared to the other treatments (B, C and D) from week 6 onwards. For the bean crop the elevated CO₂ chamber often resulted in the second tallest plants. The second difference is that there is no significant differences in plant height between the plant

grown in the ambient glasshouse (B) and those exposed to additional CO₂ (C and D). The differences in plant height for the latter three treatments for the beetroot were small. On the other hand in the bean crop, the plants of these treatments showed bigger differences in height.



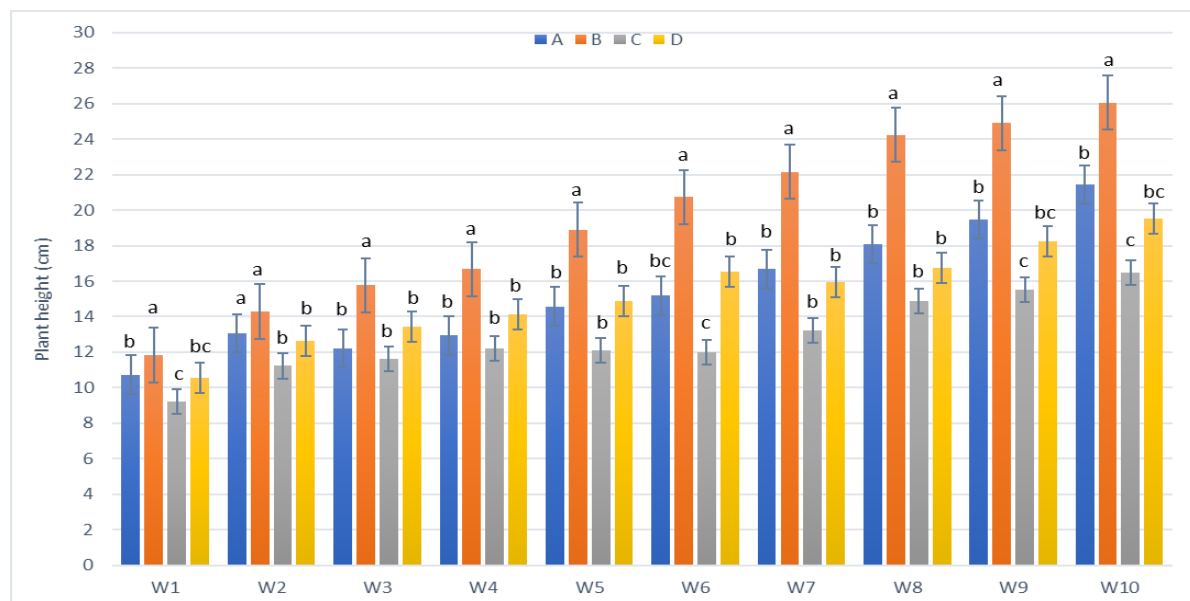
A to E represents the different treatments applied in the trial and W1-W10 represents weeks one to ten. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week. Bars of the same week and no letter indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments.

Figure 2.12: Weekly changes in plant height of beetroot plants treated with carbon dioxide.

Swiss chard

Throughout the trial, the Swiss chard plants (Figure 2.13) grown in the glasshouse at ambient CO₂ (B) were significantly taller than the plants in any of the other treatments. The plants exposed to the high (8 gram) CaCO₃ treatment (C) resulted in the shortest plants but from week 3 to 8 they were not significantly shorter than those grown in the elevated CO₂ chamber (A). The plants in treatment C for most of the trial were not significantly shorter than those treated with the low CaCO₃ (D) treatment. Initially (week 1 and 2) the plants grown in the elevated CO₂ chamber (A) were taller than those treated with the low CaCO₃ (D) treatment, however this from week 3 to 6 the plants of treatment D were taller. In the last four weeks of the trial this changed again with the plants in treatment A being taller than those of treatment D, however the differences in height was never significant. All three crops (beans, beetroot and Swiss chard) had the tallest plants when they were grown in the ambient CO₂ glasshouse (B). Both the beans and Swiss chard treated with the high (C) and low (D) CaCO₃ treatment

had similar reactions in terms of plant height (with the treatment D having slightly but not significantly taller plants than those of treatment C).



A to E represents the different treatments applied in the trial and W1-W10 represents weeks one to ten. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week. Bars of the same week and no letter indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments.

Figure 2.13: Weekly changes in plant height of Swiss chard plants treated with carbon dioxide.

2.3.7 Stomatal density

The stomata (Figure 2.14) on the bean leaves were more abundant closer to the leaf veins whereas the stomata of the beetroot and Swiss chard leaves were scattered throughout the leaf. The beetroot stomata tended to be small and further apart than that of the Swiss chard.

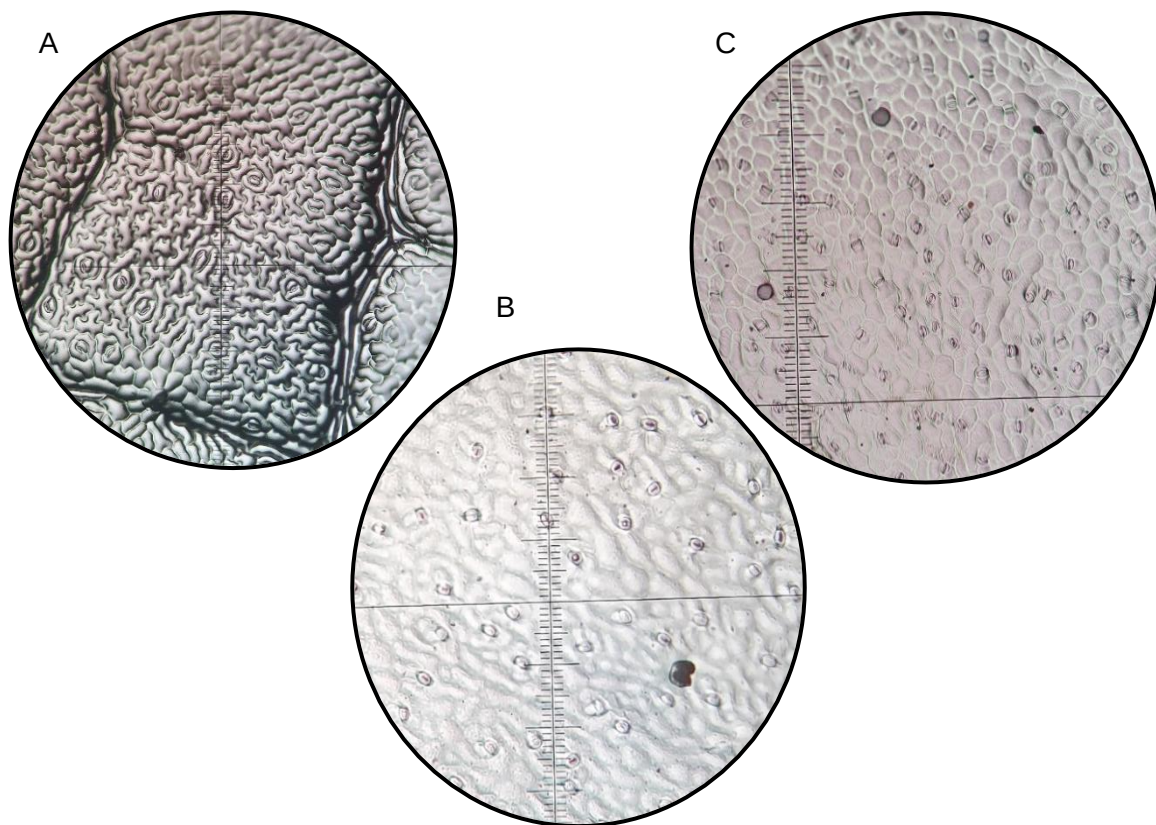
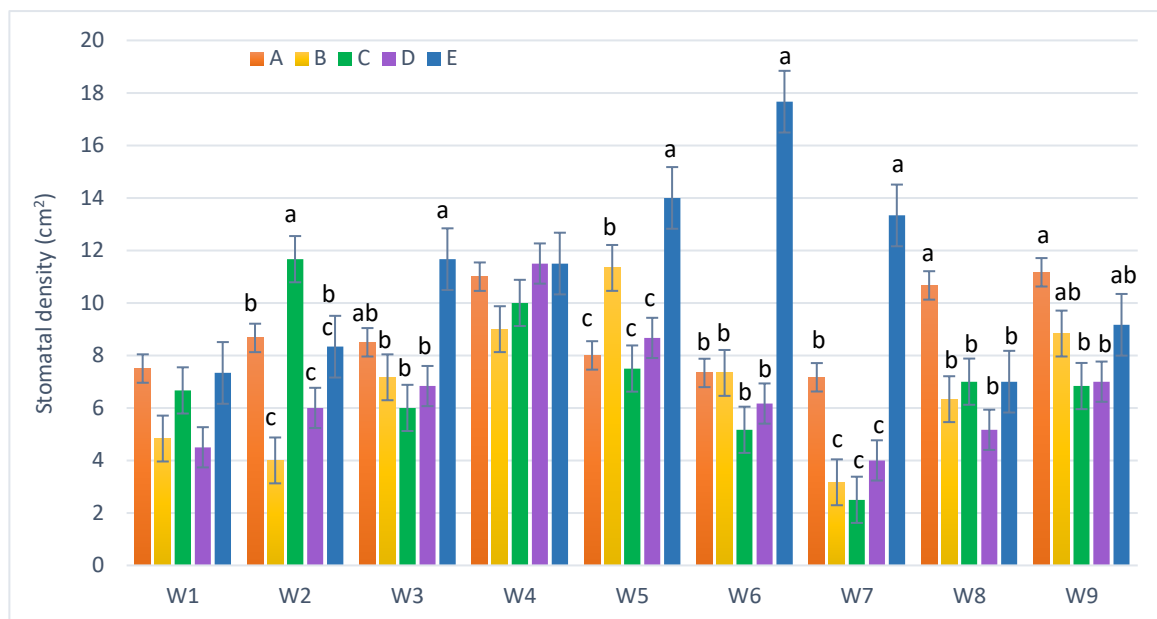


Figure 2.14: The stomatal density of beans grown in an in elevated CO₂ chamber (A), high CaCO₃ treated beetroot(B) and ambient Swiss chard (C).

Beans

The effects of the treatments on stomatal density fluctuated much over the duration of the trial (Figure 2.15). There were treatments that stood out, namely the elevated CO₂ chamber (A), the ambient CO₂ chamber (E) and to a lesser extent the ambient CO₂ glasshouse (B). The two CaCO₃ treatments (C and D) showed no significantly different results, except in week three, but there was not one treatment that gave consistently better results than the other.



A to E represents the different treatments applied in the trial and W1 to W9 represents week 1 to 9. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars of the same week and no letters indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments.

Figure 2.15: Weekly changes in stomatal density as effected by different carbon dioxide treatments on beans.

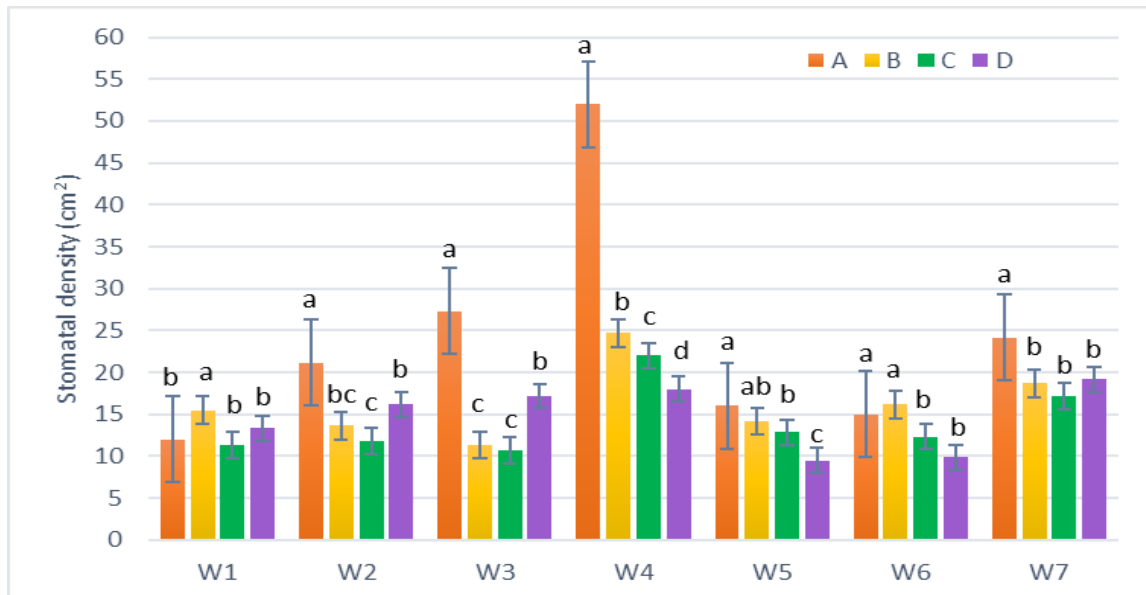
Beetroot

The elevated CO₂ chamber (A) consistently resulted in the two highest stomatal densities between treatments throughout the trial (Figure 2.16). The stomatal densities of the plants in the ambient CO₂ glasshouse (B) were higher than that of the plants treated with the high and low CaCO₃ treatments (C and D respectively), except in week 2 and 3, but lower than the plants grown in the elevated CO₂ chamber (A) in week 1 and 6. The two CaCO₃ treatments (C and D) again had fluctuating results throughout the trial, with not one treatment consistently out-performing the other.

Swiss chard

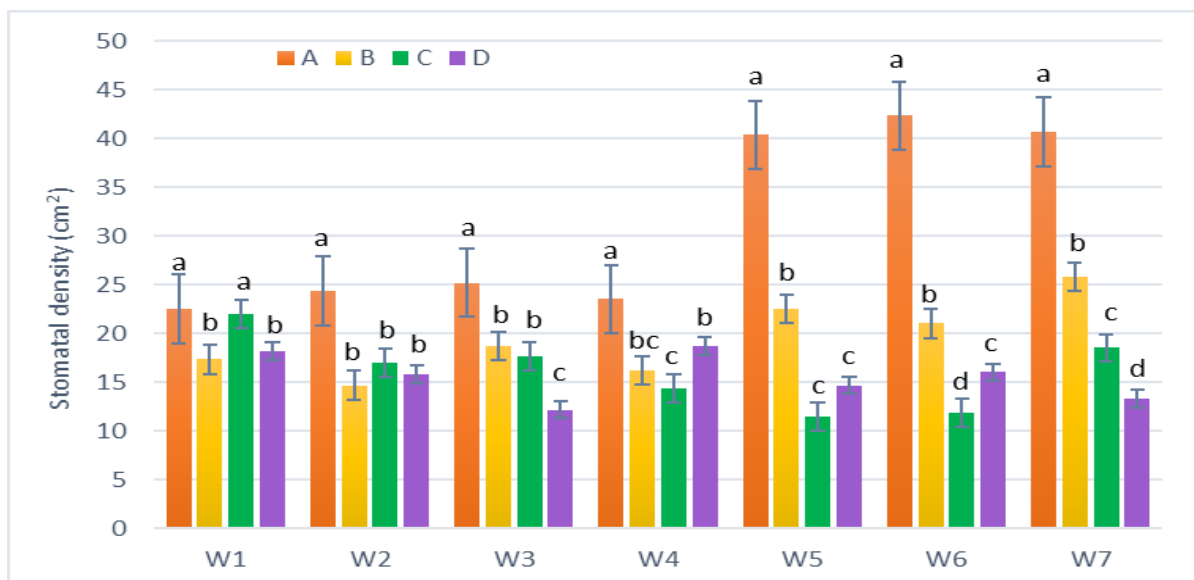
The reaction of the Swiss chard followed a similar pattern as that of beetroot in terms of the stomatal density (Figure 2.17), although the stomatal density on Swiss chard leaves were less. The Swiss chard plants showed a more clear pattern of increase in stomatal density in the plants grown in the elevated CO₂ chamber (A) and the ambient CO₂ glasshouse (B). Similar to the CaCO₃ treatments of the beetroot, these treatments (C and D) also resulted in

fluctuating results throughout the trial with no treatments consistently out-performing the other in the Swiss chard plants.



A to E represents the different treatments applied in the trial and W1 to W9 represents week 1 to 9. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars of the same week with different letters indicates a significant difference between treatments.

Figure 2.16: Weekly changes in stomatal density as effected by different carbon dioxide treatments on beetroot.

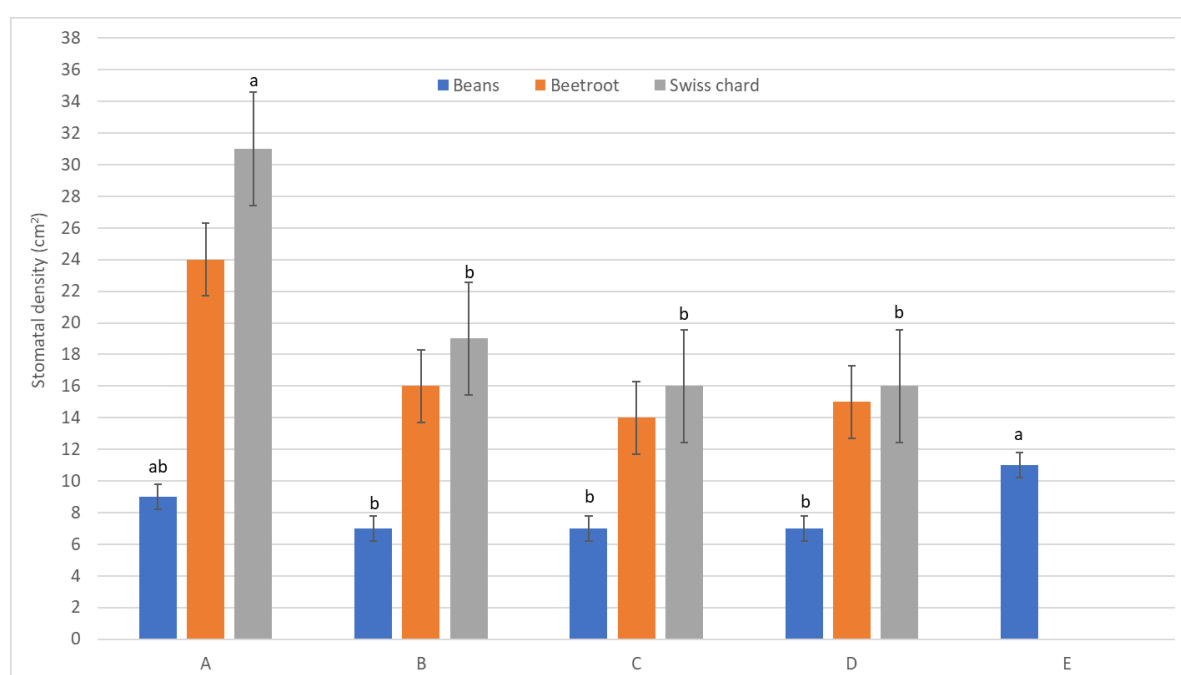


A to E represents the different treatments applied in the trial and W1 to W9 represents week 1 to 9. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars of the same week with different letters indicates a significant difference (P, 0.05) between treatments.

Figure 2.17: Weekly changes in stomatal density as effected by different carbon dioxide treatments on Swiss chard.

Overall effect on stomatal density of all three crops

Both beetroot and the Swiss chard that grew in the elevated CO₂ chamber (A) resulted in the highest stomatal densities between treatments (Figure 2.18). The trend in stomatal densities in the other treatments of beetroot and Swiss chard followed a similar trend. The beans that grew in the ambient CO₂ chamber (E) had the highest stomatal densities followed by those grown in the elevated CO₂ chamber (A). The plants exposed to the CaCO₃ treatments (C and D) did not significantly increase the stomatal densities.



A to E represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars of the same colour and different letters indicates a significant difference between treatments ($P < 0.05$) whereas those without letters indicates no significant difference.

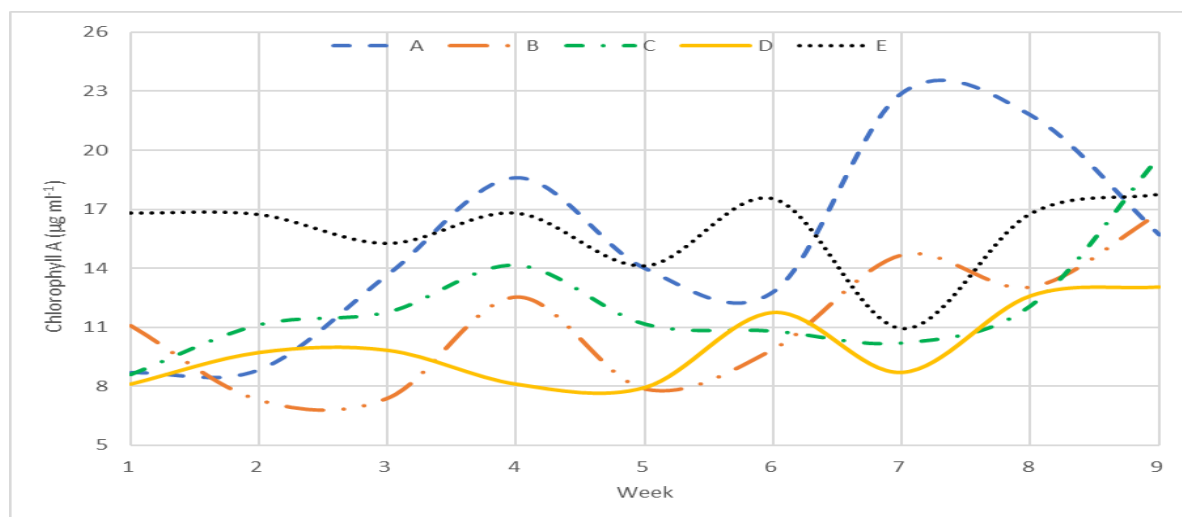
Figure 2.18: Effect of the different carbon dioxide treatments on the stomatal density of all three crops on average.

2.3.8 Bean photosynthetic pigments

Chlorophyll A

Initially the bean plants (Figure 2.19) that grew in the ambient CO₂ chamber (E) had the highest chlorophyll A (chl A) concentration (17 $\mu\text{g ml}^{-1}$). The plants that were grown in the glasshouse at ambient CO₂ (B), those that received the high (8 g) and low (4 g) CaCO₃

treatments (C and D respectively) and those that were grown in the elevated CO₂ chamber (A) had the lowest initial chlorophyll A concentrations (between 8 and 11 µg ml⁻¹). The plants in treatment D followed a similar trend to the plants in treatment E. The plants grown in the ambient CO₂ glasshouse (B) followed a similar changes in chl A than the plant grown in the elevated CO₂ chamber (A). The plants of treatment C had more gradual increases in the concentration of chlorophyll A (week one to four) followed by a slight decrease (week five to eight).



A to E represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm).

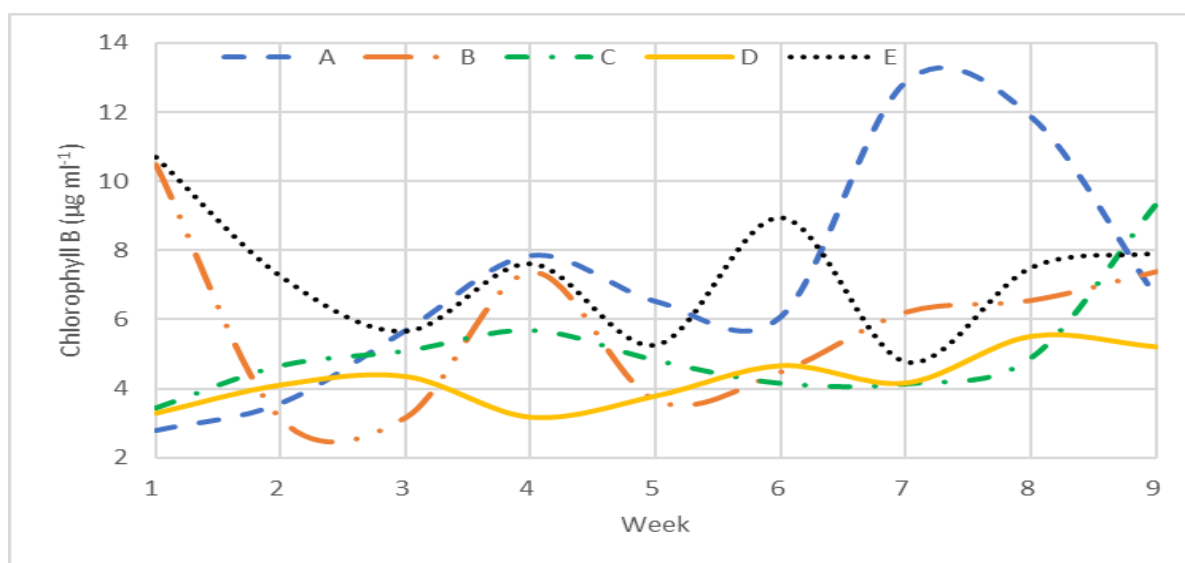
Figure 2.19: Effect of carbon dioxide on the concentration of chlorophyll A in bean plants.

Chlorophyll B

The concentration of chlorophyll B [chl B] in figure 2.20 in the beans that grew in the ambient CO₂ chamber (E) and those that were grown in the glasshouse at ambient CO₂ (B) were the highest (10 - 11 µg ml⁻¹). The [chl B] of the plants in treatment E had similar fluctuations throughout the trial than the plants in treatment B. The plants grown in the ambient CO₂ glasshouse (B) followed a similar but opposite pattern than the plants that were treated with the low CaCO₃ treatment (D). The weekly changes in [chl B] of the plants that received the high CaCO₃ treatment (C) had more gradual increases and decreases in the trial. The [chl B] of all the treatments followed similar fluctuations throughout the trial as compared to their corresponding [chl A].

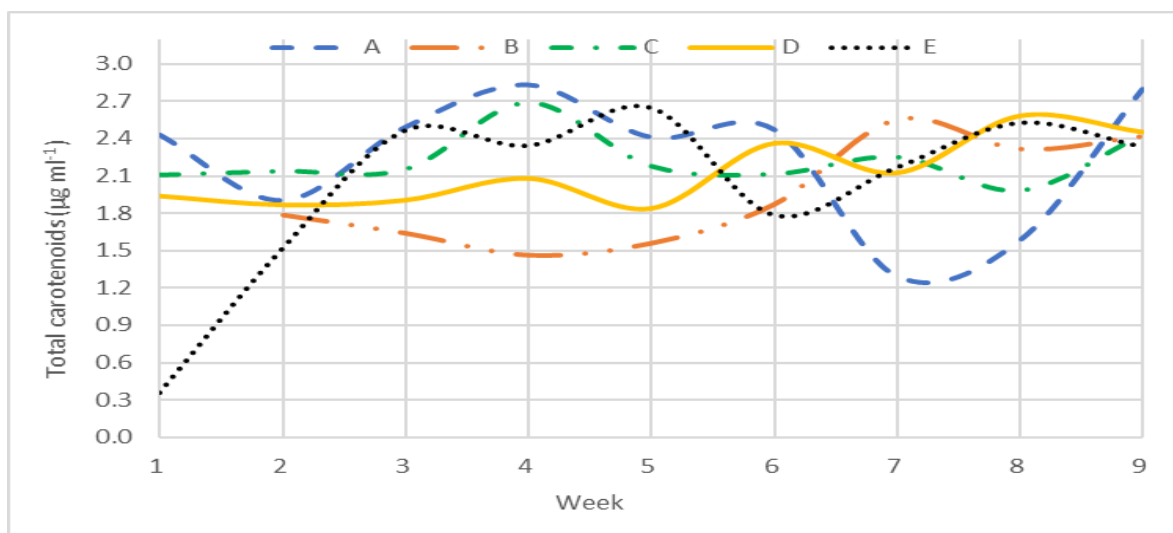
Total carotenoids

The total carotenoid concentration (Figure 2.21) for the plants of treatment E was the lowest in the first week of the trial ($0.4 \mu\text{g ml}^{-1}$), followed by those of treatment A, B, C and D (between 1.8 and $2.4 \mu\text{g ml}^{-1}$). The total carotenoid concentration of the plants in treatment E followed similar but opposite fluctuations than those of the bean plants treated with the low CaCO_3 treatment (D). The changes in the total carotenoid concentration of the plants in treatment D also followed a similar but opposite trend to that its corresponding [chl A] (Figure 19). The total carotenoid concentration of the plants grown in the ambient CO_2 glasshouse (B) gradually decreased from week two to four, whereafter it increased exponentially until week seven. During the first six weeks of the plants exposed to the high CaCO_3 treatment (C) followed a similar pattern to those of the plants grown in the elevated CO_2 chamber (A), whereafter treatment C followed a similar pattern to that of treatment B. The plants in treatment A in the last few weeks of the trial (week 7 to 9) followed a pattern opposite of the fluctuations in treatment B and C.



A to E represents the different treatments applied in the trial. A = elevated CO_2 chamber (700 ppm); B = ambient CO_2 (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO_2 chamber (418 ppm).

Figure 2.20: Effect of carbon dioxide on the concentration of chlorophyll B in bean plants.



A to E represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm).

Figure 2.21: Effect of carbon dioxide on the total carotenoid concentration in bean plants.

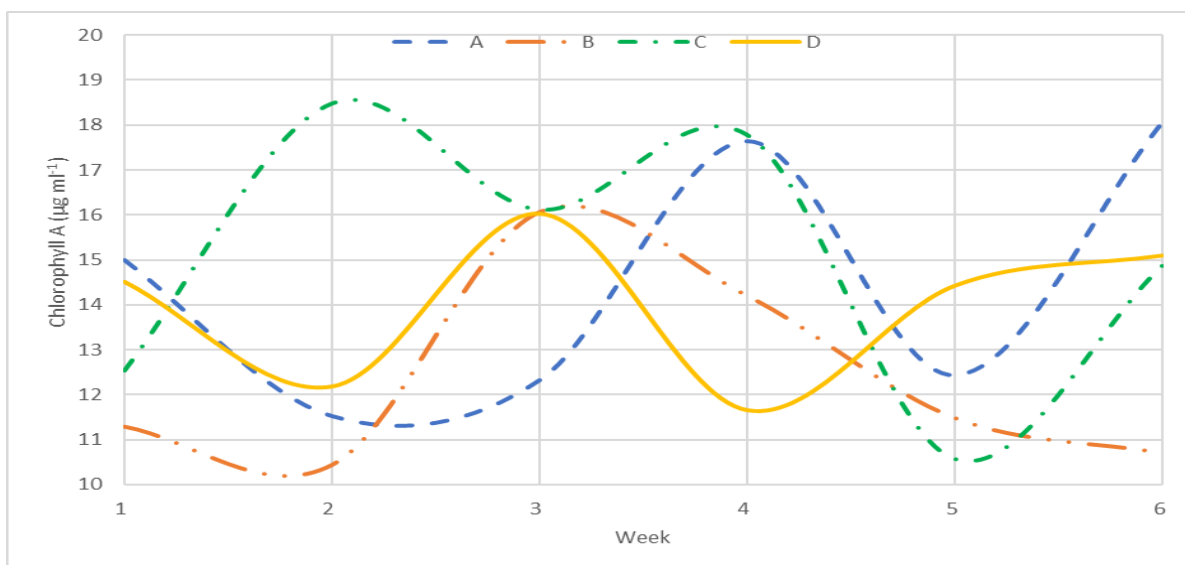
2.3.9 Beetroot photosynthetic pigments

Chlorophyll A

The beetroot plants (Figure 2.22) that were grown in the CO₂ chamber (A) followed a similar but opposite pattern than the plants treated with the high CaCO₃ (C) from week three to six. In the initial weeks (1-3) treatment A decreased from 15 µg ml⁻¹ to 12 µg ml⁻¹. The plants exposed to the low CaCO₃ treatment (D) followed a similar but opposite pattern than those in treatment C. The [chl A] of the plants grown in the ambient glasshouse (B) had a similar pattern than that of the plants in treatment A.

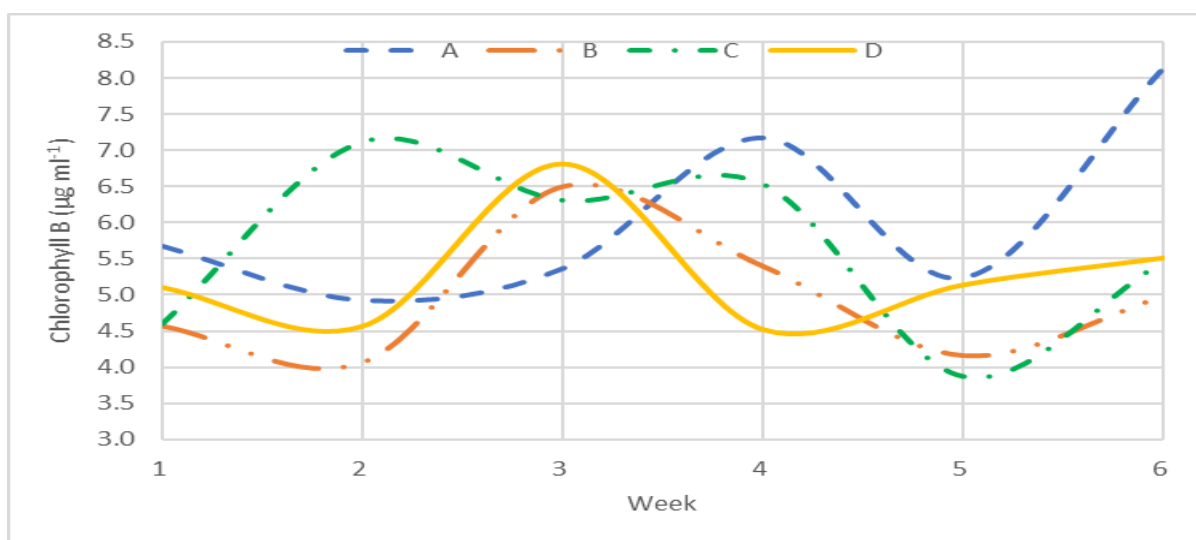
Chlorophyll B

The changes in the concentration of chlorophyll B (Figure 2.23), in the beetroot plants, of the various treatments (A to D) followed a similar pattern than those in chlorophyll A (Figure 22). The only deviations from the patterns in chlorophyll A was in the first week where the concentration of treatment B was only 0.5 µg ml⁻¹ lower than that of treatment D, the difference between the chlorophyll B in treatment A and D was larger than in [chl A]. The second deviation was in the concentration of chl B in the last week of the trial that increased whereas it decreased in the [chl A]. The only other difference is that the concentration of chlorophyll A ranged between 10 µg ml⁻¹ and 19 µg ml⁻¹ and [chl B] between 3.5 µg ml⁻¹ and 8.2 µg ml⁻¹.



A to D represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week.

Figure 2.22: Effect of carbon dioxide on the concentration of chlorophyll A in beetroot plants.



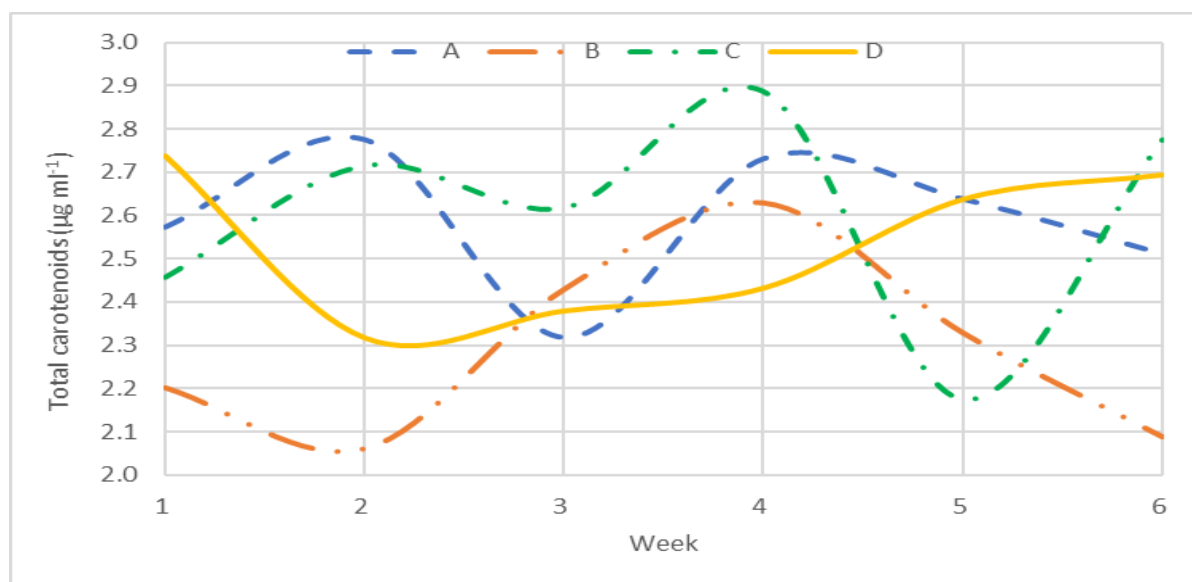
A to D represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week.

Figure 2.23: Effect of carbon dioxide on the concentration of chlorophyll B in beetroot plants.

Total carotenoid

The changes in the total carotenoid concentration (Figure 2.24) of the plants that were grown in the glasshouse at ambient CO₂ (B) followed a similar pattern to the treatment's corresponding [chl A] (Figure 2.22). The only difference being that chlorophyll A ranged

between $10 \mu\text{g ml}^{-1}$ and $16 \mu\text{g ml}^{-1}$ whereas the carotenoid concentration ranges between $2 \mu\text{g ml}^{-1}$ and $2.7 \mu\text{g ml}^{-1}$. The changes in the carotenoid concentration of the plants that received the high (8 g) CaCO_3 treatment (C) generally followed the same fluctuations than those in chlorophyll A of the same treatment, however the first increase of the carotenoid concentration was not as steep as the increase that occurred in the [chl A] of treatment C in figure 22. The plants that received the low (4 g) CaCO_3 treatment (D) initially had the highest carotenoid concentration in week one but decreased from $2.75 \mu\text{g ml}^{-1}$ to $2.3 \mu\text{g ml}^{-1}$ in week two. From this week until the end of the trial the concentration of carotenoids in this treatment increased gradually until the end of the trial. The plants that grew in the CO_2 chamber (A) followed a similar but opposite pattern to the [chl B] of the plants in treatment D.



A to D represents the different treatments applied in the trial. A = elevated CO_2 chamber (700 ppm); B = ambient CO_2 (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week.

Figure 2.24: Effect of carbon dioxide on the total carotenoid concentration in beetroot plants.

2.3.10 Swiss chard photosynthetic pigments

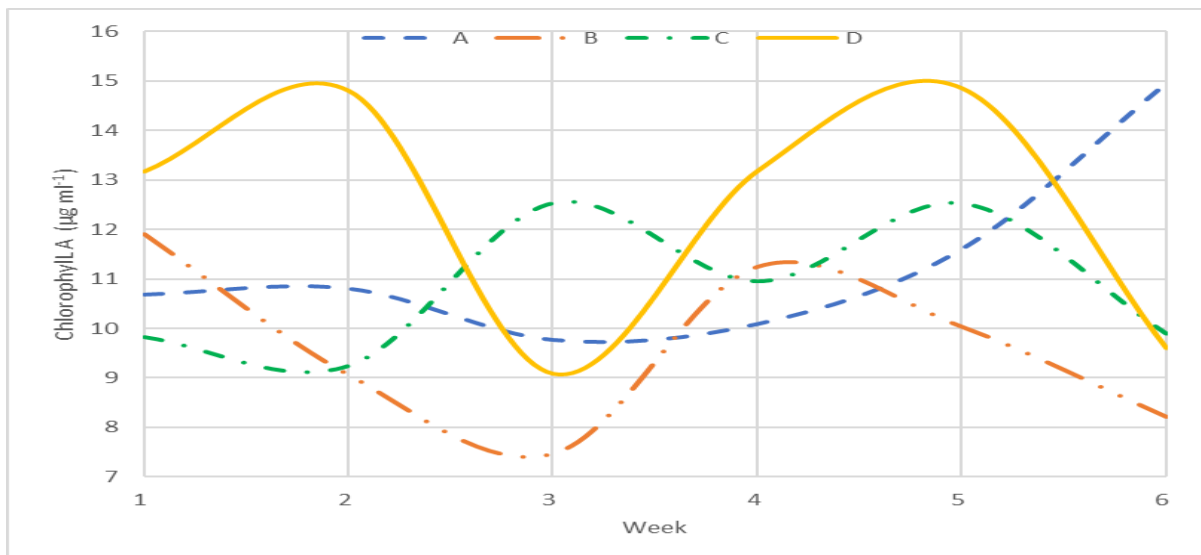
Chlorophyll A

The plants treated with the low CaCO_3 treatment (D) had a [chl A] pattern similar but opposite to that of the [chl A] of the beetroot plants (Figure 2.25). The plants that were grown in the elevated CO_2 chamber (A) had the second lowest chlorophyll A concentration in week one and increased exponentially until the end of the trial. The changes in the concentration of chlorophyll A of the plants that received the 8 g CaCO_3 treatment (C) had the lowest

chlorophyll A concentration initially. Throughout the trial the [chl A] fluctuated in a similar but mirrored pattern of the [chl B] of beetroot of the same treatment (Figure 16).

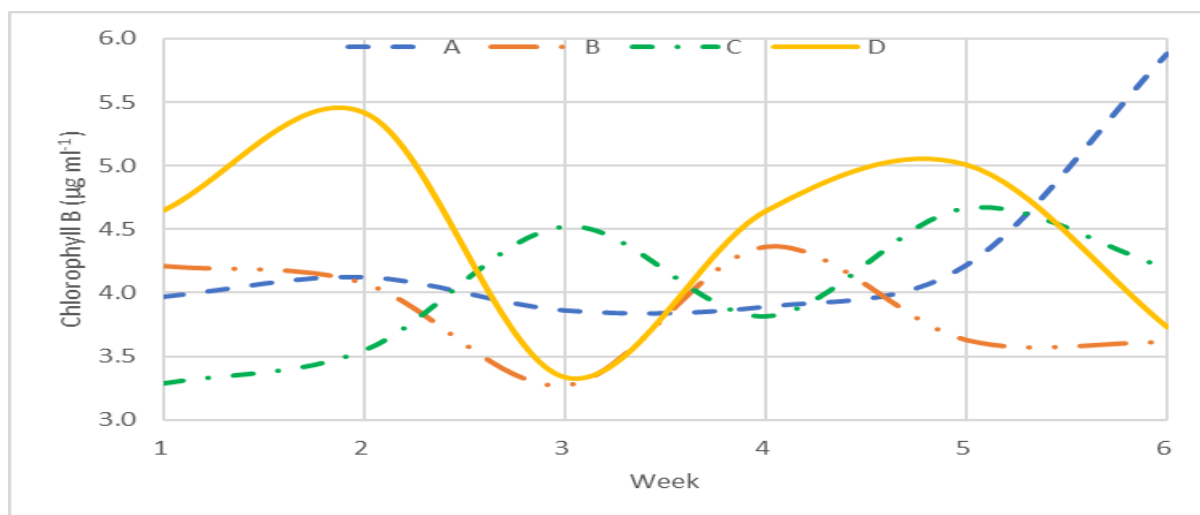
Chlorophyll B

The changes in the concentration of chlorophyll B (Figure 2.26) in the different treatments followed similar patterns to their changes in chlorophyll A (Figure 2.25) however the change in chlorophyll A occurred between $7.5 \mu\text{g ml}^{-1}$ and $15 \mu\text{g ml}^{-1}$ the changes in chlorophyll B ranged between $3 \mu\text{g ml}^{-1}$ and $5.5 \mu\text{g ml}^{-1}$. The deviations in the patterns of chlorophyll A from chlorophyll B occurred in the curve of treatment A overlapping with that of treatment C in the fourth week of the trial.



A to D represents the different treatments applied in the trial. A = elevated CO_2 chamber (700 ppm); B = ambient CO_2 (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week.

Figure 2.25: Effect of carbon dioxide on the concentration of chlorophyll A in Swiss chard plants.

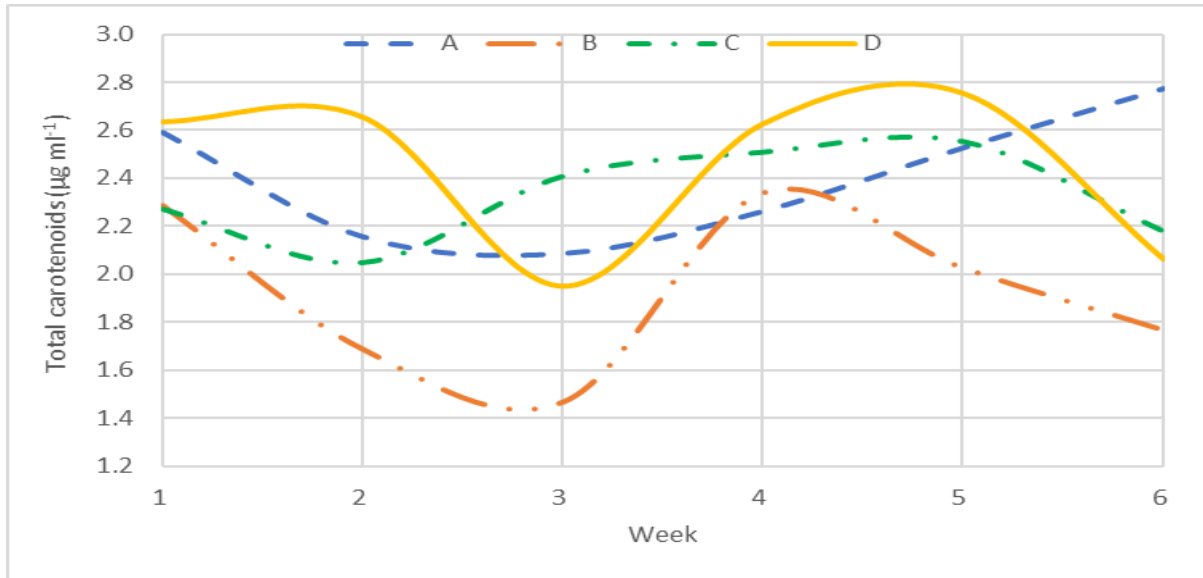


A to D represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week.

Figure 2.26: Effect of carbon dioxide on the concentration of chlorophyll B in Swiss chard plants.

Total carotenoid

The total carotenoid content of Swiss chard plants (Figure 2.27) that were grown in the glasshouse at ambient CO₂ (B) started at about 2.3 µg ml⁻¹ decreased over two weeks to 1.4 µg ml⁻¹, increasing to approximately 2.6 µg ml⁻¹ before gradually decreasing to 1.8 µg ml⁻¹ over two weeks. The plants that were grown in the CO₂ chamber (A) initially had a high total carotenoid concentration (2.6 µg ml⁻¹) that exponentially decreased to 2.1 µg ml⁻¹ over two weeks whereafter the concentration increased gradually from week three to week six (2.6 µg ml⁻¹). The total carotenoid concentration of the plants that received the 8 g CaCO₃ treatment (C) started at the same concentration as treatment B, decreased slightly to 2 µg ml⁻¹ increased in concentration over the next three weeks to 2.6 µg ml⁻¹ before decreasing (week five to six) to 2.2 µg ml⁻¹. The plants that received the 4 g CaCO₃ treatment (D) had decreases and increases in the same weeks that the chlorophyll A and B had, however the increases and decreases were not as large.



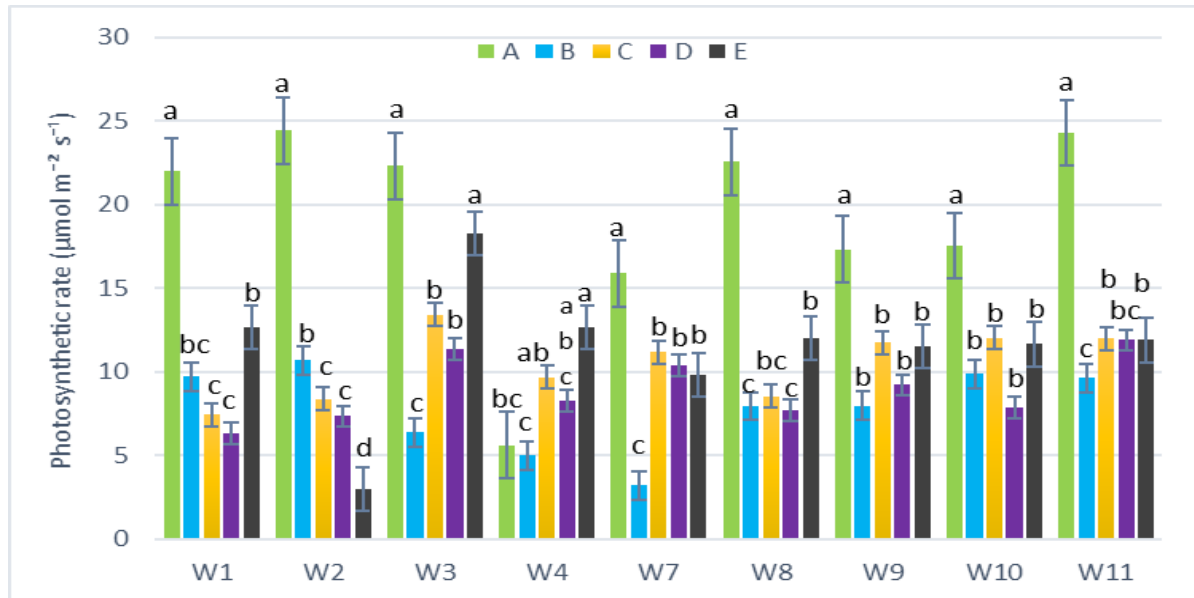
A to D represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week.

Figure 2.27: Effect of carbon dioxide on the total carotenoid concentration in Swiss chard plants.

2.3.11 Photosynthesis

Beans

Throughout the trial the bean plants that grew in the CO₂ chamber (A) had the highest photosynthetic rate (Figure 2.28), except in week four. The plants that grew in the ambient CO₂ chamber (E) had the second highest photosynthetic rate throughout the trial. The photosynthetic rates of the two CaCO₃ treatments (C and D) fluctuated throughout the trial, but the plants treated with the high CaCO₃ (C) treatment consistently had a higher rate than treatment D. Throughout most of the trial the plants grown in the ambient CO₂ glasshouse (B) had the lowest photosynthetic rate among all the treatments.

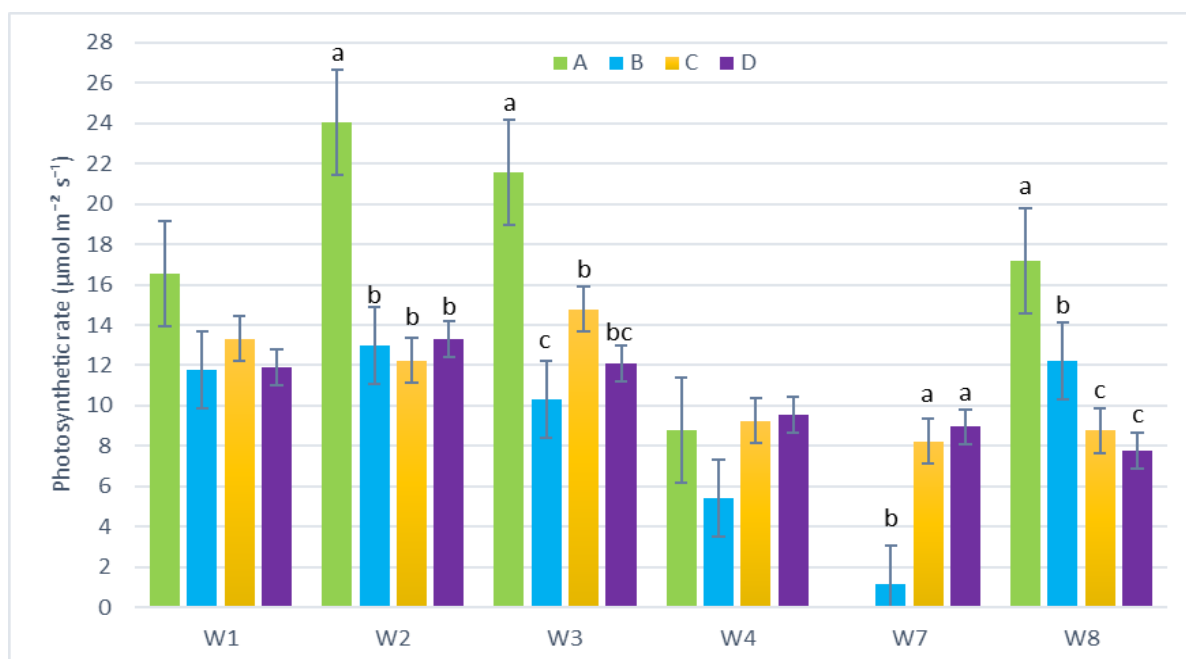


A to E represents the different treatments applied in the trial and W1-W11 represents the week 1 to 11. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars of the same week with different letters indicates a significant difference ($P < 0.05$) between treatments.

Figure 2.28: Weekly changes in photosynthetic rates as effected by different carbon dioxide treatments in beans.

Beetroot

The beetroot plants grown in the elevated CO₂ chamber (A) throughout the trial had the highest photosynthetic rate (Figure 2.29) except in week 4 and 7. The photosynthetic rate of the beetroot plants in treatment A were significantly higher than any of the other treatments throughout the trial, except in week 3 and 4. In week five the plants in treatment A did not have a photosynthetic measurement, this is due to an error when taking the measurements. The plants grown in the ambient CO₂ glasshouse (B) and those treated with the high and low CaCO₃ treatments (C and D, respectively) fluctuated throughout the trial. The plants in treatment B throughout most of the trial had a photosynthetic rate lower than treatments C and D. Between treatments C and D no treatments had a photosynthetic rate consistently higher or lower than the other.

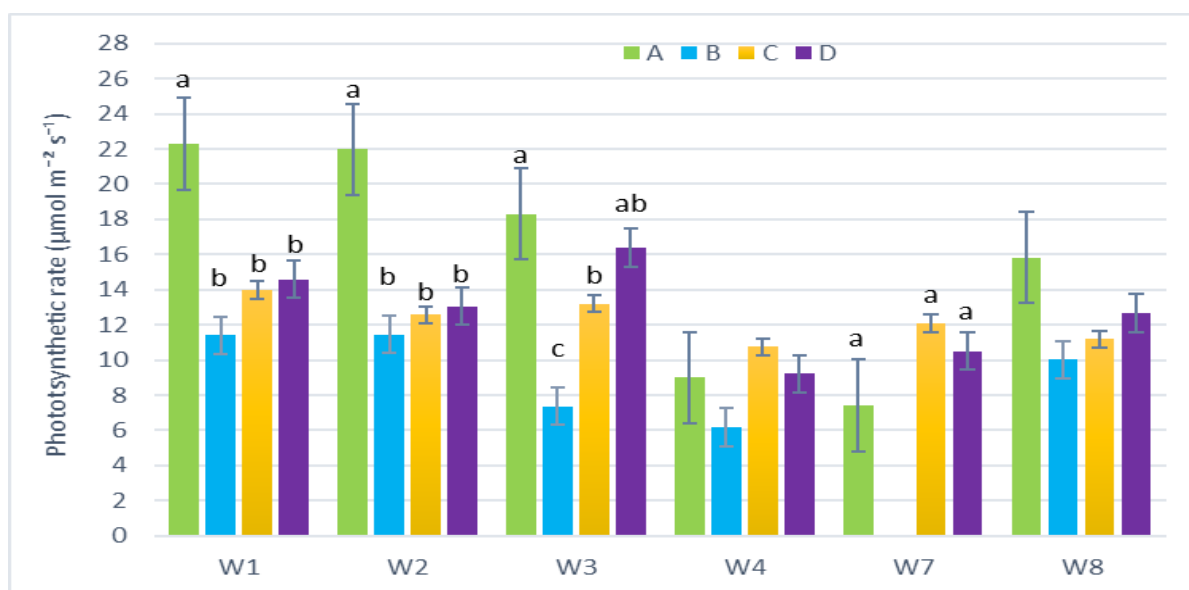


A to E represents the different treatments applied in the trial and W1-W8 represents the week 1 to 8. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week. Bars of the same week with different letters indicates a significant difference ($P < 0.05$) between treatments.

Figure 2.29: Weekly changes in photosynthetic rates as effected by different carbon dioxide treatments in beetroot.

Swiss chard

The photosynthetic rates of the Swiss chard plants (Figure 2.30) had a similar trend in photosynthetic rate than the beetroot plants, with the plants grown in the elevated CO₂ chamber (A) having the highest rates throughout most of the trial. The plants that grew in the ambient CO₂ glasshouse (B) consistently had photosynthetic rates that were lower than the plants that were treated with the high and low CaCO₃ treatments (C and D, respectively). From week 1 to 5 the plants in treatment B decreased in terms of photosynthetic rate and in week 7 there wasn't a measurement due to an error. The photosynthetic rate of the plants in treatment C and D fluctuated throughout the trial. Throughout most of the trial the plants that were treated with the low CaCO₃ treatment (D) had a higher photosynthetic rate than those in treatment C.



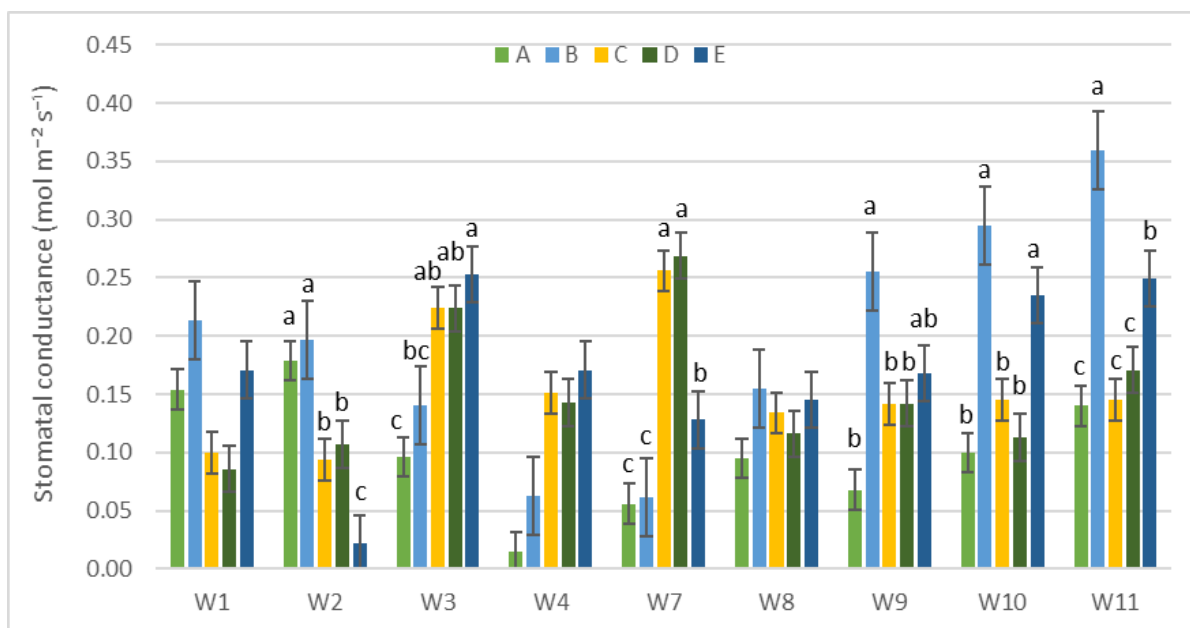
A to E represents the different treatments applied in the trial and W1-W8 represents the week 1 to 8. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week. Bars of the same week with different letters indicates a significant difference ($P < 0.05$) between treatments.

Figure 2.30: Weekly changes in photosynthetic rates as effected by different carbon dioxide treatments in Swiss chard.

2.3.12 Stomatal conductance

Beans

Throughout the trial the plants that grew in the elevated CO₂ chamber (A) had the lowest stomatal conductance (Figure 2.31) whenever the photosynthetic rate was highest (Figure 2.28). Throughout most of the trial the plants that grew in the ambient CO₂ glasshouse (B) had a stomatal conductance that fluctuated weekly, and a similar pattern was seen with its photosynthetic rate. Whenever the stomatal conductance lowered so did the photosynthetic rate and vice versa when the stomatal conductance increased. The stomatal conductance of the plants that grew in the ambient CO₂ chamber (E) fluctuated a lot in the first month of the trial, whilst from week 7 to 11 an increase in stomatal conductance occurred whilst the photosynthetic rate during the same period stayed more or less the same. The stomatal conductance of the two CaCO₃ treatments (C and D) fluctuated throughout the trial, with no treatment consistently having a higher conductance than the other.



A to E represents the different treatments applied in the trial, W1-W11 represents week 1 to 11. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber. Bars of the same week with different letters indicates a significant difference ($P < 0.05$) between treatments.

Figure 2.31: Stomatal conductance in beans effected by different carbon dioxide treatments.

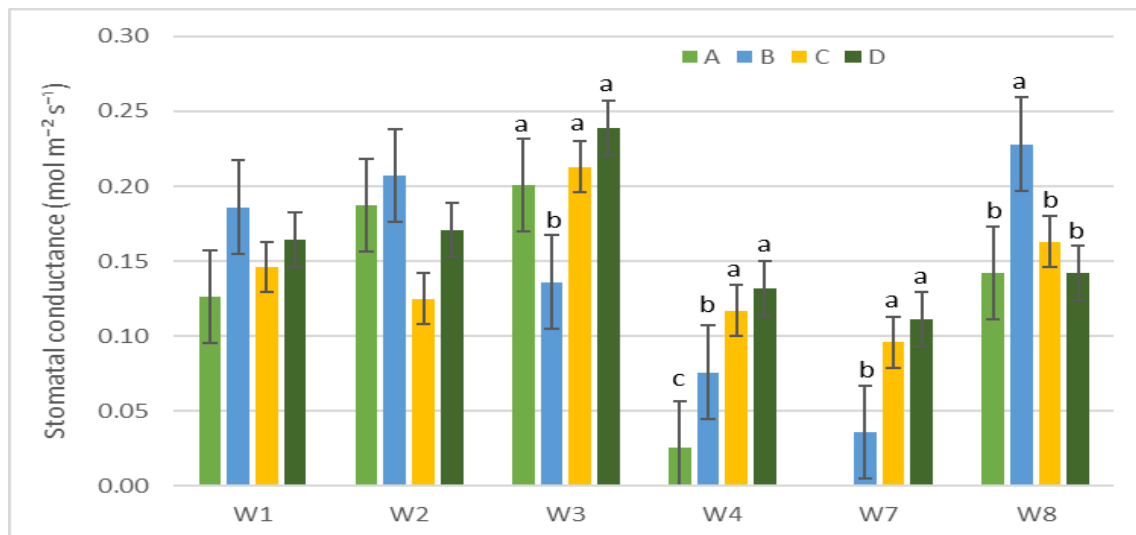
Beetroot

The stomatal conductance of the beetroot plants (Figure 2.32) grown in the elevated CO₂ chamber (A) had the lowest stomatal conductance except in week 2 and 3 with week 7 having no measurement due to an error when taking the measurements. The plants grown in the ambient CO₂ glasshouse (B) and those treated with the high and low CaCO₃ treatments (C and D, respectively) had stomatal conductances that fluctuated throughout the trial. Between treatments C and D the latter treatment had a stomatal conductance that was consistently higher than treatment C except in week 8. The plants in treatment B throughout most of the trial had not only a stomatal conductance lower than treatments C and D but also a photosynthetic rate (Figure 2.29).

Swiss chard

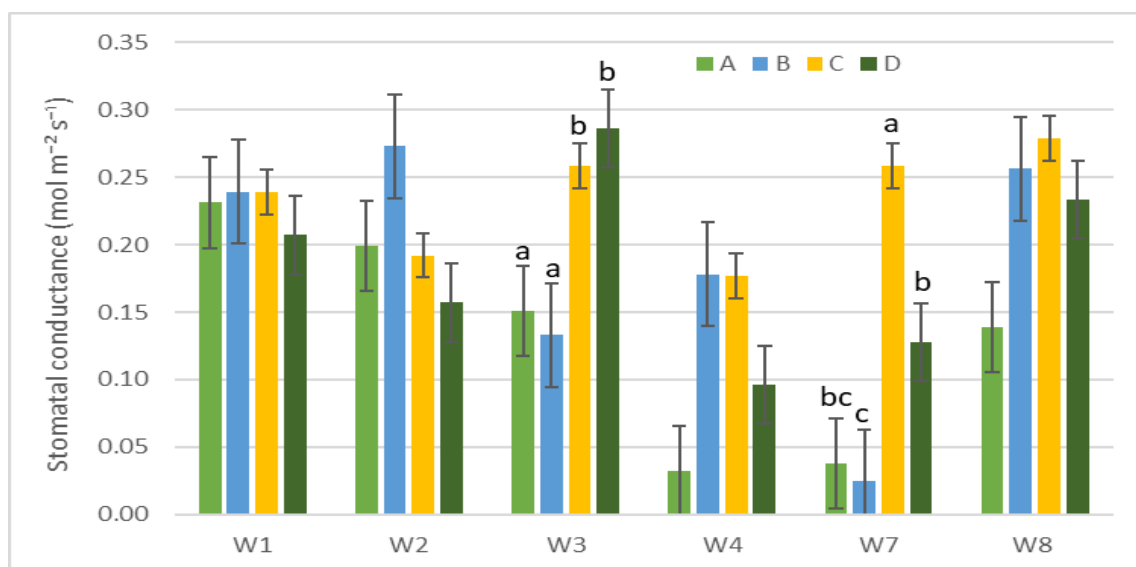
Throughout most of the trial the stomatal conductance of the Swiss chard plants (Figure 2.32) did not differ significantly between treatments except week 3 and 7. During the first month of the trial, the stomatal conductance of the plants grown in the elevated CO₂ chamber (A) decreased and increased in the last two weeks of the trial. The plants treated

with the low CaCO_3 treatment (D) fluctuated throughout the trial. When comparing the latter treatment to the plants treated with the high CaCO_3 treatment (C) the latter treatment consistently had a higher stomatal conductance than that of treatment D except in week 3.



A to D represents the different treatments applied in the trial, W1-W8 represents week 1 to 8. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week. Bars of the same week with different letters indicates a significant difference (P < 0.05) between treatments.

Figure 2.32: Stomatal conductance in beetroot plants effected by different carbon dioxide treatments.



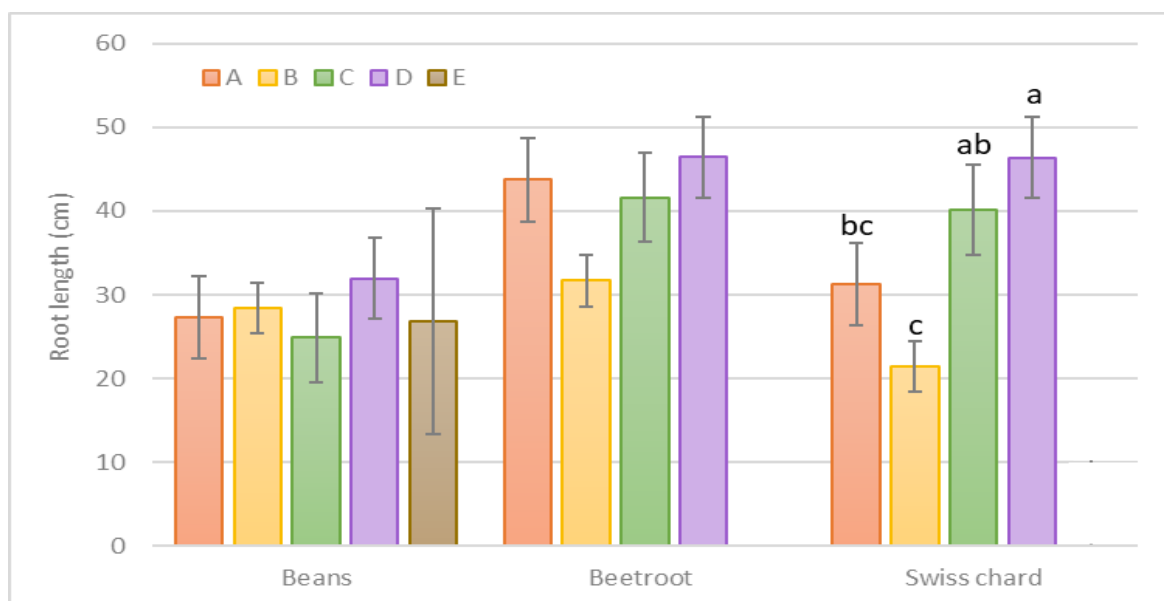
A to D represents the different treatments applied in the trial, W1-W8 represents week 1 to 8. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week. Bars of the same week with different letters indicates a significant difference (P < 0.05) between treatments.

Figure 2.33: Stomatal conductance in Swiss chard plants effected by different carbon dioxide treatments.

2.3.13 Roots and biomass of beans, beetroot and Swiss chard

Root length

The beans and beetroot plants (Figure 2.34) did not have any treatment that resulted in significantly longer roots. All three crops treated with the low (4 g) CaCO_3 treatment (D) resulted in the longest roots and only with the Swiss chard plants was the difference significant. The plants treated with the high (8 g) CaCO_3 treatment (C) had shorter roots than that of treatment D but the difference was not significant. The beetroot and Swiss chard plants grown in the ambient glasshouse (B) had the shortest roots but they were not significantly shorter than those grown in the elevated CO_2 chamber (A). The root length of the beans in the ambient glasshouse were slightly longer than those in the elevated CO_2 chamber (A) and ambient CO_2 chamber (E). The treatment in Swiss chard that resulted in the tallest plants resulted also in the shortest roots (treatment B). Treatment C and D had the two shortest Swiss chard plants but the longest roots. The beetroot plants grown in the elevated CO_2 chamber had the second longest roots but the shortest plants. The opposite occurred in the beetroot plants grown in the ambient glasshouse (B) i.e. they had the tallest plants but shortest roots. At the end of the trial the plants treated with the low CaCO_3 treatment (D) had longer roots than those treated with the high CaCO_3 (C) but their height did not differ significantly. The root lengths of the beans did not differ significantly between treatments nor did the plant heights of all the treatments except those that grew in the ambient CO_2 chamber (E).



A to E represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars of the same crop and no letter indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments.

Figure 2.34: Effect of different carbon dioxide treatments on the root length of beans, beetroot and Swiss chard.

Root types

Both beans and beetroots have a taproot system, whilst the Swiss chard plants, although closely related to beetroot, has a fibrous root system. The finer roots seen in B are the lateral roots and looks more similar to the roots of the Swiss chard plant.

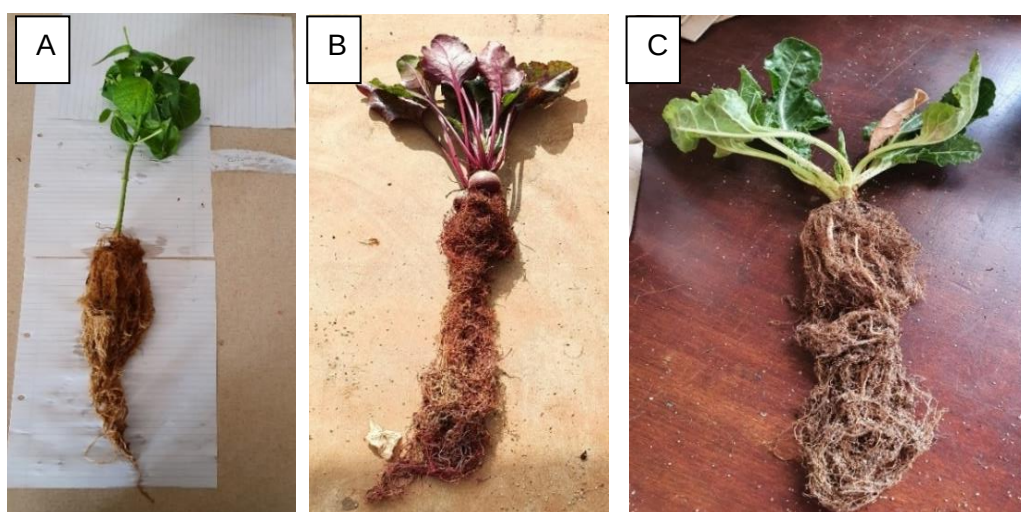
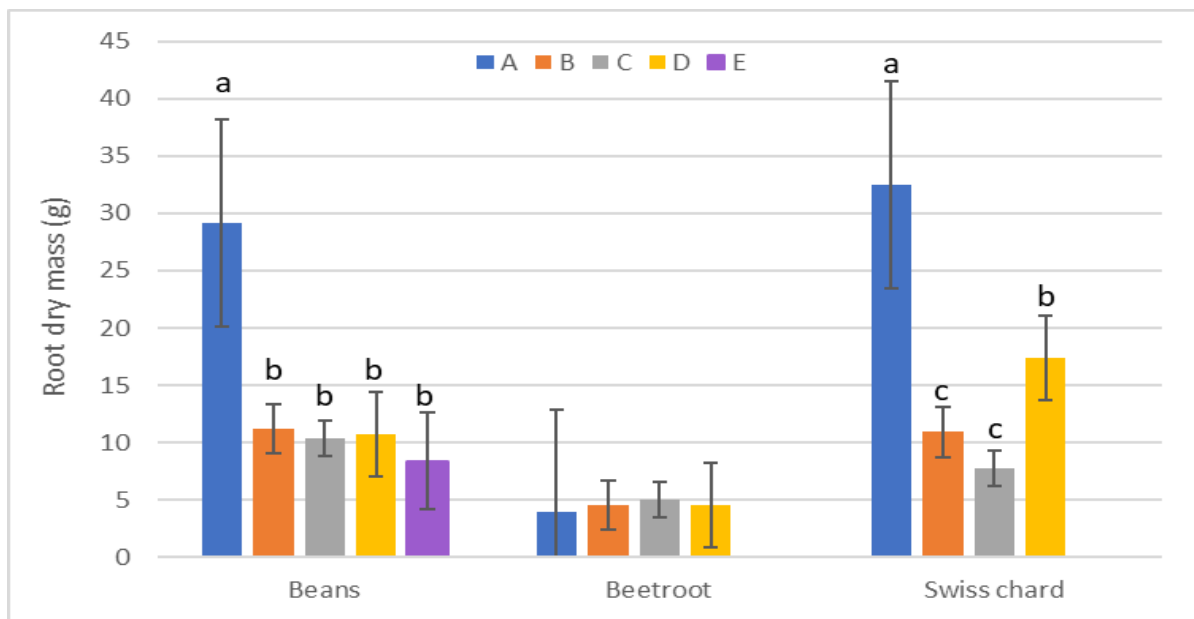


Figure 2.35: Root types of bean (A), beetroot (B) and Swiss chard (C) plants grown in the elevated CO₂ chamber.

Root dry mass

The root growth of the beetroot plants did not differ significantly between the treatments (Figure 2.36). The beans and Swiss chard plants that were grown in the elevated CO₂ chamber (A) resulted in a significant increase of the root mass. Although there was a slight reaction of the bean roots to the lower CaCO₃ (D) treatment, it was not significantly different from the ambient (B) or higher CaCO₃ treatments (C). The roots of the Swiss chard plants were the only crop that reacted significantly and positively to the lower CaCO₃ concentration (D).



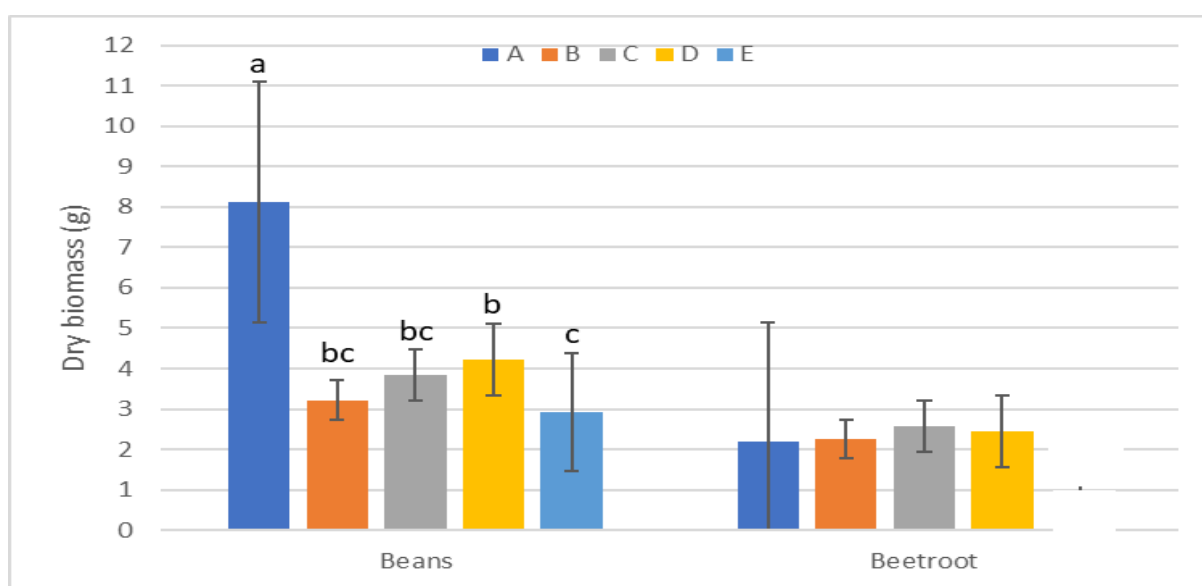
A to E represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars of the same crop and no letter indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments.

Figure 2.36: Effect of different carbon dioxide treatments on the root dry mass of beans, beetroot and Swiss chard.

Dry biomass

Similar to the root dry mass of the beans (Figure 2.37), the dry biomass of plants in the elevated CO₂ chamber (A) was significantly heavier than the dry biomass of the beans in any of the other treatments. The biomass of the beans grown in the ambient CO₂ chamber (E) was significantly lighter than the biomass of treatment D. The dry biomass from the plants that grew in the glasshouse at ambient CO₂ was not significantly heavier than the plants in treatment E and those that received the high (8 g) CaCO₃ treatments (C). The dry mass of the bean biomass treated with the low CaCO₃ treatment (D) was not significantly heavier than

those in treatments B or C. The dry leaf mass of the beetroot plants did not differ significantly between treatments. As with the beetroot plant height, the leaf dry mass followed a similar trend with the elevated CO₂ chamber (A) plants having the lighter weight of leaves. The other three treatments, however, did not differ from the elevated CO₂ chamber results nor each other. The beans in the elevated CO₂ chamber (A) did not have plants that were significantly taller than those in treatments B, C and D. The dry biomass of the plants in treatment A was significantly heavier than any of the other treatments. The bean plants in treatment E had the shortest plants and dry biomass.



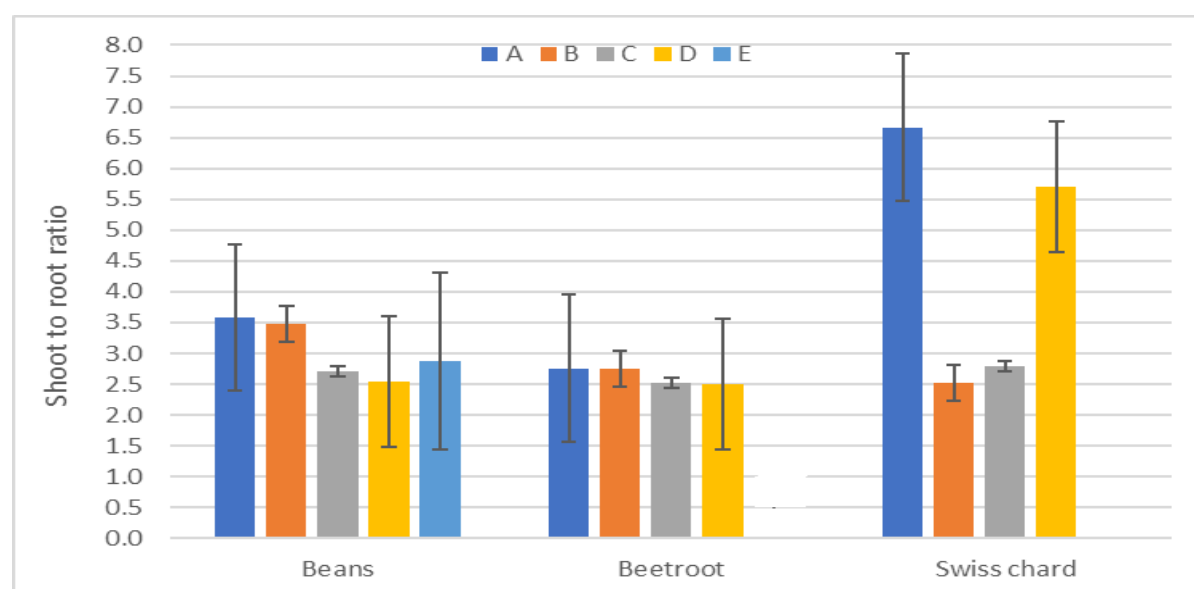
A to E represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars of the same crop and no letter indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments.

Figure 2.37: Effect of different carbon dioxide treatments on the dry biomass of beans and beetroot.

Root to shoot ratio

The root of the beans grown in the elevated CO₂ chamber (A) as well as those grown in the ambient CO₂ glasshouse (B) grew 3.5 g of roots for every gram of biomass (Figure 2.38). The plants treated with the high and low CaCO₃ treatments (C and D respectively), had the lowest shoot to root ratio with 2.7 g and 2.5 g of roots for every gram of biomass, respectively. The shoot to root ratio for the beans grown in the ambient CO₂ chamber (E) was slightly higher than those of treatment C and D at 2.8 g of roots for every gram of biomass. The shoot to root ratio of the beetroot follows a similar trend to those of the beans, with both treatment A and B being the highest (at 2.8) followed closely by treatment C and D (at 2.5).

The Swiss chard on the other hand, had significant root growth (6.7 g) for every gram of biomass (shoot mass). The shoot to root ratio for the Swiss chard plants in treatment C was a bit higher than in treatment B. The shoot to root ratio of the Swiss chard plants treated with the low CaCO_3 (D) treatment had significant root growth (5.7 g) for every gram of shoot growth.



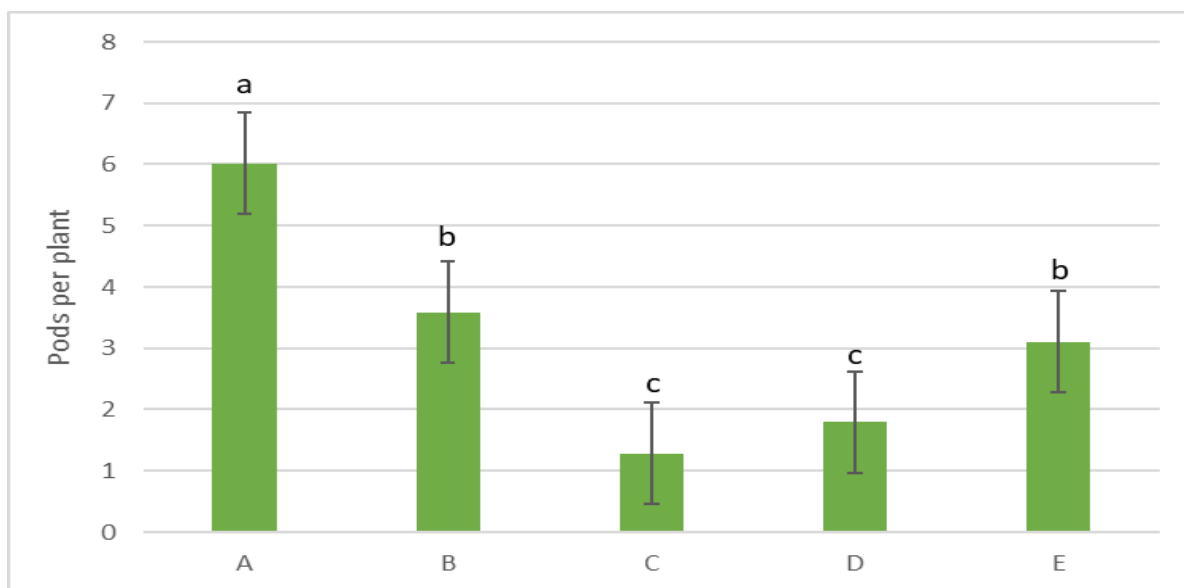
A to E represents the different treatments applied in the trial. A = elevated CO_2 chamber (700 ppm); B = ambient CO_2 (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO_2 chamber (418 ppm). Bars of the same crop and no letter indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments. Not including the pods for the shoot aspect of the bean plants or the beet mass for beetroot root aspect.

Figure 2.38: Effect of different carbon dioxide treatments on the root to shoot ratio of beans, beetroot and Swiss chard.

2.3.14 Bean pods

Average number of pods per plant

The beans that grew in the elevated CO_2 chamber (A) had the highest number of pods on average (Figure 2.39) followed by the plants grown in the ambient CO_2 glasshouse (B). The number of pods per plant grown in the ambient CO_2 chamber (E) on average were close to those grown in treatment B. The plants expose to the high CaCO_3 treatment (C) had the lowest number of pods per plant on average (approximately 1) and the plants from the low CaCO_3 treatment (D) had a slightly higher number of pods per plant (2).

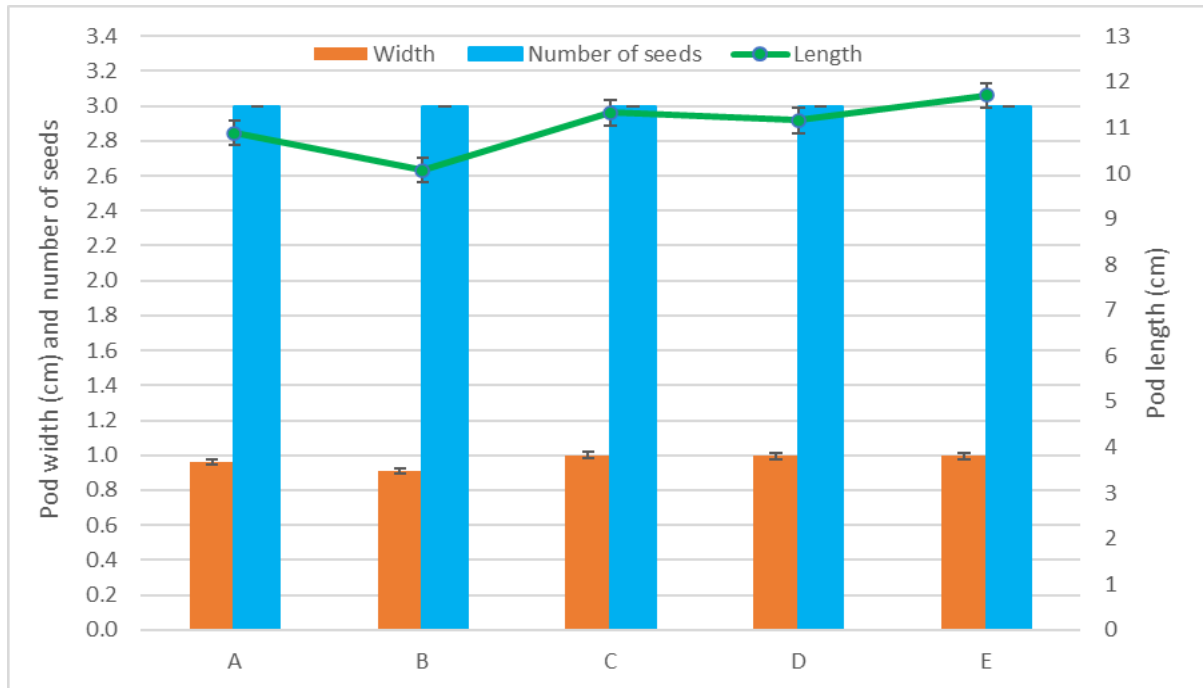


A to E represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars with different letter indicates significant differences between treatments ($P < 0.05$).

Figure 2.39: Effect of different carbon dioxide treatments on the average number of bean pods per plant.

Pod length, width and number of seeds.

Nether the pod lengths, widths or number of seeds differed between treatments (Figure 2.40). The average pod lengths of the beans grown in the ambient CO₂ glasshouse were the shortest at approximately 10 cm and those grown in the elevated CO₂ chamber (A) were only slightly longer (approximately 11.2cm). The pod lengths of the plants exposed to the high CaCO₃ treatment (C) resulted in the second longest pods and the pods of the plants grown in the ambient CO₂ chamber (E) had the longest pods. The pods of the plants exposed to the high CaCO₃ treatment (D) were only slightly shorter than the pods in treatment C. Regardless of pod length, width and treatment the number of seeds in the pods did not differ.



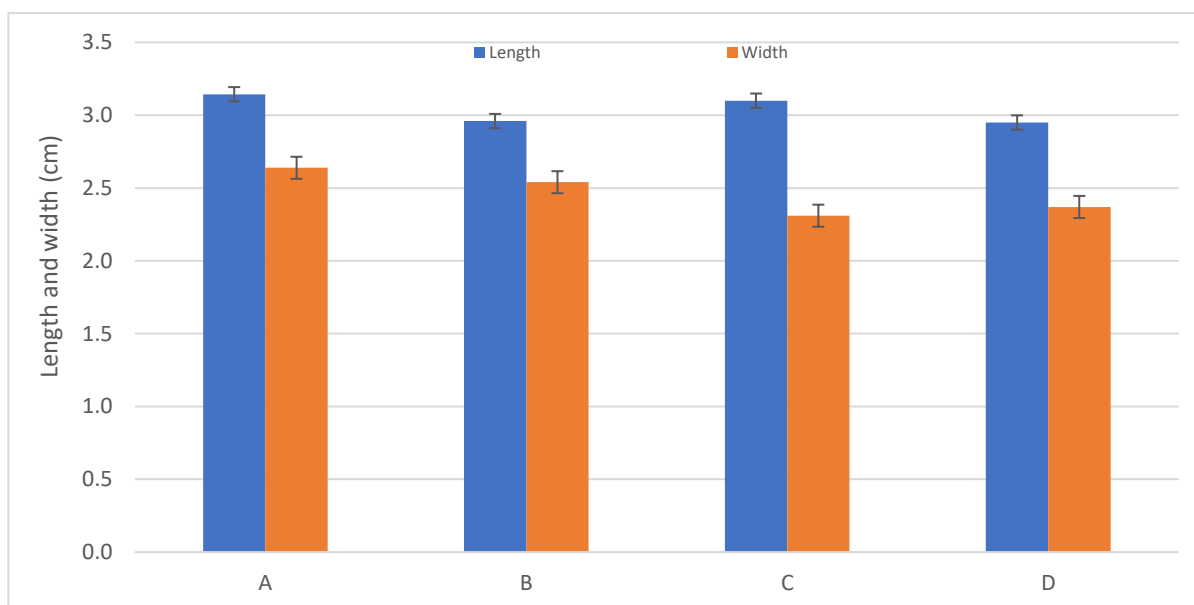
A to E represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars/lines of the same colour and no letter indicates no significant difference between treatments ($P < 0.05$).

Figure 2.40: Effect of different carbon dioxide treatments on bean pod length, width and number of seeds on average.

2.3.15 Beets

Length and width

There is no significant difference in the beet lengths, widths or dry mass between the treatments (Figure 2.41). The beets were approximately 3 cm long and 2.5 cm wide. While the lengths were not much affected by the treatments, the width tended to be a bit smaller with the addition of CaCO₃ (C and D).



A to D represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week. Bars of the same crop and no letter indicates no significant difference between treatments ($P < 0.05$) whereas bars with different letters indicates a significant difference between treatments.

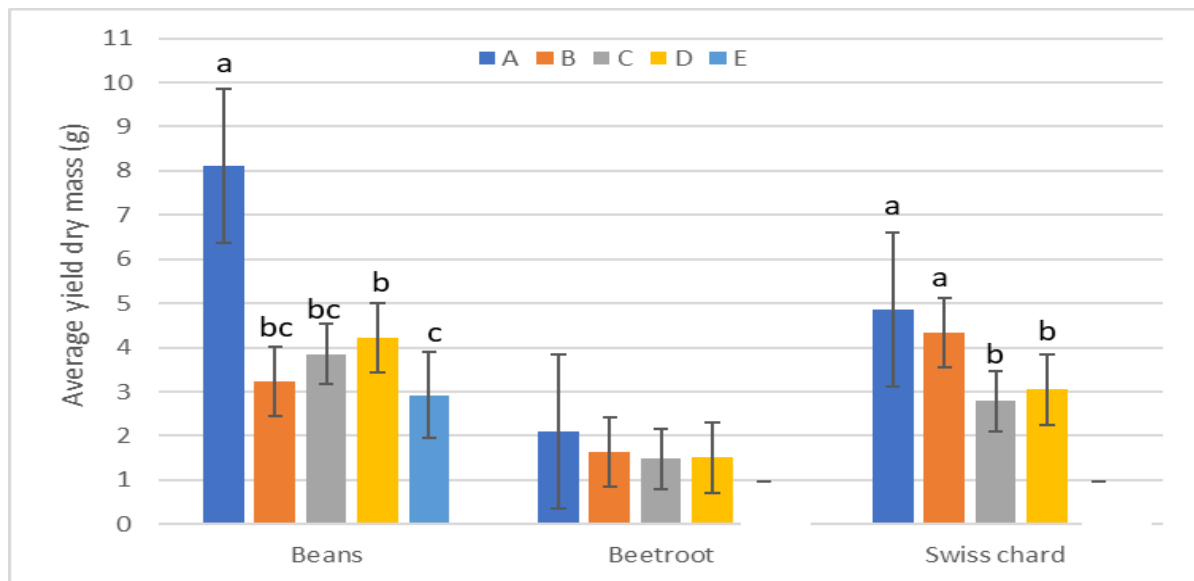
Figure 2.41: Effect of different carbon dioxide treatments on the length and width of beets.

2.3.16 Yield dry mass

Beans, beetroot and Swiss chard

The total yield dry mass (Figure 2.42) of the beans grown in the elevated CO₂ chamber (A) and those in the ambient CO₂ glasshouse were the highest (approximately 55g) followed by those in the ambient CO₂ chamber (E) at approximately 36 grams. The dry yield mass of the plants exposed to the low CaCO₃ treatment (C) was slightly higher than those exposed to with the high CaCO₃ treatment (D), however these treatment overall had the lowest yield. The yield dry mass of treatment A, B and E followed the same trend as its corresponding shoot to root ratio. The trend in the total yield dry mass of the beans in treatment C and D were opposite to the trend in the shoot to root ratio. The beetroot plants grown in the elevated CO₂ chamber had the heaviest yield dry mass but did not differ significantly from any of the other treatments. The yield of the beetroot plants that received additional CO₂ did not significantly affect the yield dry mass. The Swiss chard plants that grew in the CO₂ chamber (A) was significantly heavier than the dry biomass in any of the other treatments except from the plants grown in the glasshouse at ambient CO₂ (B). The dry leaf mass of the Swiss chard plants in the latter treatment was significantly heavier than those in treatments C and D (8 g and 4g of CaCO₃, respectively), however the leaf mass of the latter treatments did not differ significantly between one another. The dry biomass of the Swiss chard plants grown in the ambient CO₂ glasshouse

(B) was not significantly heavier than those grown in the elevated CO₂ chamber (A), however in terms of plant height the opposite was true.



A to E represents the different treatments applied in the trial. A = elevated CO₂ chamber (700 ppm); B = ambient CO₂ (in a glasshouse); C = 8 g of calcium carbonate and 16 ml HCl, three times a week; D = 4 g of calcium carbonate and 8 ml HCl, three times a week; E = ambient CO₂ chamber (418 ppm). Bars from the same crop with no letter indicates no significant difference between treatments (P < 0.05), whereas bars with different letters indicates a significant difference between treatments.

Figure 2.42: Effect of different carbon dioxide treatments on the average yield dry mass of the beans, beetroot and Swiss chard plants per treatment.

2.4. Discussions and conclusions

Preliminary trial

For the first trial with only the beans, those plants that received additional CO₂ from the CO₂ tablets or the CaCO₃ reactions did not significantly affect the height of the plants. The roots of the plants grown in the CO₂ chamber at 700ppm CO₂ did not have significantly longer roots than those grown at ambient CO₂ (in a glasshouse), which is supported by a study by Salsman et al. (1999) on root length under elevated CO₂ who also found that the roots of *P. vulgaris* was not affected by elevated CO₂ concentration. The root lengths and mass (fresh and dry) of the plants that received additional CO₂ from the CO₂ tablets or the CaCO₃ reactions had root lengths significantly shorter than any of the other treatments due to the fact that the treatments damaged the roots to such an extent that the plants died. The mass of the roots of the plants grown at 700ppm CO₂ were notably less than those of the plants that grew in ambient CO₂ glasshouse and is contradictory to what other studies have found. Numerous studies (approximately half of all studies with root data) however, have evaluated the root dry mass of plants grown under elevated CO₂ and found that root mass increases under elevated CO₂ concentrations (Wittwer, 1978; Rogers et al., 1983a; Imai et al., 1985; Norby et al., 1992). The heavier root mass of the plants grown at ambient CO₂ could be attributed to the application of additional nitrogen in the soil during the trial as studies by Tian et al. (2008) and Gaudin et al. (2011) have shown that root growth is significantly affected by nitrogen (N) fertilization and that low N levels results in root elongation.

Rogers et al. in 1997 did a review of available studies on crop species responses to carbon dioxide enrichment and found that the root to shoot ratio typically increases (approximately 60% of the cases), occasionally decreases (approximately 38% of cases) but it was seldom unaffected (3%). In this study the plants grown in the CO₂ chamber at 700 ppm as well as the plants that received additional CO₂ from the CO₂ tablets or the CaCO₃ reactions had a higher shoot to root ratio than the plants grown at ambient CO₂ that had a low shoot to root ratio. Studies have found that a gain in dry mass is more frequently exhibited in roots than other plant organs (Imai and Murata 1976, Wittwer 1978, Rogers et al. 1983, Imai et al. 1985, Hocking and Meyer 1991, Norby et al. 1992) similar to what was found with the roots of the plants grown at ambient CO₂. Other studies on root growth have showed the importance of root growth and its effect on not only the shoot growth but yield as well (Vamerali et al. 2003, Zhang et al. 2009, Wang et al. 2014, Ota et al. 2020). This was also found in this study as the plants grown at ambient CO₂ not only had the heavier root mass than any of the other

treatments but the beans harvested did not differ from those of the beans in the CO₂ chamber in terms of mass, pod length, pod width nor number of seeds per pod.

Studies have found that a reduction in stomatal density is considered to be a common reaction to increases in CO₂ levels (Woodward 1987, Lin et al. 2001, Teng et al. 2009, Xu et al. 2016). In this study the stomatal density of the bean plants did not differ significantly between treatments nor decreased. There are studies in which the stomatal densities were unchanged (Tricker et al. 2005).

Second pot trial

Beans

The beans in this trial that received additional CO₂ through the CaCO₃ treatments were initially significantly shorter than the plants in both the CO₂ chamber (700 ppm CO₂), and the ambient CO₂ glasshouse and halfway through the trial they were no longer significantly shorter. The increases in height of the plant that received additional CO₂ through CaCO₃ can be attributed to the slight increases in photosynthesis as a result of increased CO₂ exposure. The plants that grew in the CO₂ chamber at 700 ppm also had remarkably high photosynthetic rates throughout the trial which is supported by the studies done by Björkman (1981), Chagvardieff et al. (1994), Dorais (2003), Ainsworth and Long (2005) and Van der Kooi et al. (2016). These researchers found that increases in carbon dioxide causes not only photosynthesis to increase but also positively affected plant growth. The plant that received the additional CO₂ through the CaCO₃ reactions did not have higher photosynthetic rates nor were the biomass significantly heavier than the plants grown at ambient CO₂ in the glasshouse. This is contradicting to what Björkman (1981), Chagvardieff et al. (1994), Dorais (2003), Ainsworth and Long (2005) and Van der Kooi et al. (2016) found, however many other studies have found that the root dry mass of plants grown under elevated CO₂ will increase (Rogers et al. (1994). Rogers et al. (1997) did a review of available studies on crop species responses to carbon dioxide enrichment and found that the root to shoot ratio typically increases (approximately 60% of the time/cases), occasionally decreases (approximately 38% of cases) but it was seldom unaffected (3%). In this study the bean plants grown in the elevated CO₂ chamber did not have a shoot to root ratio greater than those of the plants grown at ambient CO₂. The plants that received additional CO₂ from the CaCO₃ treatments had the lowest shoot to root ratios among all the treatments. Studies have found that a gain in dry mass is more frequently exhibited in roots than other plant organs (Imai and Murata 1976, Wittwer 1978, Rogers et al. 1983, Imai et al. 1985, Hocking and Meyer 1991, Norby et al. 1992) similar to what was found with the roots in this trial. The

root dry mass as well as the shoot dry mass of the plants grown at 700 ppm CO₂ were significantly heavier than any of the other treatments, which Wittwer (1978), Rogers et al. (1983), Imai et al. (1985) and Norby et al. (1992) also found in plants grown at elevated CO₂. Widuri et al. (2018) found that root growth of the common bean, directly affects plant growth. In this study the plants grown at 700 ppm CO₂ had the heaviest root and shoot dry mass. The plants that received additional CO₂ from the CaCO₃ treatments had heavier shoot dry masses than those grown at ambient CO₂ but the difference was not significant as the root dry masses between the treatments also did not differ significantly. Even though there was a significant difference in the root dry mass between the treatments, there was no significant difference in terms of the root length between the treatments. This could be attributed to the fact that the plants were grown in pots which may restrict plant root length (Poorter et al. 2012). Carbon dioxide had no effect on the length, width or number of seeds in the pods that were harvested.

The plants grown at 700 ppm CO₂ and those that received the additional CO₂ from the CaCO₃ reactions have harvests that were notably lighter than those that grew in the glasshouse at ambient CO₂. The lower harvest dry mass could be attributed to the aphids and red spider mites that were found on the plants of the previously mentioned treatments. The plants grown at ambient CO₂ and those grown in the ambient CO₂ chamber did not have any pests to affect the harvest. The plants that were treated with the 4 grams of CaCO₃ had the fewest pods to harvest (resulting in the lightest harvest dry mass) throughout the trial. In general, it has been found that the stomatal conductance of plants generally reduces under elevated CO₂ (Medlyn et al. 2001, Ainsworth and Rogers 2007, Gao et al. 2015) and in this study similar results were found in the plants that grew in the elevated CO₂ chamber and those receiving additional CO₂ from CaCO₃.

Beetroot

Studies by Rogers and Dahlman (1993), Allen and Amthor (1995) and Wittwer (1995) found that plants grown under elevated CO₂ frequently show not only increased plants growth but also increased water use efficiency. In this study however, the beetroot plants that grew in the CO₂ chamber at 700 ppm resulted in the shortest plants throughout the trial whereas the plants that received the CaCO₃ treatments and those that grew in the glasshouse at ambient CO₂ did not differ significantly from one another. The shorter plants in the CO₂ chamber could be attributed to the differences in humidity between the experiment locations. The humidity in the CO₂ chamber were kept at 50% whereas the humidity in the glasshouses were 60 – 90% during the day and between 20 – 65% at night (depending on the weather).

Gislerød and Nelson (1989), Mortensen and Gislerød 1990 found that plants exposed to high relative humidity show enhanced growth due to the higher stomatal aperture and thus increased CO₂ uptake, similar to what was found in this trial.

As previously mentioned, root length and number is the most commonly evaluated parameter (45% of studies) in horticultural crops. The majority of these studies (61 -100%) found that root length increases as CO₂ increases (Rogers et al. 1994, Nie et al. 2013). In this study the plants that grew in the CO₂ chamber at 700 ppm as well as those that received additional CO₂ through the CaCO₃ treatments had root length longer than those of the plants that grew in at ambient CO₂. The root lengths (including beet lengths) of the plants that received the additional CO₂ and grew under elevated CO₂ were not significantly longer than those in/of the ambient CO₂ plants. The experimental data of studies by De Graaff et al. (2006) and Luo et al. (2006) have shown that variability in root length under different CO₂ concentrations could occur due to differences in environments. Of all the plant organs, Wittwer (1978), Imai and Murata (1979), Rogers et al. (1983), Imai et al. (1985), Hocking and Meyer (1991) and Norby et al. (1992) in their studies found that the roots tended to exhibit the greatest increase in mass under elevated CO₂. In this study however, there was no significant difference in the root dry mass including the beets of the beetroot plants between the treatments. Plant biomass is expected to increase under elevated CO₂ due to the increase in photosynthesis (Björkman 1981, Chagvardieff et al. 1994, Dorais 2003, Reyes-fox et al. 2014). The photosynthetic rates of the beetroot plants in the different treatments in this study have been fairly consistent throughout the trial. The shoot to root ratios did not differ between the treatments and studies by Imai and Murata (1976), Wittwer (1978), Rogers et al. (1983), Imai et al. (1985), Hocking and Meyer (1991), Norby et al. (1992) and Rogers et al. (1997). Other studies on root growth have showed not only its importance in shoot growth but also yield as well (Vamerali et al. 2003, Zhang et al. 2009, Wang et al. 2014, Ota et al. 2020).

The plants that were grown in the CO₂ chamber had the highest photosynthetic rate throughout the trial whereas the photosynthetic rates of the plants in the other treatments for most of the trial did not differ significantly. Whenever an increase or decrease in the photosynthetic rates occurred in the different treatments a corresponding increase or decrease occurred in the stomatal conductance of that same treatment. Comparable results were found in a study by Farquhar and Sharkey (1982) where low stomatal conductance resulted in low net photosynthetic rates, however, when stomatal conductance is high, the net photosynthetic rate will also be high. Whenever stomatal conductance is low (and therefore photosynthesis), CO₂ uptake is restricted but when stomatal conductance is high it is advantageous to photosynthesis but at a larger cost of water loss through transpiration

(Lawson and Blatt 2014). Even though the photosynthetic rates between the treatments differed significantly the dry mass of the biomass did not. The beetroot plants in both the glasshouse and CO₂ chamber were infected with powdery mildew closer to the end of the trial. The plants were treated with Efecto Funginex once the first symptoms were seen but it was difficult to eradicate. It is known that the photosynthetic rate of plants decline with the invasion of most pathogens (Walters et al. 2008, Kretschmer et al. 2019). Various authors have monitored the effect of pathogens on the photosynthetic rate of the host plant and they have discovered an incredibly significant phenomenon. They found that a large number of pathogens in the initial infection stages of infection results in the photosynthetic capacity of the host plant to always decrease (Scharte et al. 2005, Bonfig et al. 2006, Swarbrick et al. 2006, Chen et al. 2015, Hu et al. 2020).

Swiss chard

The photosynthetic rates of the Swiss chard plants in the different treatments in this study have been fairly consistent throughout the trial. The plants that were grown in the CO₂ chamber had the highest photosynthetic rate throughout most of the trial, whereas the photosynthetic rates of the plants in the other treatments (for most of the trial) did not differ significantly from one another. They were initially, however, significantly lower than those of the CO₂ chamber. Even though the photosynthetic rates of the plants that received additional CO₂ were not significantly higher than those of the ambient CO₂, the biomass of the plants were significantly lower than those of the ambient treatment. This is contrary to studies what other studies have observed. Numerous studies done by Björkman (1981), Chagvardieff et al. (1994), Dorais (2003) and Reyes-fox et al. (2014) found that plant biomass typically increases under elevated CO₂ due to the increase in photosynthesis (Björkman 1981, Chagvardieff et al. 1994, Dorais 2003, Ainsworth and Long 2005, Reyes-fox et al. 2014, Van der Kooi et al. 2016). Whenever an increase or decrease in the photosynthetic rates occurred in the plant of the different treatments a corresponding increase or decrease occurred in the stomatal conductance of that same treatment. Comparable results in a study by Farquhar and Sharkey (1982) were found. They found that a low stomatal conductance resulted in low net photosynthetic rates, however, when stomatal conductance is high, the net photosynthetic rate will also be high. Whenever stomatal conductance is low (and therefore photosynthesis), CO₂ uptake is restricted but when stomatal conductance is high it is advantageous to photosynthesis but at a larger cost of water loss through transpiration (Lawson and Blatt 2014).

Even though the photosynthetic rates between the treatments in the elevated CO₂ chamber were significantly higher among the treatments, the dry biomass was not. The

biomass of the plants that grew in the CO₂ chamber at 700 ppm and those of the ambient CO₂ treatment were both significantly higher than the biomass of the plants that received additional CO₂ through CaCO₃. The Swiss chard plants in all the treatments were infected with powdery mildew halfway through the trial. The plants were treated against the disease with Efecto Funginex once the first symptoms were seen but it was difficult to eradicate. It is possible that the decreases in photosynthesis was due to the presence of the disease. The photosynthetic rate of plants decline with the invasion of most pathogens as studies by Walters et al. (2008) and Kretschmer et al. (2019) found. Various authors have monitored the effect of pathogens on the photosynthetic rate of the host plant and they have discovered an incredibly significant phenomenon. They found that a large number of pathogens in the initial infection stages of infection results in the photosynthetic capacity of the host plant to always decrease (Scharte et al. 2005, Bonfig et al. 2006, Swarbrick et al. 2006, Chen et al. 2015, Hu et al. 2020). Studies have found that a reduction in stomatal density is considered to be a common response to increases in CO₂ levels (Woodward 1987, Lin et al. 2001, Teng et al. 2009, Xu et al. 2016). In this study the stomatal density of the Swiss chard plants in the CO₂ chamber (700 ppm) and in the ambient glasshouse were consistent in the beginning of the trial but increased in the last few weeks. The stomatal densities of the other treatments were relatively constant throughout the trial. There are studies in which the stomatal densities did not change (Tricker et al. 2005) nor increased (Reid et al. 2003) when plants were grown in CO₂ enriched environments.

As previously mentioned, root length and number is the most commonly evaluated parameter (45% of studies) in horticultural crops under elevated CO₂. The majority of these studies (61 -100%) found that root length increases as CO₂ increases (Rogers et al. 1994, Nie et al. 2013). In this study the Swiss chard plants that grew in the CO₂ chamber at 700 ppm as well as those that grew at ambient CO₂ had root lengths significantly shorter than the plants that received additional CO₂ through the 8 g CaCO₃ treatment. The shoot to root ratio of the plants grown under elevated CO₂ were significantly greater than those that received additional CO₂ via CaCO₃, but not those in the ambient CO₂ glasshouse. Studies by George et al. (2003), Iversen et al. (2008) and Pritchard et al. (2008) have shown that elevated CO₂ strongly influences root growth and distribution, however, the experimental data of other studies have shown it varies between species and the environments (De Graaff et al. 2006, Luo et al. 2006). In this study the root length did not differ between the CaCO₃ treatments. The root dry mass of the plants in the CO₂ chamber (700 ppm) on the other hand, had roots significantly heavier than those in any of the other treatments. The roots of the plants that received additional CO₂ through the 8 g CaCO₃ treatment had roots significantly heavier than those that received the 4 g CaCO₃ treatment and those in the ambient glasshouse. The root dry mass of the latter

treatments did not differ significantly from one another. Studies by other researchers have shown that root growth affects both shoot growth and yield (Imai and Murata 1976, Wittwer 1978, Rogers et al. 1983, Imai et al. 1985, Hocking and Meyer 1991, Norby et al. 1992, Rogers et al. in 1997, Vamerali et al. 2003, Zhang et al. 2009, Wang et al. 2014, Ota et al. 2020).

Chlorophyll concentrations of all three crops

Studies by Mamatha et al. (2014), Dey et al. (2017), Dong et al. (2018) and Song et al. (2020) found that elevated CO₂ could increase the chlorophyll content. In this trial the beans that grew in the ambient CO₂ chamber had a higher initial chlorophyll a content than the plants in any of the other treatments. The chlorophyll A and B content of the beans in all the treatments increased throughout the trial except the chlorophyll B content of the plants that grew in the ambient CO₂ chamber or in the glasshouse at ambient CO₂. The carotenoid content of the plants that grew in the ambient chamber had the biggest increase whereas those of the other treatments only had slight increases. Overall the plants that received additional CO₂ from the 8 g of CaCO₃ had the highest chlorophyll A and B content at the end of the trial and the plants in the elevated chamber (700 ppm) had the highest carotenoid content.

Chl A and B content in the beetroot plants that grew in the CO₂ chamber (700 ppm) increased, whereas the carotenoid content decreased slightly. Chl A and the carotenoid content decreased slightly in the beetroot plants grown in the glasshouse at ambient CO₂, whereas Chl B content increased slightly. The photosynthetic pigments of the beetroot plants treated with the CaCO₃ treatments remained relatively unaffected by the additional CO₂

In this trial, the chlorophyll A, B and carotenoid content increased in the Swiss chard plants that grew in the CO₂ chamber (700 ppm) when comparing the initial content to the content at the end of the trial. The Swiss chard that grew in the glasshouse at ambient CO₂ and those that received additional CO₂ from the 8 g CaCO₃ had a decrease in Chl A, B and carotenoid content. A study by Mamatha et al. (2014) found that when CO₂ is applied appropriately, can contribute to chlorophyll accumulation. The plants that received additional CO₂ from the 4 g CaCO₃ treatment had no change in Chl A, B and carotenoid content from the beginning to the end of the trial. The Chl A, B and carotenoid content of the bean plants in the same treatment decreased which is contradictory to the finding in the study by Mamatha et al. 2014.

From the pot trials it was clear that elevated CO₂ could positively effect plant growth and yield. It was also evident that the different vegetable types (root, leaf and fruiting) will react differently

to a rise in CO₂ levels, which could be exploited in intensifying vegetable production per unit area. More research is, therefore needed with various vegetable species for making accurate predictions of how yield would be affected.

CHAPTER 3

Effect of applying hydrogen peroxide as a foliar spray on the growth and yield of three vegetable crops

3.1 Introduction

Plants naturally have to withstand a continuously changing environment in terms of water, temperature, radiation etcetera, which causes plants to struggle in reaching their genetic growth potential. This genetic growth potential can be reached with the release of plant growth regulators at the maximum concentration (depending on plant age and treatment mode). Not only plant growth but yield are also seriously affected by an increase in the number of both biotic and abiotic stress combinations as a result of global warming and the climatic anomalies connected to it (Mittler 2006, Prasad et al. 2011, Atkinson et al. 2013, Narsai et al. 2013, Prasch and Sonnewald 2013, Suzuki et al. 2014, Mahalingam 2015, Pandey et al. 2015a, Ramegowda and Senthil-Kumar 2015). These simultaneous stresses have significant impact on crop production, for example simultaneous heat and drought stresses have proven to be more damaging to crop yield than that of each stress separately (Mittler 2006, Prasad et al. 2011, Fahad et al. 2017). Plant physiology is also affected by the interactions between various dynamic factors, especially plant photosynthesis as mesophyll conductance is dependent on both the concentration of carbon dioxide and irradiance (Kaiser et al 2015). Conversely the positive effect that elevated temperatures have on the photosynthetic efficiency of plants is diminished during a drought (Pfannschmidt and Yang 2012).

The relevant works of numerous researchers have established the role of hydrogen peroxide (H_2O_2) as a secondary messenger molecule (Orozco-Cárdenas et al. 2001, Neill et al. 2002, Veal et al. 2007, Quan et al. 2008) and its role in regulating plant physiology under both biotic and abiotic stress conditions (Orozco-Cárdenas et al. 2001, Małolepsza and Różalska 2005). A number of studies have shown that the exogenous application of H_2O_2 can improve plant tolerance to different abiotic stresses such as drought (He et al. 2009, Sun et al. 2016), heavy metals (Hu et al. 2009, Nazir et al. 2019) and heat (Gao et al. 2010). This occurs either through the regulation of the reactive oxygen species (ROS) detoxification, gene expression or the control of several stress-response pathways (Abass and Mohamed 2011, Li et al. 2011, Terzi et al. 2014, Hossain et al. 2015).

Initially hydrogen peroxide was used to sterilize seeds in agriculture (Massee 1913). Later on it was discovered that it can also improve seed germination (Ching 1959). Recently the application of H_2O_2 to various crops has resulted in the improvement of the physiological performance of plant as well as plant growth metabolism (Ishibashi et al 2011, Deng et al.

2012, Kao 2014, Maswada and Abd El-Rahman 2014, Sun et al. 2016). Unfortunately the degradation of chlorophyll can also occur (Hasan et al 2016). Other studies have shown that seedling growth has improved in numerous plants when the seeds had been soaked in H₂O₂ (Liu et al. 2012, Rajashekar and Baek 2014) before sowing. It is, however, dependent on the H₂O₂ concentration (Razem 2008). Generally H₂O₂ is applied in low concentrations (≤ 2.5 mM) as higher concentrations (5 mM maximum) stimulates cell senescence and cell death (Deng et al. 2012).

As a strong oxidising agent hydrogen peroxide is mainly applied to plants through fertigation in order to inhibit the growth of plant pathogens, despite having a strong effect against established pathogens (Shetty et al. 2007, Raudales et al. 2014, Ali 2018). Hydrogen peroxide can also be used in controlling pests, such as nematodes, which are sensitive to oxygen (Blum and Fridovich 1982). A study by Caixeta et al. (2018) found that the application of hydrogen peroxide has the ability to trigger a phytotoxic response in plants. When it is applied at lower concentrations than recommended for pathogen control (lower than 100 mg L⁻¹), it can improve plant growth. Hydrogen peroxide may positively affect the germination of lettuce seeds and prevent the presence of adult flies. When hydrogen peroxide is injected into the soil it can result in increased biomass production and water use efficiency (Gil et al. 2009). Injecting H₂O₂ into saturated heavy clay soils, via the irrigation system, have shown an increase not only in the biomass of crops such as soybeans, cotton and zucchinis but also their yield (Bhattarai et al. 2004).

Hydrogen peroxide has a promising role as a reactive oxygen species in not only plant growth but also plant metabolism (Hasan et al. 2016). Numerous studies have found that with the application of H₂O₂ at low concentrations, plant growth can be improved (Lin and Kao 2000, Foreman et al. 2003, Liao et al. 2009, Li and Jai 2013, Ma et al. 2014, Ahmad et al. 2014, Hernández-Barrera et al. 2015, Hasan et al. 2016). The application of H₂O₂ to crops could therefore potentially increase not only crop growth but also crop yield. It is however expected that different crops will respond differently to the foliar application of H₂O₂ (thus increased O₂ levels), therefore a leafy, fruiting and rooting vegetable crop were studied. The aim of this study was to investigate the effect of hydrogen peroxide on plant growth and yield when applied as a foliar spray. Swiss chard, beetroot and beans were grown in an open field at plot scale. The objectives are to determine the reaction of the three vegetable crops as influenced by hydrogen peroxide in terms of the stomatal density, chlorophyll content, leaf area, growth and yield. It was expected that Swiss chard and beetroot will show an increase in leaf area, have a greater stomatal density, show an increase in chlorophyll content, exhibit increased growth and produce higher yields than the plants not treated with hydrogen

peroxide. It is expected that the beans will produce higher yields than the plants receiving no treatment.

3.2 Methodology

3.2.1 Experimental location

This study was conducted at Innovation Africa @UP (Previously known as the Hatfield Experimental farm, University of Pretoria (25°45'06.4"S 28°15'33.5"E). A 270m² plot in an open field equipped with an irrigation system was utilized for this trial. The plot had an east-west orientation. Some small game animals such as guinea fowl, bunnies, cats, Hyraxes, Duiker and muskrats' etcetera were present on the farm.

3.2.2 Experimental design

This experimental study was done in a randomised group design. It consisted of three vegetable crops namely Swiss chard (Fordhook Giant), beetroot (Crimson Globe) and beans (Star 2000). Five treatments were applied, namely: A (hydrogen peroxide concentration 0.05 ml L⁻¹ once a week), B (hydrogen peroxide concentration 0.05 ml L⁻¹ twice a week), C (no treatment, just water), D (hydrogen peroxide concentration 0.1 ml L⁻¹ once per week), E (hydrogen peroxide concentration 0.1 ml L⁻¹ twice per week). The days on which the treatments were applied varied as weather conditions (rain and wind speed etc.) was taken into consideration. Each treatment had 32 replicates per crop.

Field layout

The plot consisted of three large blocks (8 m by 9 m). Each large block consisted of four sub-blocks (2 m by 9 m). The sub-blocks were divided into three sub-sub-blocks. Each crop was then randomly allocated to a sub-sub-block. Each of the sub-sub-blocks had two ridges (0.8 m apart) in the middle. The plot was not perfectly level lengthwise. A soil analysis was conducted before the seedlings were planted to determine whether any fertilizer should be added as well as whether the soil pH was conducive to plant growth. The soil analysis concluded that the soil was a bit low in nitrogen, therefore three grams of (Limestone Ammonium Nitrate) LAN fertilizer were applied every 25 cm, approximately 10 cm deep at the point where the seedlings would be planted. The fertilizer was then covered with approximately 1-2 cm of soil and irrigated every second day, over a period of 10 days for 45 minutes before planting. Approximately 250 seedlings of beetroot and Swiss chard seedlings were purchased, whereas the beans were cultivated from seeds. The seedlings were planted on 22 of November 2021 and the seeds on the 23 November 2021. The plot was then covered with a

net for two weeks until the beans germinated and the Swiss chard and beetroot seedlings established. The net prevented Guinea fowl, Hyraxes, rabbits, mongoose etc. from entering the plot and damaging the seedlings. The net was removed on 10 December 2021 and the seedlings left for another 2 weeks to give the beans time to grow. The first measurements and treatments were applied in the week of the 26 December 2021.

Soil analyses

Throughout the 270 m² plot, 45 sampling sites were chosen, the “V” method was used to take the soil samples, the sample area was cleared of any surface litter and two hand shovels (15 cm blade) were used to make a “V” shape, 15 cm deep in the soil. The soil between the shovels was then put into a paper bag and labelled. The soil was dried in an oven for 24 hours at 90 °C. Once it was dried, all 45 labelled samples were sifted to remove any remaining rocks and debris. Many of the samples contained large clumps of soil that were ground and crushed with a mortar and pestle to allow for the soil to pass through the sift. The soil was then placed back into its corresponding sample bag.

Four tests were conducted on each of the sifted samples namely: a Mehlich-3 test (M3), a KCl extraction, pH and electrical conductivity (EC) test (Table 3.1). One hundred and thirty-five, 50 ml, test tubes were labelled according to the soil sample number and the test that would be conducted for e.g., samples were numbered 1-45 and given a “K” for the KCl test, pH for the pH test and “M” for the Mehlich-3 test. The same labelling system was applied for ninety (15 ml) test tubes.

Mehlich-3 tests: Three grams of soil from each sample were weighed and placed in the corresponding 50ml tube. Thereafter 45 labelled (15 ml) test tubes were placed in a test tube rack. Forty-five Whatman nr. 2 filter papers were folded three times and placed in each test tube. Thirty millilitres of the Mehlich-3 solution was then added to the soil in the 50 ml test tubes and immediately shaken for 5 minutes. After the solution was mixed, it was transferred to the filter paper in the 15 ml test tubes within three minutes. The samples were then stored at 4°C until the analysis (K, P, Cu, Fe, Mn, Mg, S, Na, Ca, Al and Zn) (Table 3.1).

KCl extraction: Two and a half grams of soil from each sample was weighed and placed in the corresponding 50ml tube. Twenty-five millilitres of 1M KCl was added to each of the 45 samples and shaken for 60 minutes in an orbital shaker. In this period 45 labelled (15 ml) test tubes were placed in a test tube rack. Forty-five Whatman nr.2 filter papers were folded three times and placed in each test tube. Once the samples were mixed in the orbital shaker, the solution was poured into the filter paper of the corresponding test tubes, until 6-10

ml of the solution was filtered. To determine the NH_4^+ in the samples, 1 ml of each of the filtered samples was pipetted into 4 ml cuvettes. One millilitre of a buffer was added to each cuvette. A new pipette tip was also required after the 0.84 ml Nitro mix and 0.42 ml of Jik were added. The samples were then left to stand for 45 minutes before a Spectrophotometer reading was done at 650 nm of NH_4^+ . In order to determine the NO_2 and NO_3 content in the samples, 0.1 ml of the filtered solution was pipetted into a 2.5 ml cuvette. Then 1.2 ml of NO_2 MIX was added into the cuvette (by pipette) and left for 20 minutes at room temperature before a Spectrophotometer reading was done at 540 nm. In order to determine the NO_3 content, 0.1 ml of NO_3 MIX was added to the NO_2 sample in the 2.5 ml cuvette. The samples were then placed in an oven for 25 minutes at 60°C , whereafter they were left to cool down for 10 minutes. Once the samples were at room temperature a Spectrophotometer reading (of NO_2 and NO_3) was taken at 540 nm (table 3.1).

The pH and EC measurements: Ten grams of the dried and sieved soil of each sample was placed in a labelled 50 ml tube to which 25 ml of de-ionized water was added. The contents were shaken rapidly for five seconds and allowed to stand for 30 minutes. When taking the measurements with the calibrated pH-meter, the first tube was shaken again, after five minutes the pH meter was placed in the tube for the last five minutes before the measurement was recorded. Five minutes after the first tube was shaken, the second tube was shaken and ready to be measured once the first measurement was recorded. Directly after the pH was measured the electrical conductivity (EC) of each samples was taken (Table 3.1). These readings were taken with a Multiparameter Photometer & pH meter.

Table 3.1: Results of the soil analyses.

		KCl test (mg kg ⁻¹)			Melich-3 (mg kg ⁻¹)										
	pH	NO ₂	NO ₃	NH ₄ ⁺	Cu	Fe	K	Mg	Mn	P	Na	Al	Ca	S	Zn
1	6.24	0.005	0.039	0.015	5.35	46.15	78.04	216.93	153.84	51.59	18.76	643.92	628.21	48.60	10.59
2	6.25	0.008	0.023	0.015	5.57	49.51	63.93	205.33	157.97	70.88	19.02	622.15	638.01	51.14	11.32
3	6.28	0.008	0.222	0.017	5.36	52.69	56.38	194.60	159.34	87.92	18.16	598.85	654.44	44.25	10.85
4	6.21	0.012	0.035	0.019	5.37	53.14	65.29	199.77	162.24	91.46	19.34	592.75	669.41	47.17	11.29
5	6.06	0.010	0.017	0.024	5.15	52.65	66.58	195.70	158.84	91.15	17.17	572.19	661.51	47.93	10.87
6	6.04	0.013	0.040	0.048	5.04	51.49	81.26	200.57	158.87	94.93	17.22	561.92	653.54	48.77	11.84
7	6.11	0.004	0.049	0.028	5.15	50.17	77.50	198.27	154.77	98.92	16.57	566.29	639.37	48.01	11.61
8	6.17	0.009	0.060	0.033	5.08	48.40	79.03	198.97	151.21	97.06	16.78	570.42	619.94	45.27	11.61
9	6.07	0.006	0.038	0.025	5.19	44.60	69.81	202.83	144.94	76.08	16.48	586.99	599.41	45.20	10.47
10	6.16	0.005	0.032	0.020	5.17	42.48	75.24	216.83	141.74	55.22	16.32	611.29	596.71	44.47	9.14
11	5.66	0.004	0.038	0.014	5.41	49.56	84.83	218.70	152.51	64.91	16.97	646.79	627.57	49.92	10.17
12	6.13	0.006	0.037	0.017	5.71	59.43	89.42	213.33	163.77	89.49	17.86	665.55	653.27	56.58	12.91
Avg.	6.11	0.01	0.05	0.02	5.30	50.02	73.94	205.15	155.00	80.80	17.55	603.26	636.78	48.11	11.06

Treatment application

The hydrogen peroxide (H_2O_2) treatments were prepared as follows: 0.5 ml and 1 ml of hydrogen peroxide was mixed with 10 L of tap water to give the respective 0.05 ml L^{-1} (low) and 0.1 ml L^{-1} (high) concentration. The treatments were applied as a fine spray with the aid of a motorised sprayer in the morning (between 10:30 and 11:30 am) to the assigned sub-blocks of all three crops. The water was applied first to the control group followed by the low H_2O_2 treatments (0.05 ml L^{-1}) and then the high H_2O_2 treatments (0.1 ml L^{-1}) treatment. Between applications of the different concentrations, the tank was flushed with clean water as well as after all the treatments were applied. The treatments were applied on days that no rain was expected and thus the treatments were not necessarily applied on the same days each week. Throughout this trial it rained a great deal and due to the fact that the plot was not levelled some parts of the plot had slight flooding. When this was detected a furrow was dug to encourage waterflow and thus minimised flooding.

Weather data

Weather data was collected from the weather station at Innovation Africa (UP) over the duration of the study as indicated in Table 3.2.

Table 3.2: The weather experienced over the period of the study.

	Total rainfall (mm)	Maximum average temperature ($^{\circ}\text{C}$)	Minimum average temperature ($^{\circ}\text{C}$)
December 2021	275.2	26.3	15.2
January 2022	155.8	31	16.3

3.2.4 Measurements

Various measurements were taken over the duration of the trial as indicated in Table 3.3. The bean plants produced bean pods over a period of time and those were harvested as they matured (**), while the beets and Swiss chard were only harvested once (*) at the end of the trial. Measurements associated with plant growth and photosynthesis were collected once a weekly (**) and every day during the first week (**/**).

In this dissertation, the term beet(s) will refer to the edible root part of the beetroot plant, while biomass will refer to the leaf (Swiss chard and beetroot) or stem and leaf material (bean plants). The stem portion of the Swiss chard and beetroot were very small at harvest and ended up being part of the leaves (Swiss chard) or beets (beetroot).

Table 3.3: All the measurements taken throughout the second trial.

	Beans	Beetroot	Swiss chard
Height	**	**	**
Chlorophyll and carotenoid content	**/** **	**/** **	**/** **
Stomatal density	**/** **	**/** **	**/** **
Pod length and width	***	NA	NA
Number of seeds per pod	***	NA	NA
Beets length and width	NA	*	NA
Pod fresh mass	***	NA	NA
Pod dry mass	***	NA	NA
Beets wet mass	NA	*	NA
Beets dry mass	NA	*	NA
Fresh biomass	*	*	*
Dry biomass	*	*	*
Leaf Area	NA	*	*
General observations	**	**	**

/ Measurements taken daily in the first week of treatments, ** measurements taken weekly, *** measurements taken per harvest and * measurements taken once trial was terminated. Blocks that have two rows of different * patterns indicates that two different rates of measurements were taken. NA (not applicable) indicates that this crop did not have that particular measurements taken.

Plant height

The plant heights were measured weekly (Table 3.3, **). This variable was measured in centimetres (cm) using a ruler, placed vertically on the surface of the soil. The measurement was taken at the highest stem meristem for the beans, from the soil surface to the tip of the longest leaf for Swiss chard and from the crown to the leaf tip of the longest leaf for the beetroot. The length and width of the bean pods and beets were measured per harvest and

at the end of the trial, respectively, with a Vernier Caliper. The root length of the beetroot plants did not include the beet part.

Sample selection and analysis

Two samples of mature leaves were taken (from 2 different plants of the same crop) from each crop and treatment (12 samples per vegetable crop) in a randomised manner. Only mature leaves were taken for the samples for further analysis. The leaf samples were collected once a week between 10:30 and 11:30 am and placed in labelled plastic bags before immediately being taken to the laboratory. Daily measurements were taken to determine if the treatments had a short term effect and the weekly measurements for the long term effect.

Chlorophyll concentration: Four discs that were 1.5 cm in diameter, were taken from each of the leaf samples and placed into 15 ml conical centrifuge tubes (labelled according to the crop, treatment and sample number) filled with 10 ml of 80% acetone. The leaf disc tissue were then homogenised and crushed with a glass rod whereafter the acetone mixture was then left in a cooling chamber for one hour. It was then centrifuged at 2500 rpm for five minutes. The samples were then taken, analysed spectrophotometrically and the absorbance determined at 470 nm, 647 nm, 663 nm wavelengths. At the end of every analyses the concentration of chlorophyll A, B and carotenoids were calculated using the following equations:

- $C_a (\mu\text{g ml}^{-1}) = 12.25(A_{663}) - 2.79(A_{647})$
- $C_b (\mu\text{g ml}^{-1}) = 21.50(A_{647}) - 5.10(A_{663})$
- $C_{x+c} (\mu\text{g ml}^{-1}) = ((1000 \times A_{470}) - (1.82 \times C_a) - (85.02 \times C_b))/198$

Where C_a represents chlorophyll A, C_b chlorophyll B, and C_{x+c} total carotenoids (i.e. carotenes plus xanthophylls).

Stomatal density: An area of approximately 2 cm^{-2} at the adaxial of the leaves were coated with clear nail polish (to get an impression of leaf epidermis), left to dry and peeled off using tweezers. The epidermal impression was then stucked to the microscope slide with a drop of glycerol (with an alcohol binding in it). The stomatal conductance was counted in three different 1 cm^{-2} squares by looking through a graticule eyepiece at 10x magnification. Stomatal densities were measured daily to determine how fast the plants reacted to the treatments as well as then over a longer period of time (weekly) if it would affect the density.

Mass measurements

The fresh and dry mass of the bean pods, beets and biomass of the plants were weighed after each harvest or at the end of the trial. The plants were placed into labelled paper bags after the fresh weight and all other measurements of the pods and beets were taken and dried in an oven at 70°C for 3-4 days. For the beets to dry, they were cut into smaller sections to dry faster. After the samples have dried they were weighed again to measure the dry mass. The biomass consisted of the above ground part of the plants that did not already contribute to crop yield. The crop yield of the beans were the bean pods, that of Swiss chard is the leaves and that of the beetroot plants the beets.

3.2.5 Insect and pathogen identification

The plants in the field, especially the beans had both aphids and caterpillars that were damaging the plants and the beetroot had white grubs that affected plant growth. The plants were then sprayed with Efeko: Aphid plus. In the first week of the second month of the trial both the beetroot and Swiss chard were covered in a whitish powder and black spots. These pathogens were later identified as powdery mildew and black spot (treated with Efecto Funginex). The outbreak of these diseases are very likely as a result of the heavy rains during the study. The plants were treated for both these pathogens successfully over two weeks, however, during week six of the study some Swiss chard and beetroot plants started to die. The Swiss chard leaves looked very wilted even after irrigating, some round spheres with a white hairy fungus could be seen (looked like nodules) on the leaf stems of the Swiss chard (Figure 3.1). The beetroot also had wilted leaves and a white, hairy fungus could be seen on the soil near the beets.



Figure 3.1: An unknown pathogen found in not only the soil but also between the leaf stems of the Swiss chard and the beets of the beetroot in the field study.

Pathogen identification

Plant material of an infected Swiss chard plant was collected and taken to a laboratory for identification. Three small pieces of infected plant material were cut with the use of a scalpel and transferred to the agar plate with forceps (these tools were cleaned by dipping them into ethanol and burned with a Bunsen burner). The plates were then closed, sealed, labelled and kept in a dark place for approximately a four days. On the fifth day, it was found that only one fungus grew on all three plates. A scalpel and forceps were sterilized again by dipping them in ethanol, burned with a Bunsen burner and left to cool. Once the scalpel was cool five small pieces (0.5 cm³) of the cultured pathogen were cut from each of the plates that contained the pathogen (with the scalpel being sterilized again between plates). A single agar piece each had approximately 50% fungi and 50% clean agar. The forceps were then used to transfer each fungi covered agar piece to new agar plate, which was then covered, sealed and labelled. The fifteen plates were again left in a dark place for a few days. After three days, about four plates had a pure culture. With the help of Prof. Kritzinger at the University of Pretoria and his colleague, Dr Mariette Truter at ARC, Roodeplaat, the pathogen was identified as *Sclerotium rolfsii*.

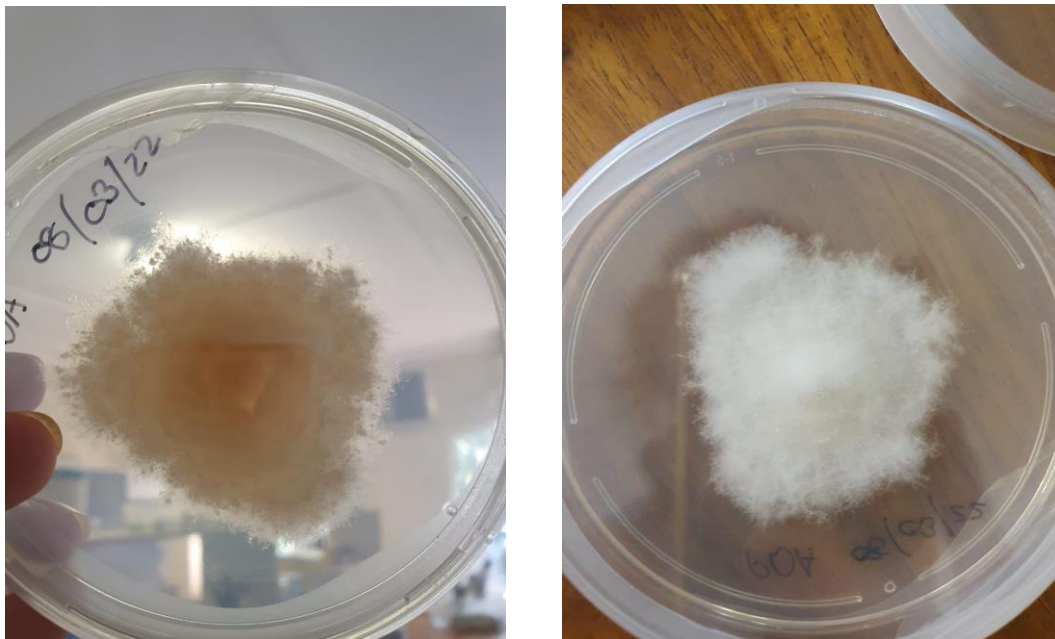


Figure 3.2: Image of the cultured fungi on the agar plate from which *Sclerotium rolfsii* was identified.

3.2.6 Leaf area index (LAI)

The leaf area of the beetroot and Swiss chard plants were measured with the use of a LI-3100C Area Meter. All the leaves of each plant was cut and passed through the machine to get the leaf area measurements. The leaf area index was later calculated as follows:

$$LAI = \frac{\text{Leaf Area (cm}^2\text{)}}{\text{Ground cover (cm}^2\text{)}}$$

3.2.7 Statistical analysis

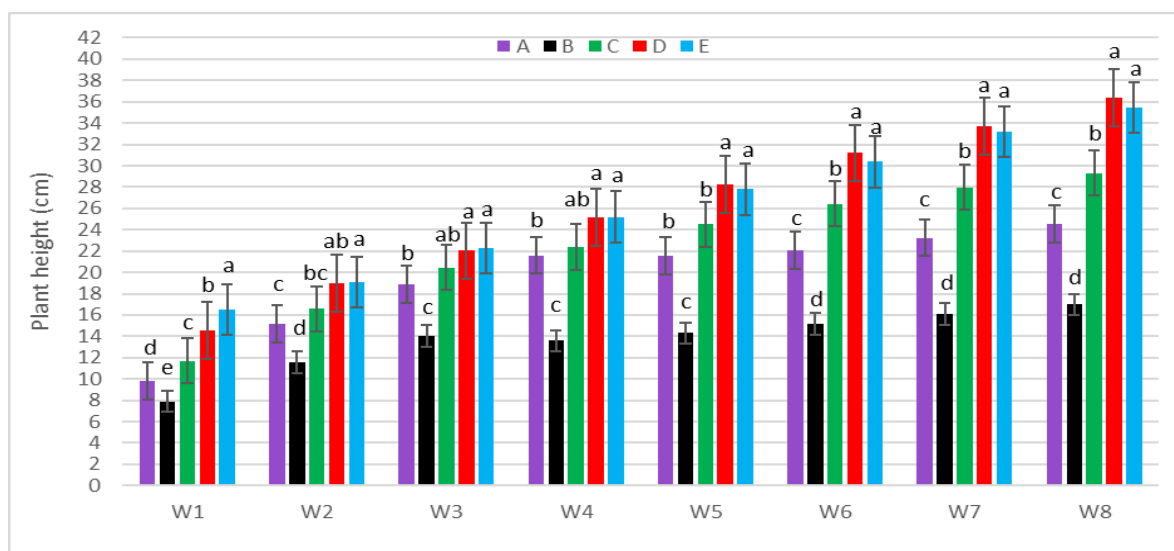
The data was analysed using the statistical package SAS (model 9.4) to test if the means were significantly ($P < 0.05$) affected by the treatments. The Tukey LSD test was used to determine if there was a significant difference at a 5% probability. The ANOVA tables are included in the Appendix C. The graphs in the results section were drawn using the means and the yield were drawn using the sum of the mass.

3.3 Results

3.3.1 Plant height

Beans

The bean plants sprayed with a hydrogen peroxide (H_2O_2) concentration of 0.05 ml L^{-1} , twice a week (B), resulted in the smallest plants throughout the eight-week study (Figure 3.3). In comparison, those that were sprayed with the same concentration of H_2O_2 but only once a week (treatment A) produced significantly ($P \leq 0.05$) taller bean plants. Compared with treatment C (control), the bean plant heights of treatments D and E (0.1 ml L^{-1} once and twice a week, respectively) resulted in the tallest plants throughout the trial. During the first month of the study, the plants in treatment E was taller than those in treatment D, however in the last month of the trial the plants in treatment D grew taller than those in treatment E. The plants treated with the low H_2O_2 treatment weekly (A) resulted in plants that initially were not significantly shorter than the plants in treatment C (control), but from week 5 to the end of the study the difference became significant. The plant heights of treatments C, D, and E show a steady increase throughout the study, whereas the heights of treatment A shows an initial increase in height whereafter it levelled off and treatment B shows a steady increase.

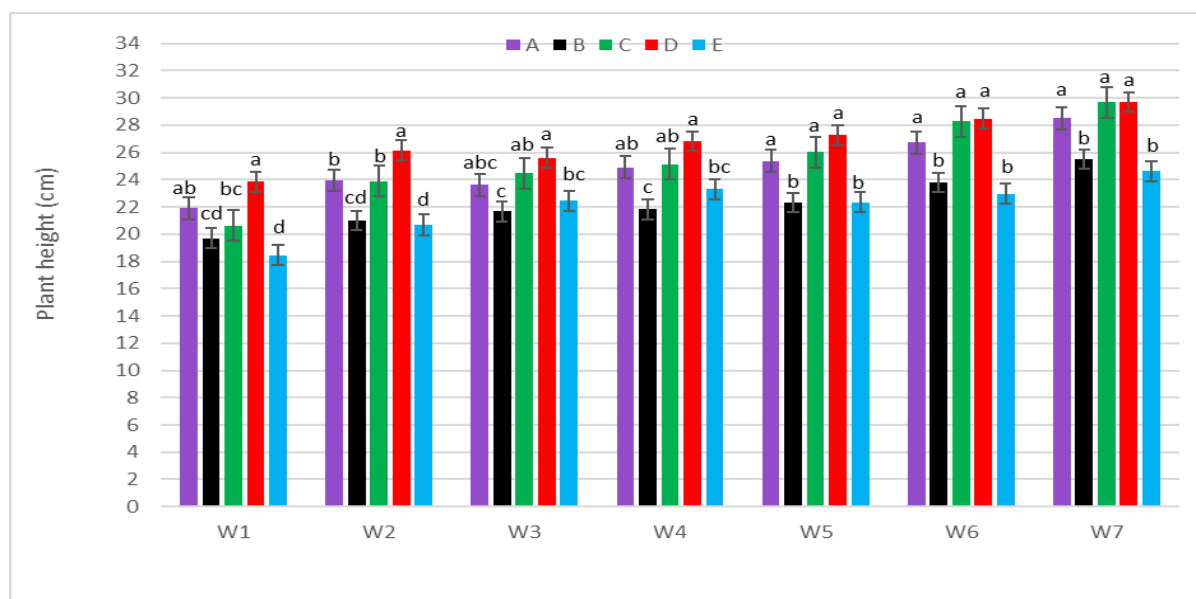


A to E represents the different treatments applied in the study and W1-W8 represents weeks one to eight. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no treatment (water); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars of the same week with different letters indicates a significant difference ($P < 0.05$) between treatments.

Figure 3.3: Weekly changes in bean plant height grown in open field conditions and treated with different foliar applications of hydrogen peroxide.

Beetroot

Throughout the study the beetroot (Figure 3.4), treated with the lower concentration of H_2O_2 weekly (A) consistently resulted in beetroot plants taller than those treated twice a week with the same concentration (B). The plants in treatment A were significantly taller than those in treatment B for most of the study, except in week 3. The weekly application of the higher concentration of H_2O_2 (D) had significantly taller plants, throughout the study, than those treated twice a week with the same concentration (E). The plant heights of treatments A, C and D did not differ significantly from each other from week 3 to week 7 and the plant heights increase throughout the study. The H_2O_2 treatments that were applied twice a week (B, 0.05 ml L^{-1} and E, 0.1 ml L^{-1}) but at different concentrations had a steady increase in height throughout the trial but ultimately resulted in the shortest plants but also did not differ significantly from each other. The beetroot height of the latter treatments (B and E) were significantly shorter than the plants in the control (C) group. The plant height of beetroot treated weekly with the lower concentration of H_2O_2 (A) throughout the trial weren't significantly shorter than the plants in the control group (C). The plants treated weekly with the high concentration of H_2O_2 (D) initially (week 1 and 2) were significantly shorter than the plants in the control group but in the last five weeks of the trial the difference became nonsignificant.

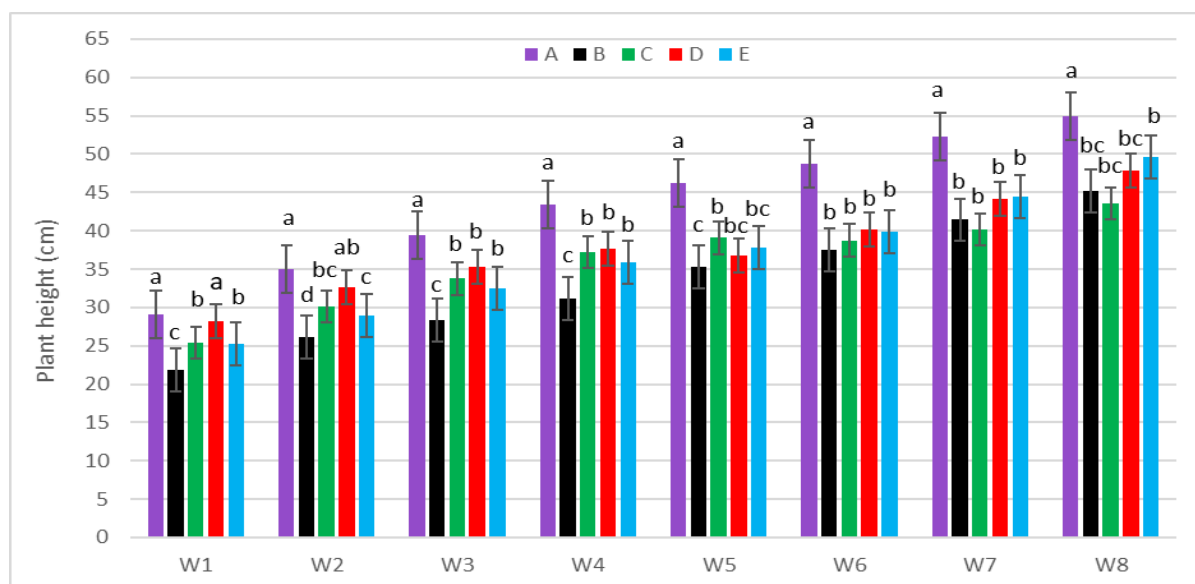


A to E represents the different treatments applied in the study and W1-W8 represents weeks one to eight. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no treatment (water); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars of the same week with different letters indicates a significant difference ($P < 0.05$) between treatments.

Figure 3.4: Weekly changes in beetroot plant height grown in open field conditions and treated with different foliar applications of hydrogen peroxide.

Swiss chard

The Swiss chard plants (Figure 3.5) treated with the low H_2O_2 concentration (0.05 ml L^{-1}) weekly (A), resulted in the plant that were significantly taller than the plants in any of the other treatments (B, C, D and E) throughout the study. The Swiss chard plants that were treated twice a week with the low concentration of H_2O_2 (B), had significantly smaller plants than those of the control group (C) during the first month of the study whereafter the difference in plant heights became nonsignificant. Initially the height of the plants treated with the high H_2O_2 concentration (D), weekly resulted in plants that were significantly taller than those that were treated with the same H_2O_2 concentration twice a week (E), but from week 3 onwards the difference became nonsignificant. Throughout the study the height of Swiss chard in treatment C, D and E did not differ significantly from one another. Throughout the study the plants in all the treatments increase in height throughout the trial. The Swiss chard was the only crop in which treatment A outperformed the control from start to finish. It was also the only crop where the low H_2O_2 concentration (A) gave better results from the beginning to the end of the study than the high concentration of H_2O_2 applied twice a week (B). The bean plants treated with the higher concentrations of H_2O_2 (C and D) gave better results from the beginning of the study until the end. The beetroot plants fared better with no treatment and/or a high concentration of H_2O_2 once a week.

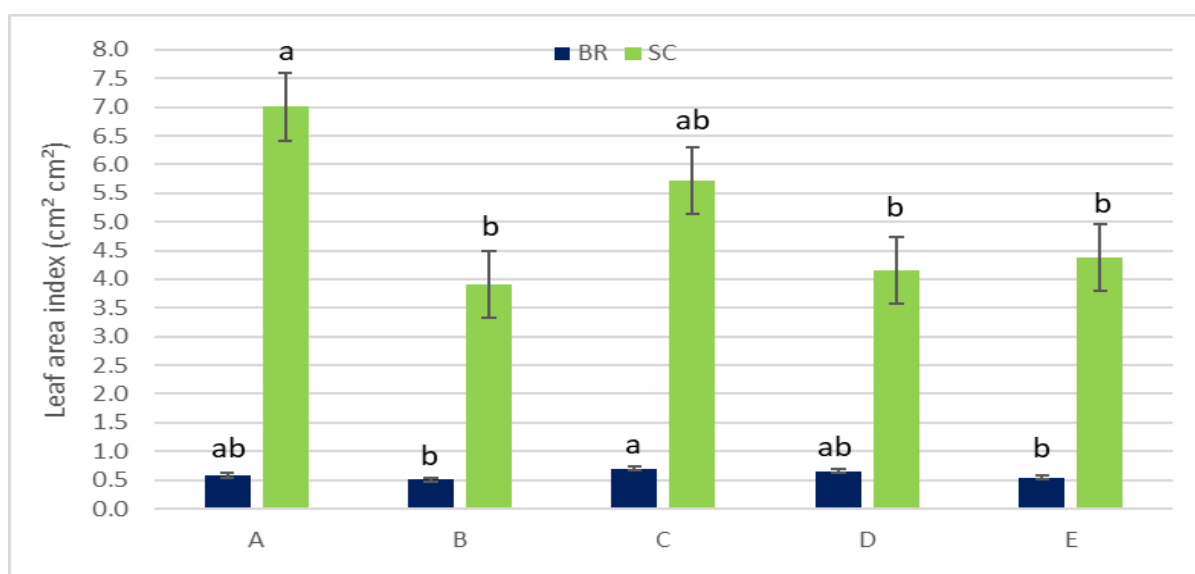


A to E represents the different treatments applied in the study and W1-W8 represents weeks one to eight. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars of the same week with different letters indicates a significant difference ($P < 0.05$) between treatments.

Figure 3.5: Weekly changes in Swiss chard plant height grown in open field conditions and treated with different foliar applications of hydrogen peroxide.

3.3.2 Leaf area index

At final harvest, most of the bean leaves were dry and brittle, therefore it could not be used to determine leaf area index. The beetroot plants (Figure 3.6) that received no treatment (C) had the highest leaf area index (LAI) and did not differ significantly from the plants treated weekly with the low H_2O_2 treatment (A, 0.05 ml L^{-1}) and those treated weekly with the high H_2O_2 treatment (D, 0.1 ml L^{-1}). The LAI of treatment C did, however, differ significantly from treatments that were applied twice a week at the lower and higher (B and E, respectively) H_2O_2 concentrations, with the latter two treatments having the lowest LAI. The Swiss chard plants treated weekly with $0.05 \text{ ml L}^{-1} \text{ H}_2\text{O}_2$ (A), resulted in plants with the highest LAI and differed significantly all the treatments, except those that received no treatment (C). The LAI of the plants treated with the low H_2O_2 concentration applied twice a week (B) and those treated with the higher H_2O_2 concentrations (D and E) did not differ significantly from one another. The Swiss chard plants in treatment B resulted in the lowest LAI for both Swiss chard and beetroot.



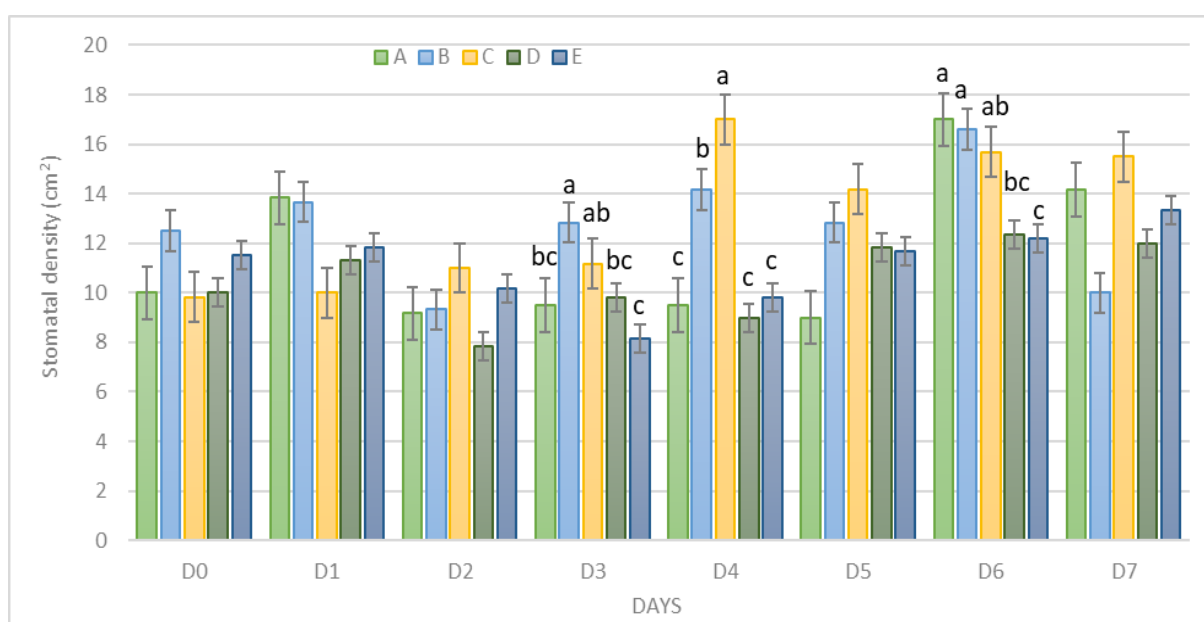
A to E represents the different treatments applied in the study and H1 to H4 represents harvest one to four. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars with the same colour and letter do not differ significantly from each other ($P < 0.05$).

Figure 3.6: The effect of different hydrogen peroxide treatments on the leaf area index of beetroot (BR) and Swiss chard (SC) plants.

3.3.3 Daily changes in stomatal density

Beans

Over a period of seven consecutive days the changes in the stomatal density were determined. The stomatal densities in the bean (Figure 3.7) plants did not differ significantly between treatments from day zero to two, day five and seven. Throughout the first week of treatments the stomatal densities fluctuated in all the treatments and no clear pattern can be discerned. The plants treated weekly with the low H_2O_2 concentration (A) throughout the trial did not consistently have a stomatal density higher or lower than that of the plants treated twice a week with the same H_2O_2 concentration (B). The plants treated weekly with the high H_2O_2 concentration (D) and twice a week (E) also did not have one treatment consistently higher or lower than the other. The plants that received no treatment (C) had the highest stomatal density during most of the first week, except on day 0, 1, 3 and 6.

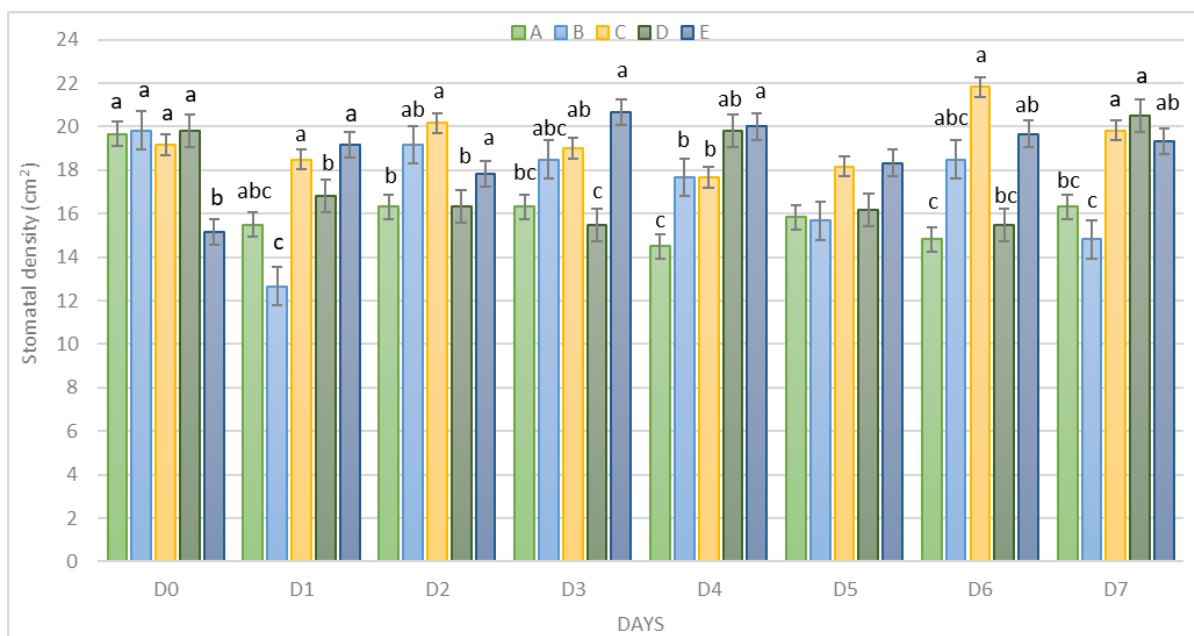


A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. D0 to D7 represent day 0 (pre-treatment) until day seven, after which all treatments for the week were applied. Bars of the same day and no letter indicates no significant difference between treatments ($P < 0.05$), whereas bars of the same day and different letters indicates a significant difference between treatments.

Figure 3.7: The daily change in the stomatal density of bean plants treated with different hydrogen peroxide treatments.

Beetroot

The stomatal density in the beetroot plants differed throughout the first week of the trial (Figure 3.8) between treatments. Similar to the stomatal densities in the beans, there is no clear pattern discernible. The plants treated weekly with the low H_2O_2 concentration (A) throughout the trial did not consistently have a stomatal density higher or lower than that of the plants treated twice a week with the same H_2O_2 concentration (B). The plants treated weekly with the high H_2O_2 concentration weekly (D) throughout most of the week had a higher stomatal density than the plants treated twice a week with the same H_2O_2 concentration (E). The plants that received no treatment (C) throughout most of the week had the highest or second highest stomatal density, except on day 4.



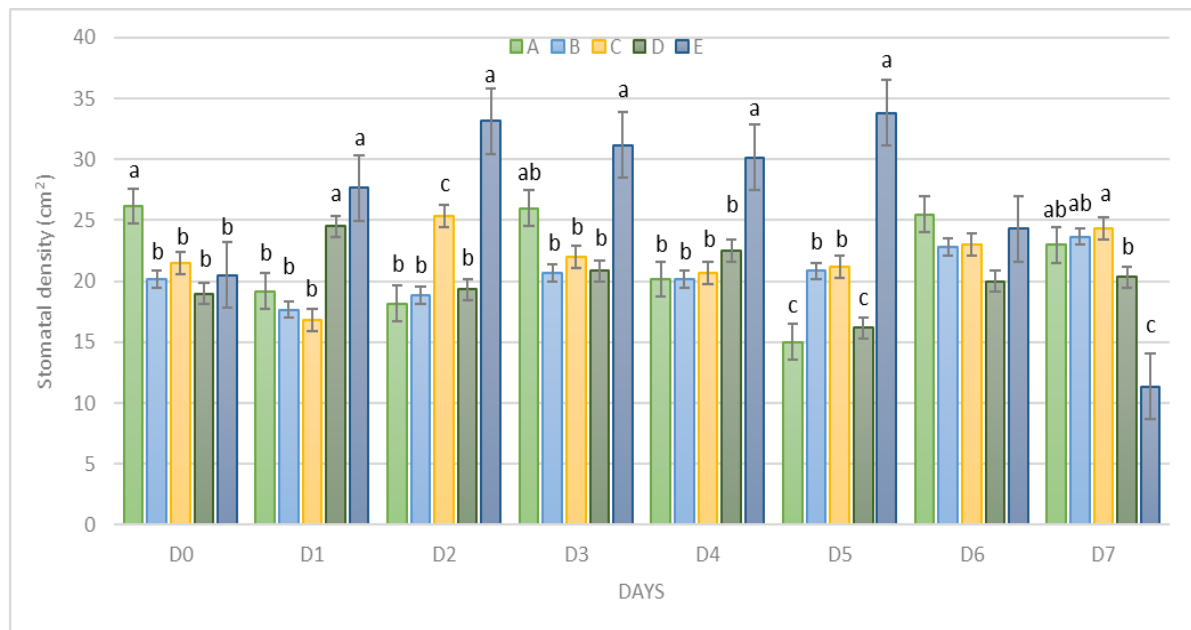
A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. D0 to D7 represent day 0 (pre-treatment) until day seven, after which all treatments for the week were applied. Bars of the same day and no letter indicates no significant difference between treatments ($P < 0.05$), whereas bars of the same day and different letters indicates a significant difference between treatments.

Figure 3.8: The daily change in the stomatal density of beetroot plants treated with different hydrogen peroxide treatments.

Swiss chard

From day two to five of the first week (Figure 3.9), the plants treated with the high H_2O_2 concentration twice a week (E), had the highest stomatal density and differed significantly from

all the other treatments, except on day three. Throughout most of the first week the stomatal density of the plants treated weekly and twice a week with the low H_2O_2 concentration (A and B, respectively) did not differ significantly from those that received no treatment (C). Between the plants treated weekly with the high H_2O_2 concentration (D) and those treated twice a week with the same H_2O_2 concentration (E), the stomatal density in the latter treatment were higher than those in treatment D.



A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. D0 to D7 represent day 0 (pre-treatment) until day seven, after which all treatments for the week were applied. Bars of the same day and no letter indicates no significant difference between treatments ($P < 0.05$), whereas bars of the same day and different letters indicates a significant difference between treatments.

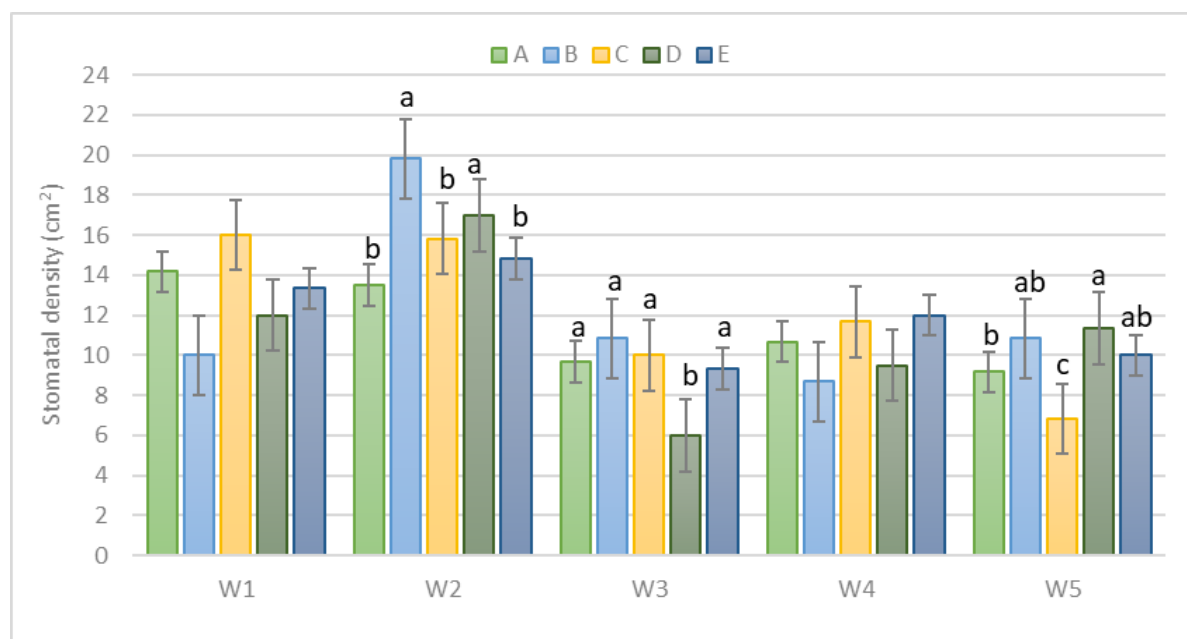
Figure 3.9: The daily change in the stomatal density of Swiss chard plants treated with different hydrogen peroxide treatments.

3.3.4 Weekly changes in stomatal density

Beans

The weekly changes in stomatal density still does not have a clear pattern that is discernible (Figure 3.10). The stomatal densities of the plants that received no treatment (C) and those treated weekly with the low H_2O_2 concentration (A) does seem to decrease throughout the trial. The stomatal densities of the two low H_2O_2 concentration treatments (A and B) showed no significantly different stomatal densities, except in week two, but there was

no one treatment that consistently had better results than the other. A similar trend occurred between that high H_2O_2 concentration treatments (D and E) than between treatment A and B.

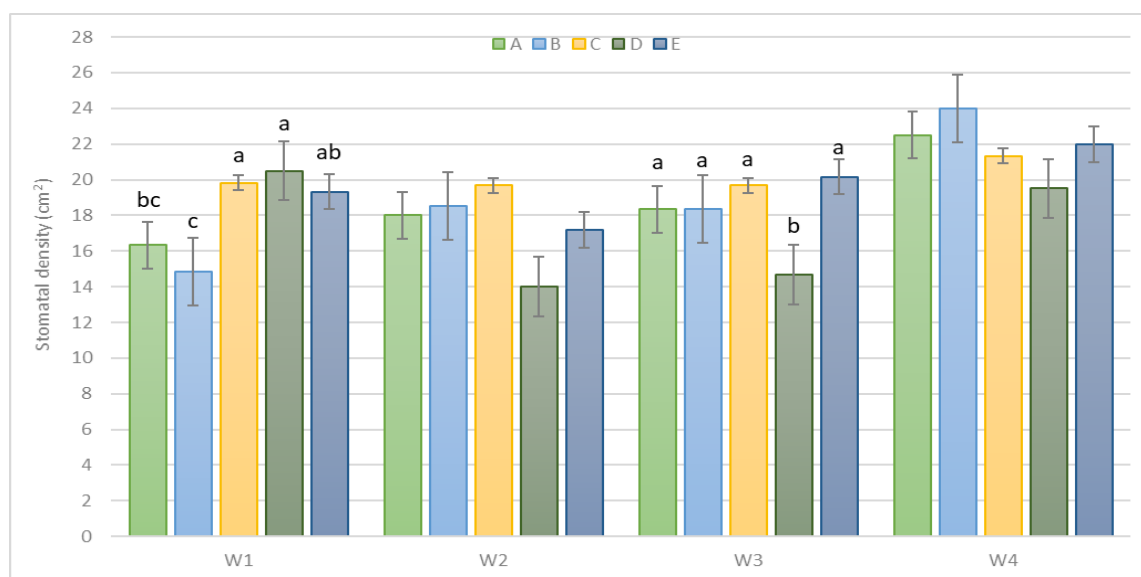


A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. W1 to W5 represent week one to week five. Bars of the same day and no letter indicates no significant difference between treatments ($P < 0.05$).

Figure 3.10: Weekly changes in the stomatal density of bean plants treated with different hydrogen peroxide treatments.

Beetroot

With the beetroot (Figure 3.11) plants, there were no significant difference between the treatments in week 2 or 4 in terms of the stomatal density. The stomatal density of the plants that received no treatment (C) remained relatively unchanged. The stomatal density of the plants treated weekly and twice a week with the low H_2O_2 concentration (A and B) increased, but there was no one treatment that consistently had better results than the other. This trend was also prevalent in the bean plants. The plants treated with the high H_2O_2 concentration weekly and twice a week (D and E, respectively) had stomatal densities that remained relatively the same.

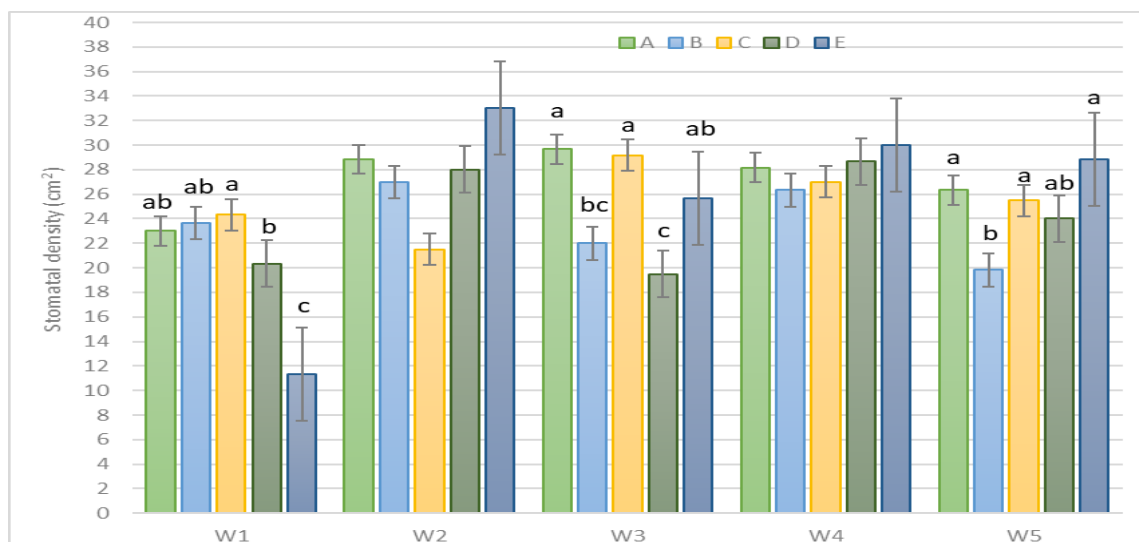


A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. W1 to W5 represent week one to week five. Bars of the same day and no letter indicates no significant difference between treatments ($P < 0.05$).

Figure 3.11: Weekly changes in the stomatal density of beetroot plants treated with different hydrogen peroxide treatments.

Swiss chard

Similar to the stomatal density of the beetroot plants that received no treatment (C), the stomatal density of the Swiss chard plants (Figure 3.12) also remained relatively unchanged. The plants treated with the low H_2O_2 concentration weekly (A) for most of the trial consistently had a higher stomatal density than the plants treated with the same H_2O_2 concentration but twice a week (B). The stomatal density of the plants treated with the high H_2O_2 concentrations weekly (D) were lower than those treated twice a week with the same H_2O_2 concentration (E). The stomatal density of the plants in treatment A, D and E fluctuated throughout the trial but tended to be higher at week 5 as compared to week 1. This was especially true for the higher level of H_2O_2 applied twice a week (E).

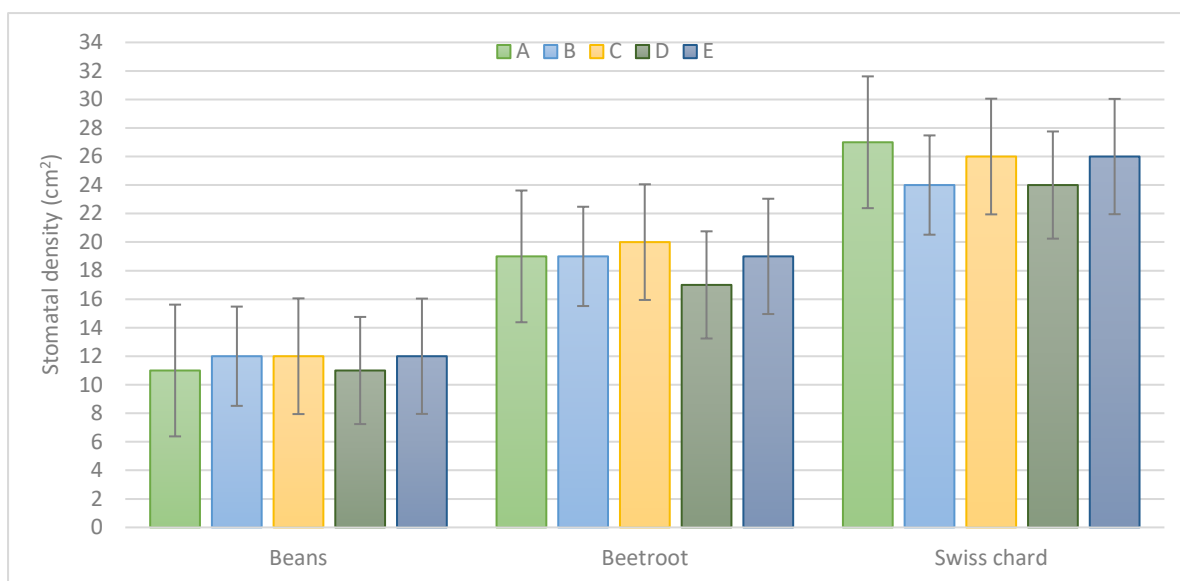


A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. W1 to W5 represent week one to week five. Bars of the same day and no letter indicates no significant difference between treatments ($P < 0.05$).

Figure 3.12: Weekly changes in the stomatal density of Swiss chard plants treated with different hydrogen peroxide treatments.

Overall stomatal density

The stomatal densities overall (Figure 3.13) did not differ significantly between treatments in beans, beetroot or Swiss chard. In all three crops the plants treated weekly with the high H_2O_2 concentration had stomatal densities consistently lower than those treated with the high H_2O_2 concentration. The bean plants treated with the low H_2O_2 concentration weekly had a stomatal density slightly lower than those treated twice a week with the same H_2O_2 concentration. The stomatal densities of the beetroot plants treated with the low concentrations of H_2O_2 were the same and both slightly less than those in the control. The stomatal density of the Swiss chard plants treated weekly with the low H_2O_2 concentration were slightly higher than those treated twice a week with the same concentration of H_2O_2 .



A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars of the same crop with no letters indicates no significant difference between treatments ($P < 0.05$).

Figure 3.13: The average overall stomatal density of beans, beetroot and Swiss chard plants treated with different hydrogen peroxide treatments.

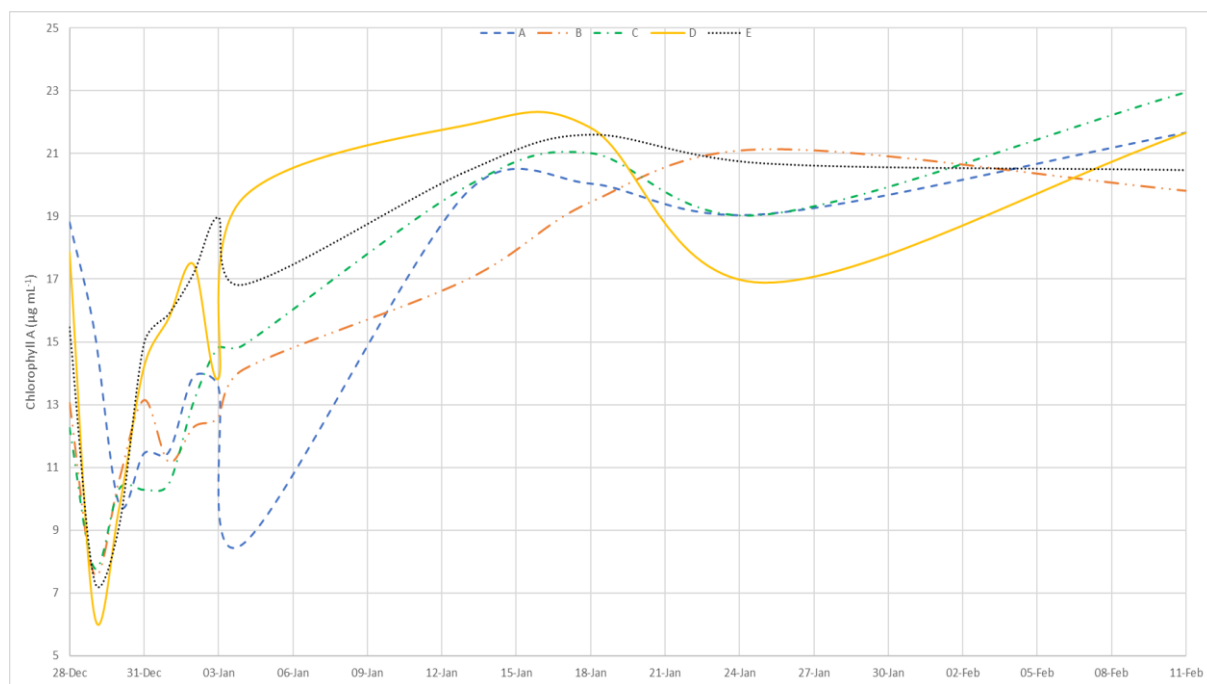
3.3.5 Changes in bean photosynthetic pigments

Chlorophyll A

In the first week (28 Dec – 4 Jan) of daily chlorophyll A concentration [chl A] readings, beans (Figure 3.14) treated weekly with the low H_2O_2 concentration (A) and those treated with the high H_2O_2 concentration twice a week (D), had the highest initial readings between $17\text{--}19 \mu\text{g ml}^{-1}$ followed by treatment E (high H_2O_2 concentration twice a week) at about $15 \mu\text{g ml}^{-1}$. The plants treated twice a week with the low H_2O_2 concentration (B) and the plants that received no treatment (C, control) had a [chl A] of $12\text{--}13 \mu\text{g ml}^{-1}$. The [chl A] of the plants in treatment D and E followed the same trend in the first week of the study, except during day six and seven when they had opposite effects. The plants in treatment A had a similar trend to that of treatment E but ultimately resulted in a decrease in [chl A] for treatment A and not treatment E. The plants in treatment B and C also had a relatively similar trend throughout the first week of the trial.

The weekly changes (4 Jan – 11 Feb) in [chl A] of the plants treated weekly with the low H_2O_2 concentration (A) followed a similar trend to the plants that did not receive treatments (C), received the high concentration of H_2O_2 weekly and twice a week (D and E, respectively). Similar to the increase in [chl A] in the first four days of the plants in treatment C, the plants

treated twice a week with the low H_2O_2 also had an increase in [chl A] in the first 5 days of week 1. From day 5 to 7 the [chl A] decreased slightly. Over the duration of the trial, plants from all the treatments showed an increase in chl A content. The control (C) had the highest content followed by the two treatments with the weekly applications (A and D). Plants from the two treatments applied twice a week (B and E) had the lowest chl A content.



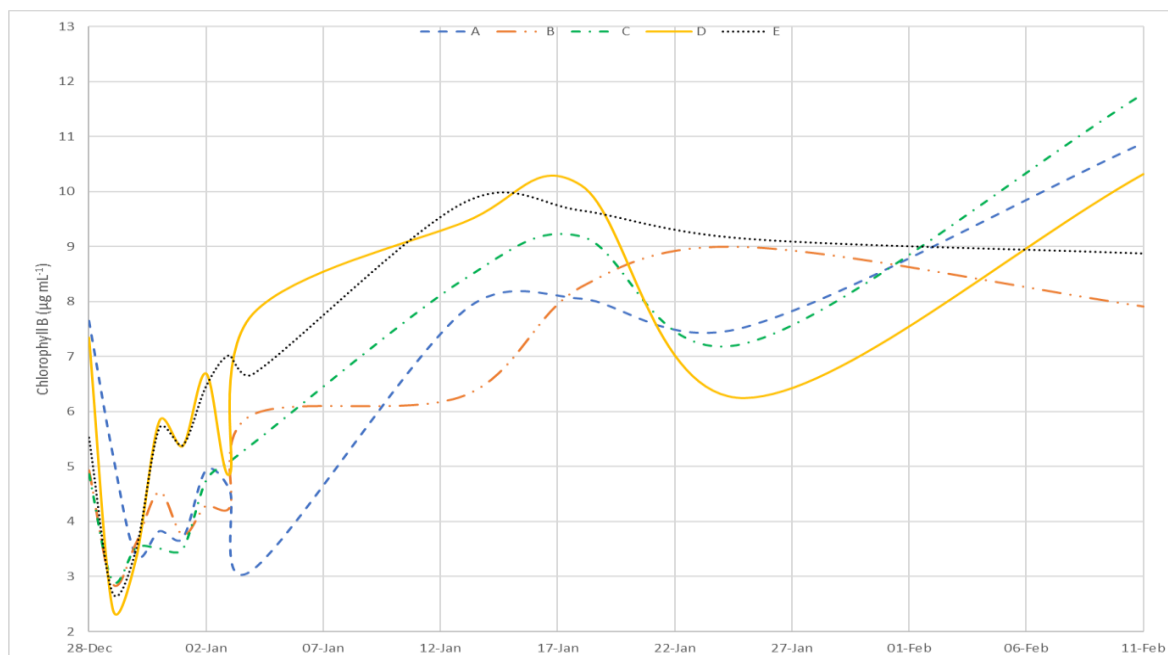
A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week.

Figure 3.14: Changes in the chlorophyll A concentration of bean plants treated with different hydrogen peroxide treatments.

Chlorophyll B

The daily chlorophyll B (Figure 3.15) concentrations in the first week (28 Dec – 4 Jan) of treatments ([chl B]) in the bean plants treated with the different H_2O_2 concentration and rates of followed trends similar to each treatments corresponding [chl A]. The only difference being the concentration of chl B was less than half of the [chl A]. The plants treated with the high H_2O_2 concentration weekly and twice a week (D and E, respectively) had the highest chlorophyll B content at the end of the study, with treatment D at almost $8 \mu\text{g mL}^{-1}$ and E just below $7 \mu\text{g mL}^{-1}$. The plants treated twice a week with the low H_2O_2 concentration (B) ended up having a slightly higher chl B concentration than that of the plants that received no treatment (C).

The weekly chlorophyll B concentration (4 Jan – 11 Feb) of the bean plants followed trends that were similar those of their [chl A]. The plants of treatment A again has a trend similar to that of treatments C, D and E. The plants that were treated twice a week with the low H_2O_2 concentration (B) initially had a trend similar to that of treatment D (4 Jan – 11 Jan) but from 19 Jan the trend of treatment B was opposite to that of treatment D. At the end of the trial the trend was the same as for chl A, with the control giving the highest values, followed by the weekly (A and D) and then the two weekly treatments (B and E).



A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week.

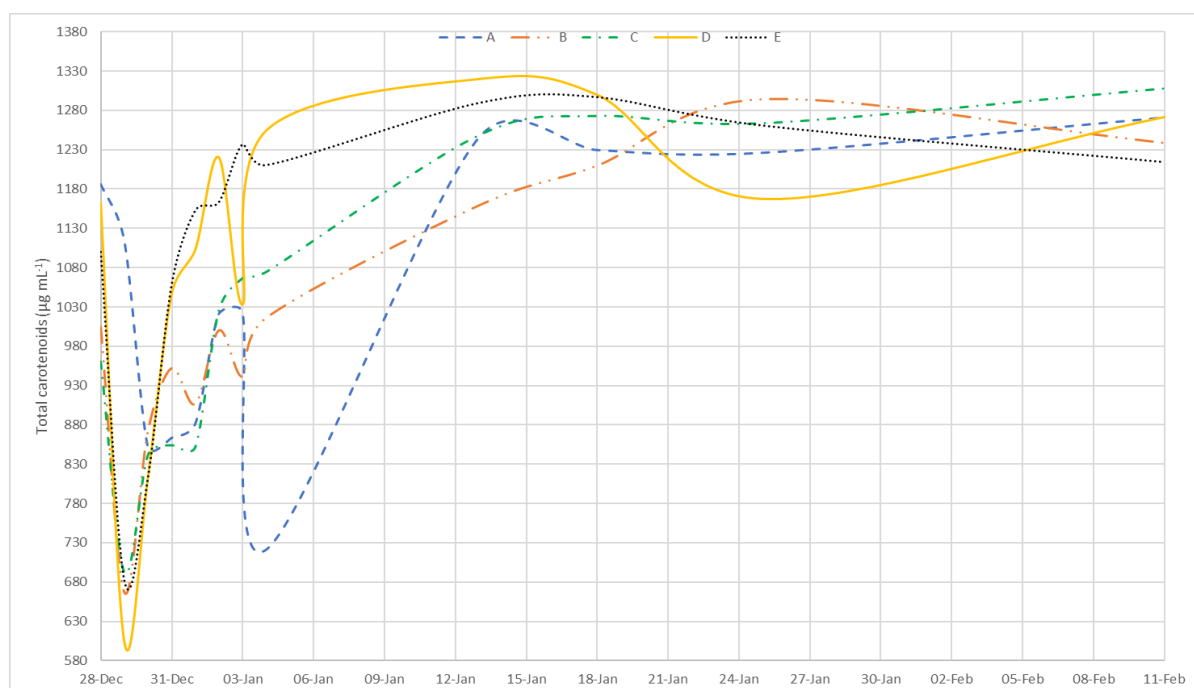
Figure 3.15: Changes in the chlorophyll B concentration of bean plants treated with different hydrogen peroxide treatments.

Total carotenoid concentration

The daily change (28 Dec – 4 Jan) in the total carotenoid concentration (Figure 3.16) of the bean plants also followed a trend more similar to the daily changes in [chl A] than [chl B]. The differences between the total carotenoid concentration from its corresponding [chl A] was that the drop in total carotenoid concentration was not as steep as the drop in [chl A] on day 2. The beans that received the high concentration of H_2O_2 weekly (D) and twice a week (E) had the highest chlorophyll A, B and carotenoid concentration when compared to the plants treated weekly with the low H_2O_2 concentration (A), which resulted in the lowest chlorophyll A,

B and carotenoid concentration. The plants in treatment B and C again had a slight increase in its total carotenoid concentration.

The weekly changes (4 Jan – 11 Feb) in the total carotenoid concentrations of the plants treated with the high H_2O_2 concentrations weekly (D) and twice a week (E) had the highest concentrations and both gradually increased until the week of the 16 Jan before decreasing. The total carotenoid concentration of the plants in treatment D had a greater decrease in the carotenoid content than those in treatment E. The plants that were treated twice a week with the low H_2O_2 concentration (B) had a trend similar to that of the plants that received no treatment (C). The plants treated weekly with the low H_2O_2 concentration (A) initially had a steep increase (4 Jan – 15 Jan) whereafter it followed a trend similar to that of treatment C. The same pairing of the control (C) being the highest followed by the weekly (A and D) and then the two weekly (B and E) treatments were observed.



A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week.

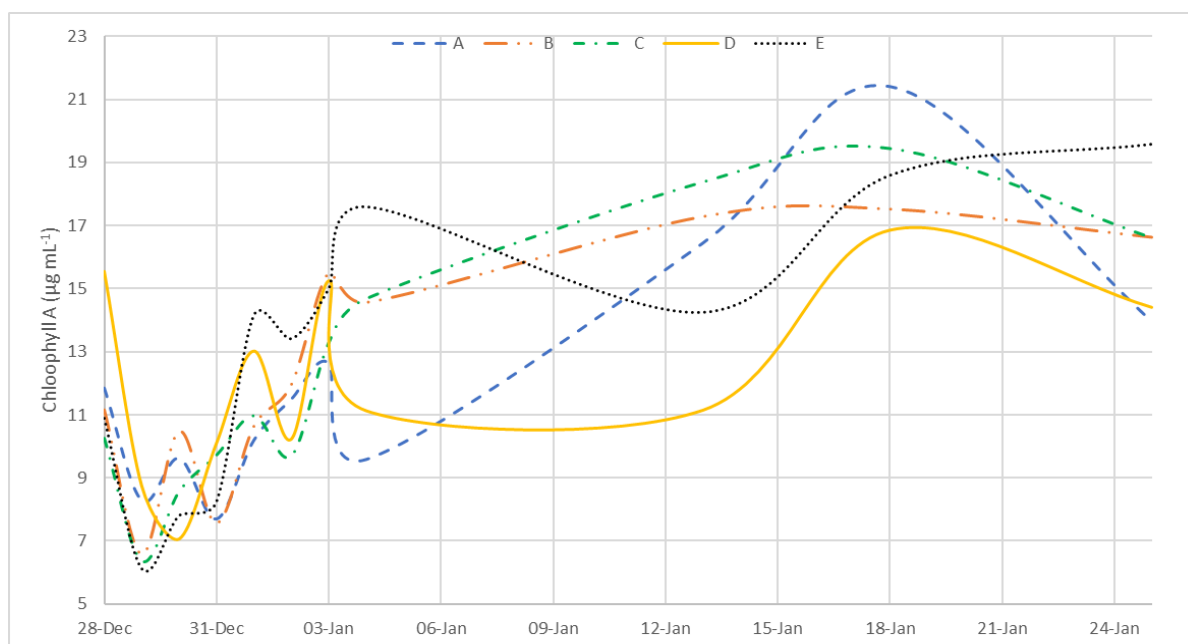
Figure 3.16: Changes in the total carotenoid concentration of bean plants treated with different hydrogen peroxide treatments.

3.3.6 Changes in beetroot photosynthetic pigments

Chlorophyll A

The daily [chl A] changes of the beetroot plants (Figure 3.17) treated with a low H_2O_2 concentration weekly (A), twice a week (B), those that received no treatment (C) and those treated with the high H_2O_2 concentration twice a week (E) had the lowest [chl A] in the first week. In contrast, the plants treated weekly with the high H_2O_2 concentration (D) started at almost $16 \mu\text{g ml}^{-1}$. Similar to the drop in [chl A] of the bean plants, the [chl A] in the beetroot plants also dropped on day one and two. The plants in treatment A and B had similar changes in [chl A] throughout the first week of treatments. The plants in treatments D and E had a similar trend until day six. When treatment E had an increase in [chl A] treatment D had a decrease. The plants in treatment C trend somewhat similar to that of treatment B.

The plants that were treated with the low and high H_2O_2 concentrations weekly (A and D, respectively) followed a similar trend in its [chl A] as that of the bean plants. Those treated with the high H_2O_2 concentration twice a week (E) initially followed the same trend as those in treatment D but in the last week the [chl A] increased in treatment E and not treatment D. The plants treated twice a week with the low H_2O_2 concentration (B) followed a similar trend in its weekly changes of [chl A] than the plants that received no treatment (C). The plants treated weekly with the low H_2O_2 concentration (A) had a steep increase in the first three weeks of the study, whereafter it followed a trend in [chl A] similar to that of the plants in treatment D. The beetroot plants clearly reacted differently than the bean plants, with the control (C) not giving the highest chl A content, but rather the treatment at the higher concentration applied twice a week (E). Even the plants treated with a lower concentration applied twice a week (B) did better than the two treatments applied only once a week (A and D).



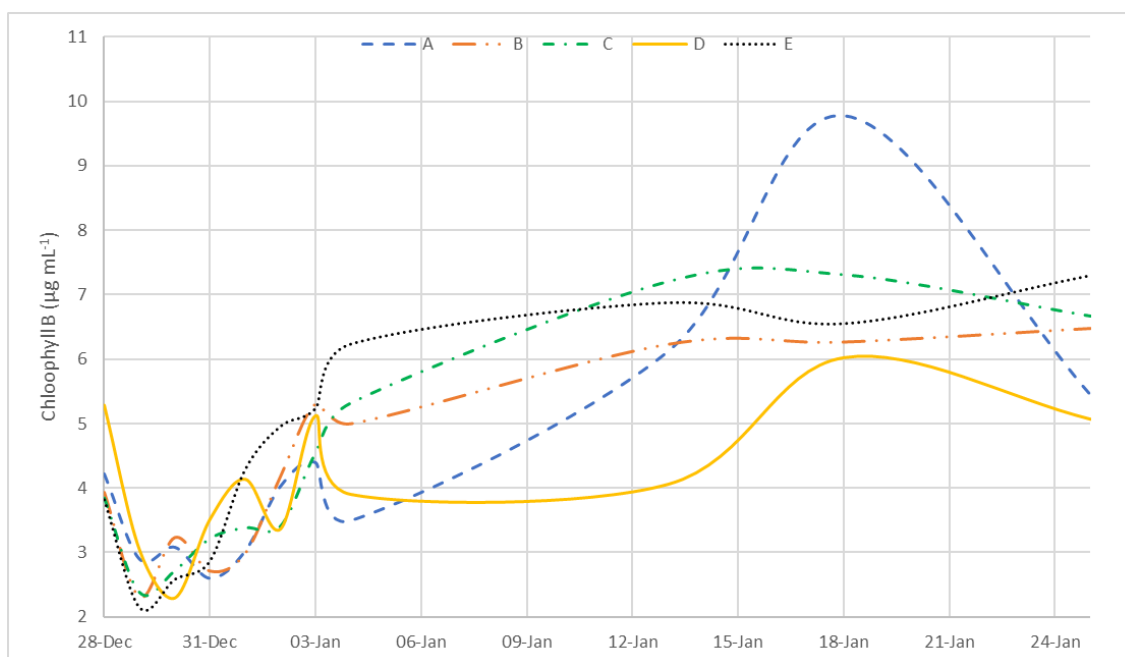
A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week.

Figure 3.17: Changes in the chlorophyll A concentration of beetroot plants treated with different hydrogen peroxide treatments.

Chlorophyll B

The daily changes in the [chl B] in treatments A to E followed trends that were similar to the corresponding treatments' daily changes in [chl A] (Figure 3.18). All the plants in treatments A-E had lower chlorophyll A and B concentrations on the same days. The plants that were treated weekly with the high H_2O_2 concentration (D) initially had the highest [chl B] but at the end of the first week, had the second lowest concentration. The plants treated twice a week with the high H_2O_2 concentration (E) had the highest [chl B] followed by treatment C, B, D and then A.

The weekly changes in [chl B] were similar to the changes that the plants, in the different treatments, had in their chlorophyll A concentrations. The only major differences were that the concentrations of chlorophyll A, in the different treatments, were higher (ranged between 9 and 22 µg mL^{-1}) than those of chlorophyll B (ranged between 3 and 10 µg mL^{-1}). The change in [chl B] of treatment D did not change initially but from week 3 it increase steeply and then had a gradual decrease. Again the two weekly treatments, regardless of concentration (B and E) resulted in higher or similar chl B concentrations than the control (C), with the two weekly treatments (A and D) giving the lowest contents.



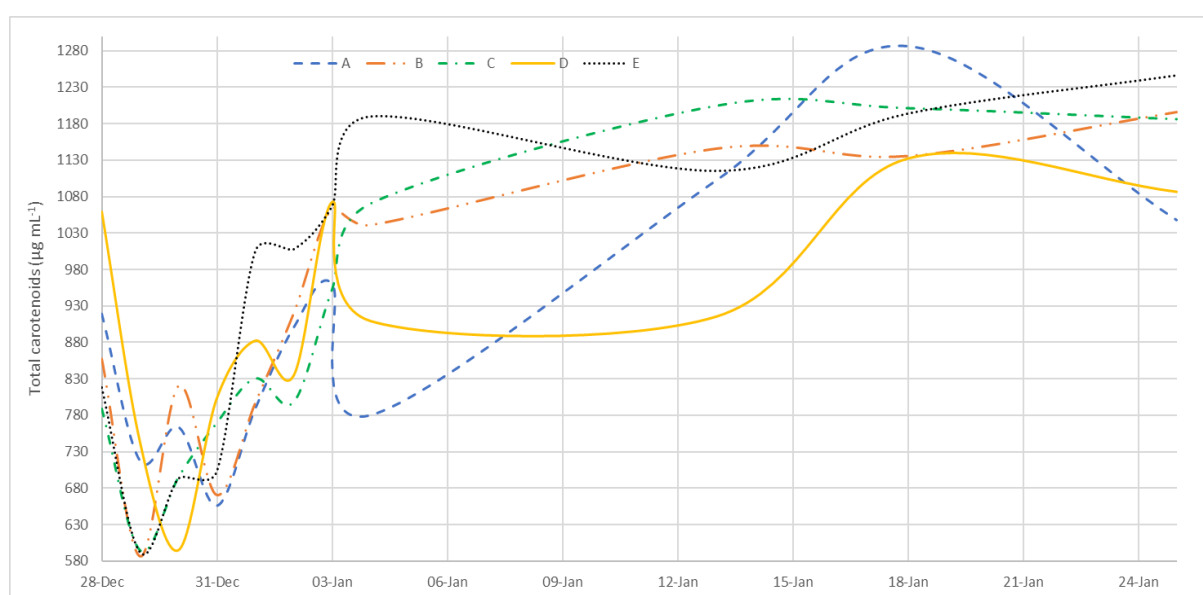
A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week.

Figure 3.18: Changes in the chlorophyll B concentration of beetroot plants treated with different hydrogen peroxide treatments.

Total carotenoid concentration

The daily changes in the total carotenoid concentrations of the beetroot plants (Figure 3.19), regardless of treatment, had trends similar to those of its [chl A] (Figure 3.17). The only difference is that the concentrations of the carotenoids were at higher concentrations (between 750 and $1050 \text{ } \mu\text{g mL}^{-1}$) than the concentration of chl A. The treatments had experienced decreases on the same days in their carotenoid concentration than those of both chlorophyll A and B. The plants that were treated weekly with the high H_2O_2 concentration (D) initially had the highest total carotenoid concentration (just above $1050 \text{ } \mu\text{g mL}^{-1}$) and at the end of the first week had the second lowest, whereas the plants of treatment E started at the second lowest concentration and ended at the highest concentration (just below $1200 \text{ } \mu\text{g mL}^{-1}$).

The weekly changes in the total carotenoid concentrations were similar to the pattern of the weekly changes of chlorophyll A concentrations. The only major differences were that the carotenoids concentrations, in the different treatments, were significantly higher (ranged between 750 and 1300 $\mu\text{g ml}^{-1}$) than those of chlorophyll A (ranged between 9 and 22 $\mu\text{g ml}^{-1}$). The only noticeable difference in the patterns of the carotenoid concentration, of the different treatments, from those of [chl A] is that the plants in treatment B had a noticeable but slight decrease in carotenoid concentration. The carotenoid concentration was also more positively affected by the applications of hydrogen peroxide twice a week (B and E) than only once a week (A and D).



A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week.

Figure 3.19: Changes in the total carotenoid concentration of bean plants treated with different hydrogen peroxide treatments.

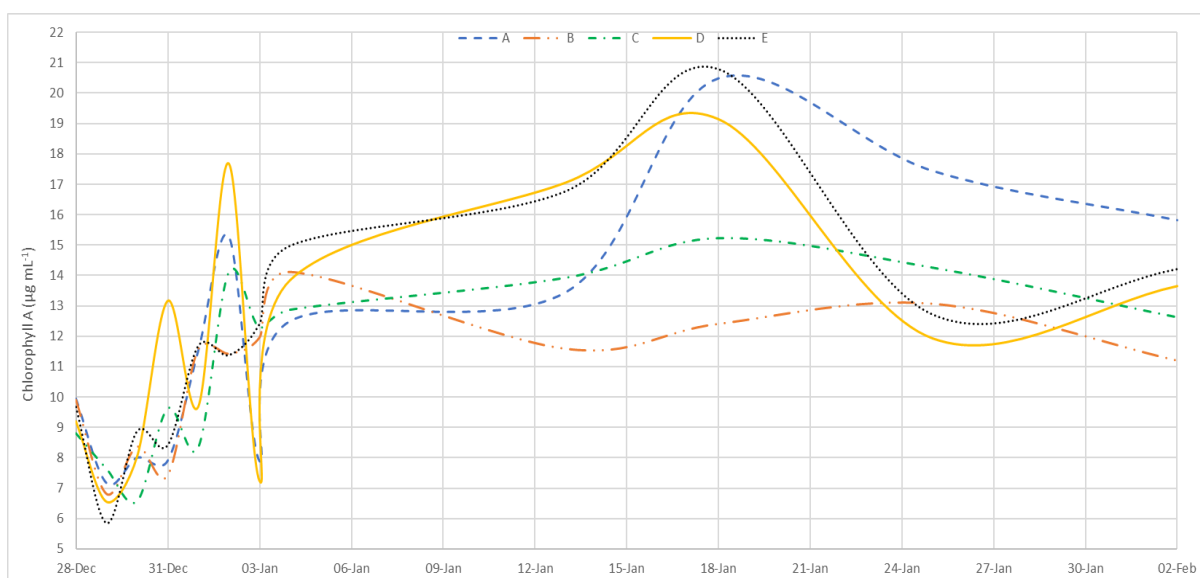
3.3.7 Changes in Swiss chard photosynthetic pigments

Chlorophyll A

The daily changes in [chl A] of the Swiss chard (Figure 3.20) plants, regardless of treatment, initially started between 9 and 10 $\mu\text{g ml}^{-1}$. The plants that were treated with the low H_2O_2 concentration weekly (A) and those that received no treatment (C) had a similar but opposite trends during the first five days of the week, whereafter they followed the same trend. The changes in treatment A was also greater than those in treatment C during the last two

days. The plants treated twice a week with the low H_2O_2 concentration (B) and twice a week with the high H_2O_2 concentration (E) followed the same trend in [chl A] throughout the first week of treatments. The plants treated weekly with the high H_2O_2 concentration (D) had [chl A] changes similar to those of treatment C.

The weekly changes in [chl A] of the Swiss chard plants treated weekly with the low H_2O_2 concentration had trend similar to the plants treated weekly and twice a week with the high H_2O_2 concentration (D and E, respectively). The plants that received no treatment (C) had a similar but opposite trend in its [chl A] than the plants treated twice a week with the H_2O_2 concentration (B). Swiss chard in terms of chl A, reacted totally different from beetroot and beans. The control (C) gave the second lowest results, only better than that of the low concentration hydrogen peroxide applied twice a week (B). The low concentration hydrogen peroxide applied once a week (A) gave the best results and that of the higher concentration were lower but similar (D and E).



A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week.

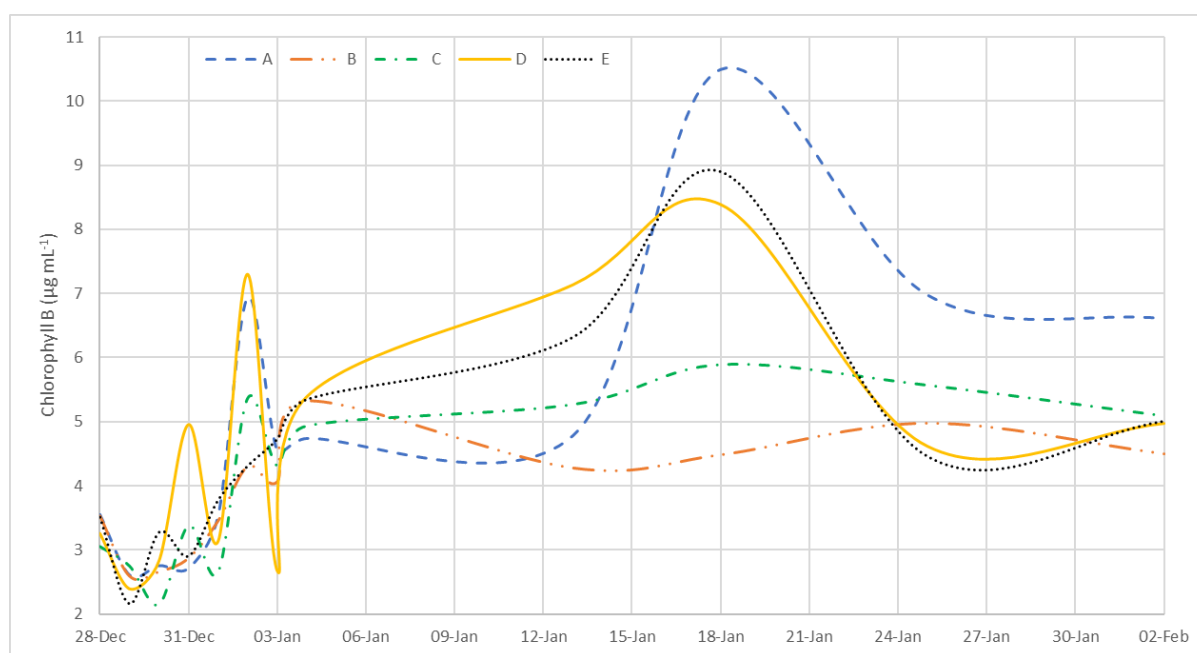
Figure 3.20: Changes in the chlorophyll A concentration of Swiss chard plants treated with different hydrogen peroxide treatments.

Chlorophyll B

The daily changes in the concentration of chlorophyll B in the Swiss chard plants (Figure 3.21), of the different treatments, during the first week of treatments followed a similar pattern than that of the [chl A]. All the treatments started at a [chl B] between 3 and $3.5 \mu\text{g ml}^{-1}$.

¹. Even though the Swiss chard that were treated weekly with the low H_2O_2 concentration (A) started at the highest concentration than the plants that were not treated (C). The plants of treatment C ended at a slightly higher [chl B] than those of treatment A. The plants that were treated with the high H_2O_2 concentration weekly (D) and twice a week (E) as well as the plants treated twice a week with the low H_2O_2 concentration (B) ended at the same chlorophyll B concentration on day seven. Throughout the week the plants in treatment A, B, D and C followed similar trends. The plants in treatment E followed the same but opposite trend as those in treatment C.

The weekly changes in the [chl B] of the Swiss chard plants followed similar trends to the weekly changes in [chl A]. The only major differences were that the [chl A], in the different treatments, ranged between 11 and 22 $\mu\text{g ml}^{-1}$, whereas [chl B] ranged between at 4 $\mu\text{g ml}^{-1}$ and ended at 10.5 $\mu\text{g ml}^{-1}$. Again the lower concentration of hydrogen peroxide applied once a week (A) gave the highest chl B content whit the other four treatments following similar trends as for chl A.



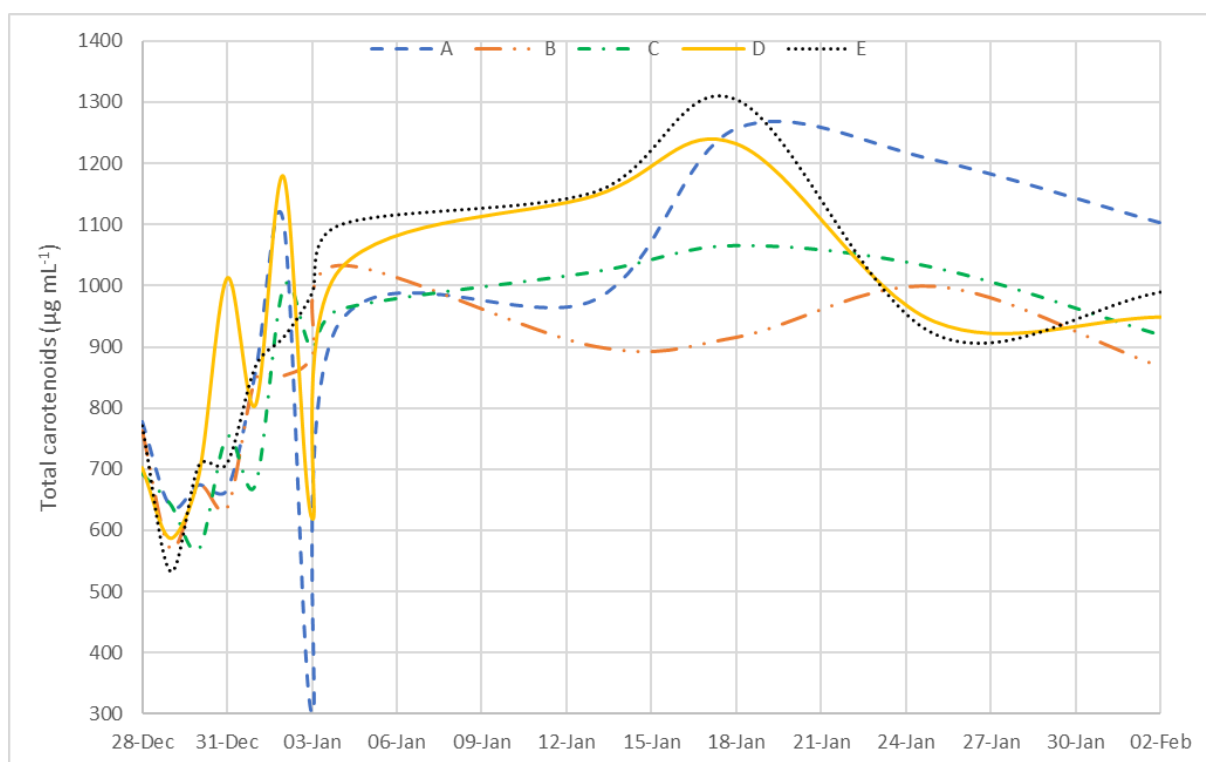
A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week.

Figure 3.21: Changes in the chlorophyll B concentration of Swiss chard plants treated with different hydrogen peroxide treatments.

Total carotenoid concentration

The total carotenoid concentration (Figure 3.22) of the plants followed similar trends to that of its [chl B]. The plants treated weekly with the low H_2O_2 concentration (A) followed similar fluctuations than those treated with the high H_2O_2 concentration weekly (D). The plants treated with the low and high H_2O_2 concentration twice a week (B and E, respectively) followed a similar but opposite trend than the plants that received no treatment (C).

The weekly changes in the total carotenoid concentrations were remarkably similar to the pattern of the weekly changes in [chl A]. The only major differences were that the concentrations of the carotenoids, in the different treatments, were significantly higher (ranged between 850 and 1350 $\mu\text{g ml}^{-1}$) than those of chlorophyll A and B. The only noticeable difference in the patterns of the carotenoid concentrations, of the different treatments, from those of [chl A] is that the plants in treatment A had a less noticeable decrease in carotenoid concentration. The second difference is that the carotenoid concentration of treatment E decreases to below the concentration of the plants in treatment D. The opposite occurred in the concentration of chlorophyll A. But the ending carotenoid values followed a similar pattern than that of the chl A, with the low concentration hydrogen peroxide applied once a week (A) gave the best results, followed by the two higher application rates of H_2O_2 (D and E), then the control (C) and lastly the twice a week low application rate of H_2O_2 (B).



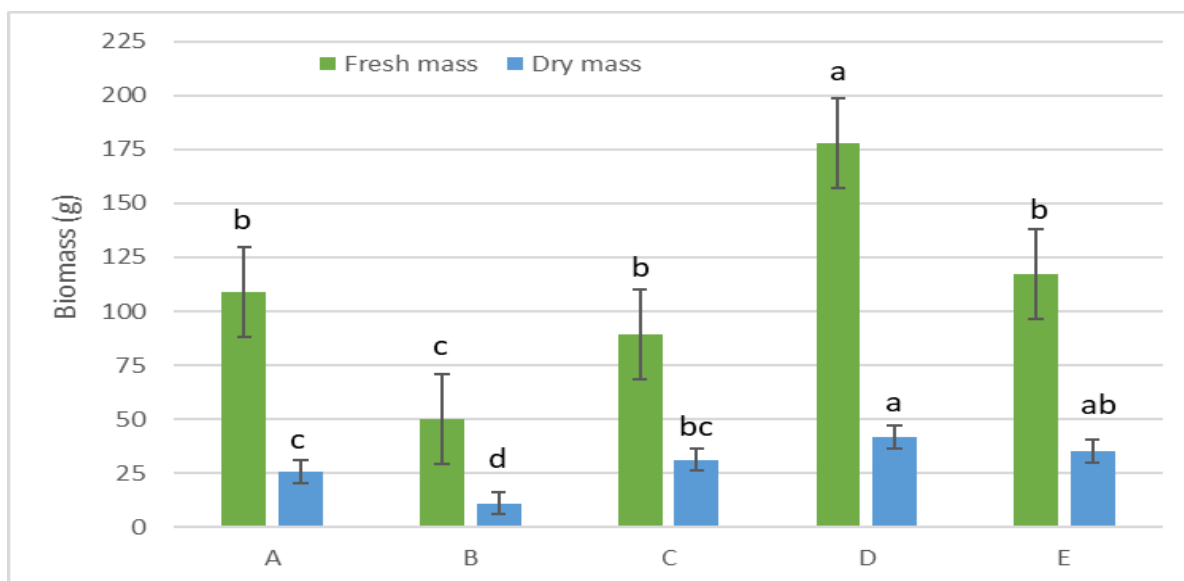
A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week.

Figure 3.22: Changes in the total carotenoid concentration of Swiss chard plants treated with different hydrogen peroxide treatments.

3.3.8 Fresh and dry biomass

Beans

The foliar application of the high H_2O_2 concentration twice a week (D) on bean plants (Figure 3.23) resulted in the greatest fresh and dry mass across all treatments. In comparison to applying no treatment (C), the application of H_2O_2 at a low concentration twice a week (B) resulted in significantly lower fresh and dry biomass. The application of the low H_2O_2 concentration weekly (A) or the high H_2O_2 concentration twice a week (E) resulted in fresh biomass that did not differ significantly from the plants in the control (C). The dry biomass had a similar trend to the fresh mass. When comparing the dry biomass of treatments D and E (high concentration H_2O_2), there was no significant difference between the treatments, nor did the dry mass of the control differ significantly from the plants in treatment E. Treatments C, D and E had significantly heavier dry mass than those of both treatments A and B. The dry mass of treatment A was also significantly heavier than that of treatment B.

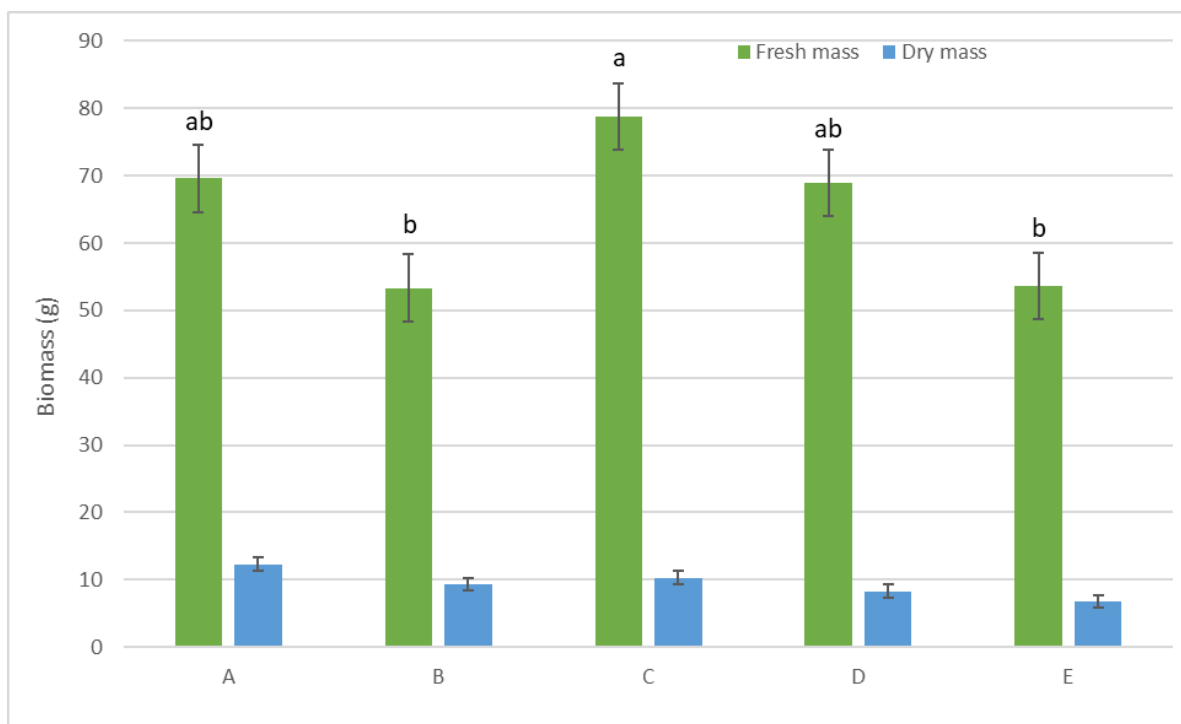


A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars of the same colour and different letters indicates a significant difference between treatments ($P < 0.05$).

Figure 3.23: The fresh and dry biomass of beans as affected by different hydrogen peroxide treatments.

Beetroot

The beetroot plants (Figure 3.24) that received no hydrogen peroxide (C) had a significantly higher fresh mass than the H_2O_2 treatments that were applied twice a week at the low concentration (B, 0.05 ml L^{-1}) and high concentration (E, 0.1 ml L^{-1}). The fresh mass of the latter treatments did not differ significantly from one another nor from the fresh mass of the beetroot plants treated weekly with the low concentration (A) and high concentration (D) of H_2O_2 . Treatment A and D did not differ significantly from the control (C). Furthermore, there was no significant difference between the dry mass of any treatments.



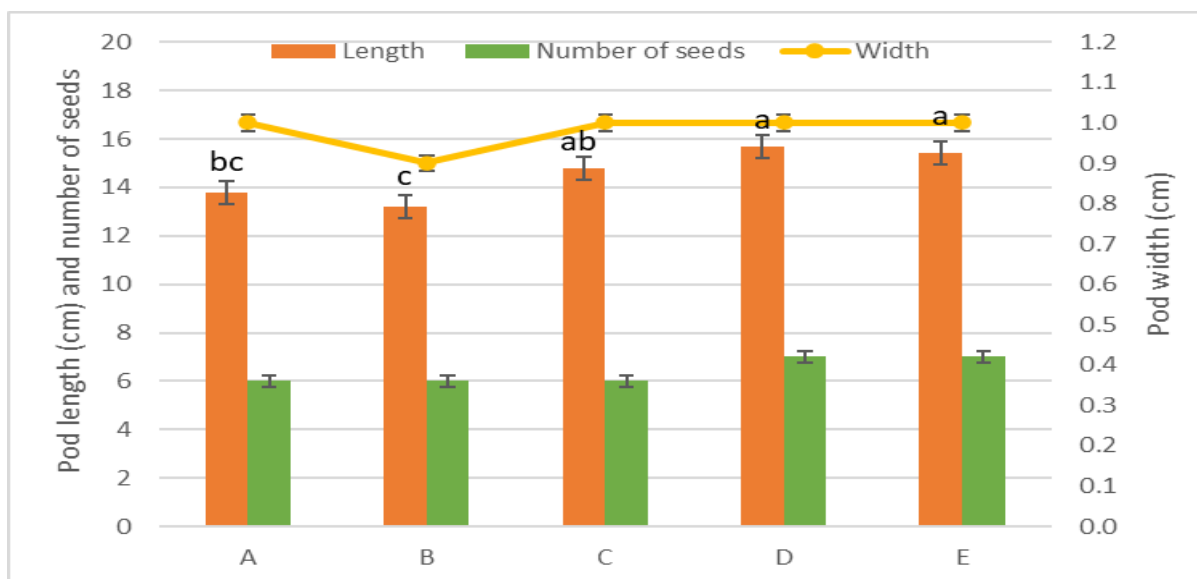
A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars of the same colour and different letters indicates a significant difference between treatments ($P < 0.05$).

Figure 3.24: The fresh and dry biomass of beetroot as affected by different hydrogen peroxide treatments.

3.3.9 Yield

Bean pod length, width and number of seeds

The beans treated weekly with the high H_2O_2 concentration (D) resulted in the longest pods on average (Figure 3.25), followed closely by those harvested from the plants treated twice a week with the same H_2O_2 concentration (E), and the control group. The plants treated with the low H_2O_2 concentration weekly and twice a week resulted in the shortest pods. The average widths of the pods on the other hand differed slightly, but not significantly between treatments. The number of seeds had a similar trend to that of the pod length between the treatments.

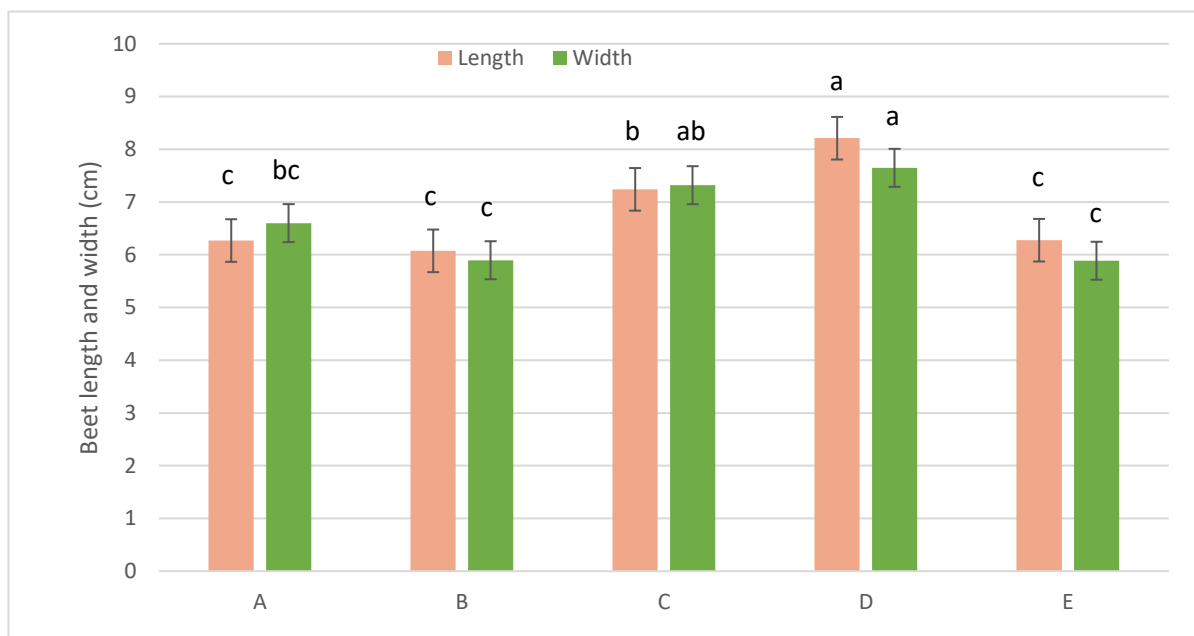


A to E represents the different treatments applied in the study and H1 to H4 represents harvest one to four. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars of the same colour with different letters indicates a significant difference ($P < 0.05$) between treatments and those with no letters indicated no significant difference between treatments.

Figure 3.25: Effect of different hydrogen peroxide treatments on the length, width and number of seeds of the bean pods.

Beet length and width

Both the length and the width of the beets follow almost the same pattern between treatments (Figure 3.26). The beet length of beetroot treated weekly, with the high H_2O_2 concentration (D), was significantly longer than the beets in any of the other treatments. The beets of the plants that were treated with the low H_2O_2 concentrations weekly (A), twice a week (B) and the plants treated with the high H_2O_2 concentration twice a week (E) did not differ significantly from one another irrespective of H_2O_2 concentration and application rate. Those that received no treatments (C) had beets that were significantly longer than those in treatment A, B and E. The beet length of plants that received no treatment (C) were significantly greater than the beet length of treatments A, B and E. The beet width of treatment A was not significantly longer than that of treatment C, nor was the beet width of treatment A significantly shorter than that of treatment C. The beet width of treatment B and E also did not differ significantly from each other but were significantly narrower than any of the other treatments, except treatment A.

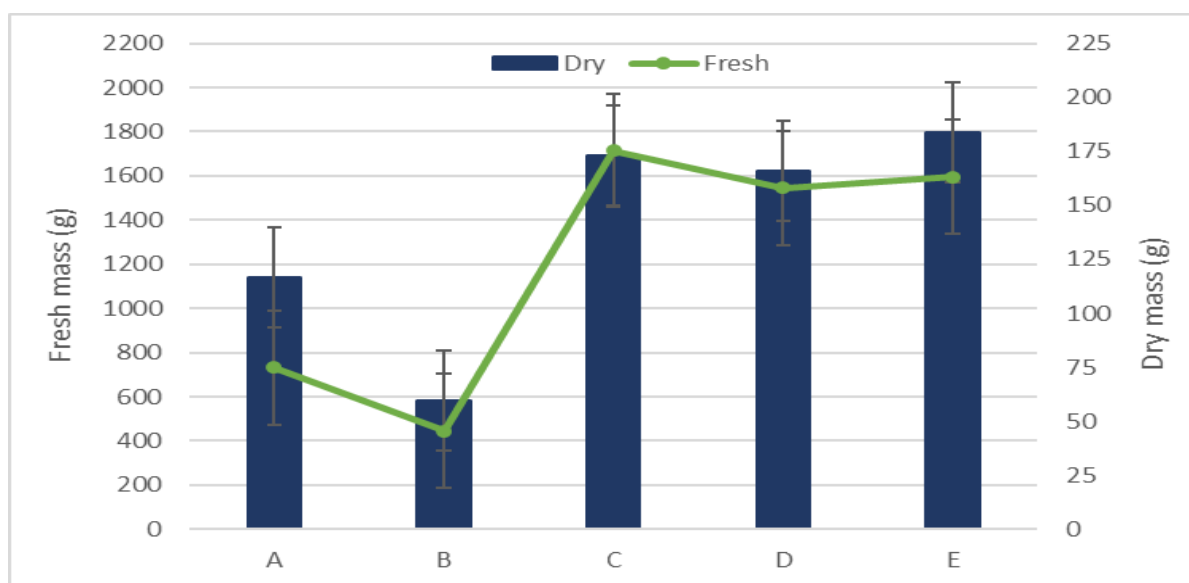


A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars with the same colour and letter do not differ significantly from each other ($P < 0.05$).

Figure 3.26: The beet length and width of beetroot, grown in open field conditions, treated with foliar applications of different hydrogen peroxide treatments.

Bean fresh and dry yield mass

During the study (Figure 3.27) the bean plants that that received no treatment (C) had the heaviest fresh and dry pod mass between treatments, followed by the pods harvested from the plants treated with the high concentration of H_2O_2 twice a week (E) and applied weekly (D). The pod fresh and dry mass of the pods harvested from the beans treated twice a week with the low concentration of H_2O_2 (B) were the lowest and those from the low H_2O_2 concentration applied weekly (A) had the second lowest.

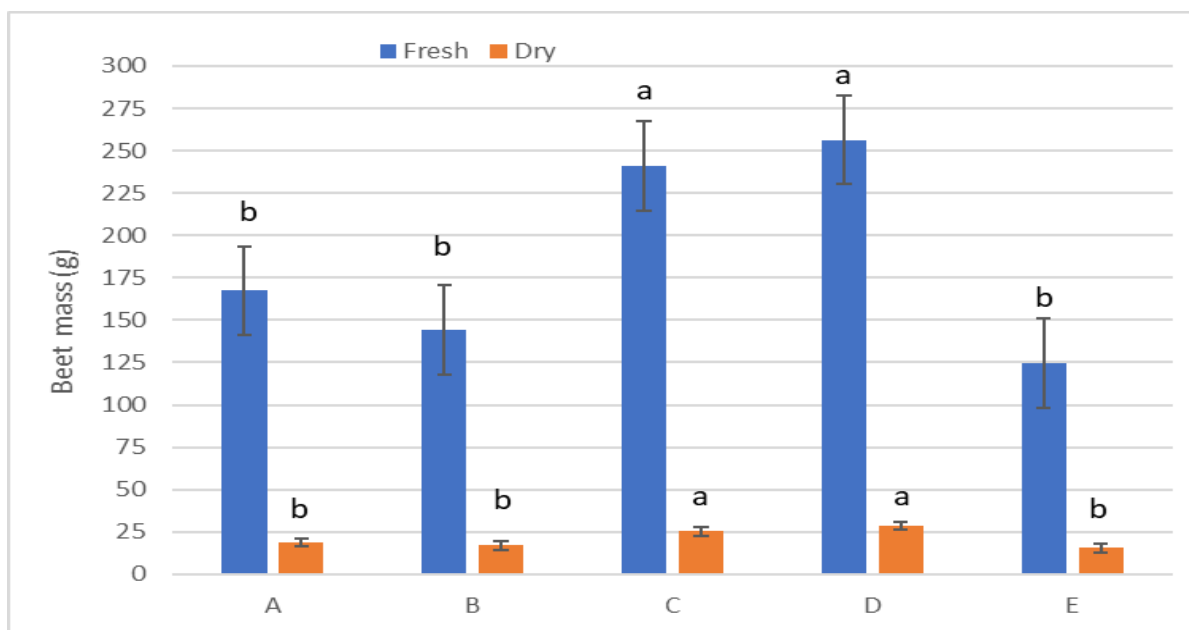


A to E represents the different treatments applied in the study and H1 to H4 represents harvest one to four. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week.

Figure 3.27: Effect of different hydrogen peroxide treatments on the total fresh and dry mass per m^2 of the bean pods harvested throughout the study.

Beet fresh and dry yield mass

The fresh and dry mass of the beets (Figure 3.28) that received no treatment (C) and those treated weekly with the high H_2O_2 concentration (D), did not differ significantly from each other and were significantly heavier than the beets in any of the other treatments. The fresh and dry mass of the treatments that received the low H_2O_2 concentrations weekly and twice a week (A and B, respectively) and those treated with the high H_2O_2 concentration (E) did not differ significantly from one another. The beet fresh mass of treatment A was heavier than those in treatment B and E, with the latter treatment being the lightest. The fresh mass of the beets followed a similar pattern to that of the beet lengths and to some degree the widths.

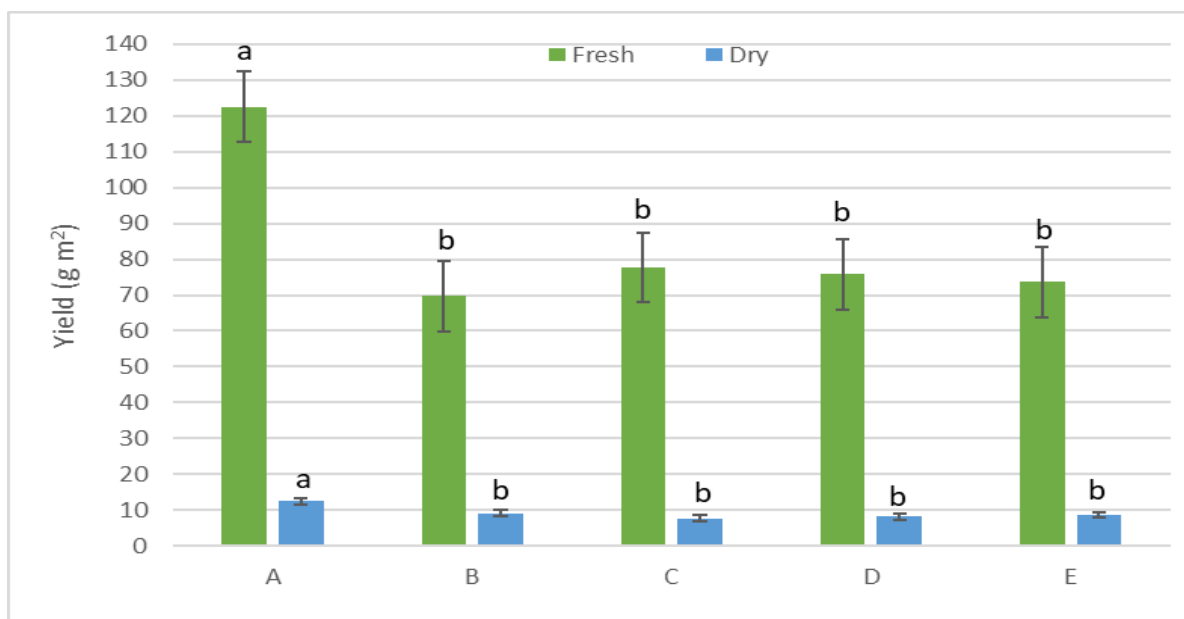


A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars with the same colour and letter do not differ significantly from each other ($P < 0.05$).

Figure 3.28: The beet fresh and dry mass of beetroot, grown in open field conditions, treated with a foliar application of hydrogen peroxide.

Swiss chard wet and dry yield

The fresh and dry yield of Swiss chard (Figure 3.25) plants that were treated weekly with the low H_2O_2 concentration (A) were significantly greater than any of the other treatments. The plants treated twice a week with the low H_2O_2 concentration (B), those that received no treatment and those treated with the high H_2O_2 concentration weekly (D) and twice a week (E) had both fresh and dry yields that did not differ between the treatments. The plants in treatment B did have the lowest fresh yield among all the treatments. When looking at all three crops in terms of fresh yield, it is unclear which one treatment resulted in a high yield. The fresh yield of the beans, beetroot and Swiss chard plants treated twice a week with the low H_2O_2 concentration (B) resulted in the lowest yields. The dry yield of beetroot and Swiss chard follows a similar trend, however, there were significant differences between the dry yield of the Swiss chard plants (treatment A were significantly greater than all other treatments) but not the beetroot. The Swiss chard plants did not have a biomass measurement as all the leaves were harvested as yield.



A to E represents the different treatments applied in the study. A = hydrogen peroxide (0.05 ml L^{-1}) applied once per week; B = hydrogen peroxide (0.05 ml L^{-1}) applied twice per week; C = no hydrogen peroxide treatment (0 ml L^{-1}); D = hydrogen peroxide (0.1 ml L^{-1}) once per week; E = hydrogen peroxide (0.1 ml L^{-1}) twice per week. Bars with the same colour and letter do not differ significantly from each other ($P < 0.05$).

Figure 3.29: The fresh and dry yield mass of Swiss chard, grown in open field conditions, treated with a foliar application of hydrogen peroxide

3.4 Discussion and conclusions

Beans

In the initial month of the field study, the control plants did not differ significantly from the beans that received the higher H₂O₂ concentrations weekly and twice a week in terms of height. In the last month of the study, however, the height of the plants that received the latter treatments were significantly taller than the plants in any of the other treatments. A study by Orabi et al. (2015) found that a low concentration of H₂O₂ (0.05 ml L⁻¹) can have a significantly positive effect on plant growth. Khandaker et al. (2012) found that with the exogenous application of H₂O₂ increased not only plant growth but also in the biochemical properties and physiological activities of plants. The plants treated weekly with the low concentration of H₂O₂ (0.05 ml L⁻¹) initially were not significantly shorter than the plants of the control group but in the second month of the study the heights increased gradually still.

The application of the low H₂O₂ concentration (0.05 ml L⁻¹) twice a week resulted in the shortest plants throughout the study. Their short height could be attributed to the fact that this sub-sub block tended to flood and the soil would remain flooded for a few days as it rained (December – January). Studies have been conducted to establish the effect of flooding duration on the different growth stages of bush beans. These studies have found that when the soil is flooded for five days or more can result in a decrease of 50% in plant dry mass afterwards (Reed et al. 2005). A study on the effect of flooding on soybeans found comparable results. This study found that in the early vegetative stages of the plants flooding for only three days killed the plants (Boru et al. 2003). When soil is flooded the oxygen balance in the soil will be disturbed, roots can drown, reduces the uptake of water by the roots thus resulting in water stress. Flooded soil also results in less oxygen exchange between the soil and atmosphere and causes root growth to be reduced (Irmak 2008). Numerous authors have stated the importance of plant roots for crop growth. They found that root growth directly affects both shoot growth and crop yield (Vamerali et al. 2003, Zhang et al. 2009, Wang et al. 2014, Ota et al. 2020). The accumulation of water in this part of the plot (even when irrigating) could have affected root growth, therefore shoot growth as well.

Ahmad et al. (2014) and Hasan et al. (2016) found that when hydrogen peroxide is applied at concentrations lower than 100 ml L⁻¹ there may be an increase in shoot weight. In this study the plants that received the low H₂O₂ concentration (0.05 ml L⁻¹) twice a week had the lowest dry biomass whereas the plants that received the high concentration H₂O₂ (0.1 ml L⁻¹) weekly had the highest fresh biomass among the treatments. The plants that received the low concentration H₂O₂ weekly and the high concentration H₂O₂ twice a week, however, had fresh biomass that were not significantly higher than those in the control treatment. The dry

biomass of the treatments had the same trend as the fresh biomass except the high H₂O₂ concentration treatment applied weekly did not differ significantly from the same concentration but applied twice a week. The low H₂O₂ concentration applied once a week were significantly lower than that of the high H₂O₂ concentration applied twice a week.

Alakhdar and Shoala (2021) found that the exogenous application of H₂O₂ positively affected the growth and yield of three different resistant bean cultivars. The higher concentration of H₂O₂ treatment (1.5 mM) on two of the three cultivars resulted in the highest number of seeds and pods. In this study the number of seeds of the plants (between the four harvests) that received the high concentration of H₂O₂ weekly and twice a week for the most part did not differ significantly from the control. The plants treated twice a week with the low concentration of H₂O₂ initially had the lowest number of seeds per harvest but midway through the study the number of seeds were significantly different from the control group. The bean plants treated weekly with the low H₂O₂ concentration showed a decrease in the number of seeds from the first harvest to the last whereas the number of seeds in the pods of the other treatments stayed relatively the same. The pod lengths followed similar trends to those of the number of seeds but the significant differences are more pronounced. The mean pod widths of each treatment remained relatively the same between harvests (results not presented). The study by Orabi et al. (2015) have shown in their study on tomato plants that a low H₂O₂ treatment could have substantial positive results on fruit yield.

Throughout most of the first week of the study the stomatal densities did not differ significantly between treatments. The stomatal densities of the beans in every treatment fluctuated daily but ultimately did increase. When looking at the weekly stomatal changes, the plants that were treated weekly with the low concentration of H₂O₂ and the plants in the control group the stomatal densities decreased. The stomatal densities in the beans of the other treatments fluctuated but stayed relatively the same. Overall the stomatal densities did not differ between treatments in any of the crops. A study by Salisbury (1928) found that the stomatal density tends to be greater in smaller leaves and less on larger leaves. In this study it was not clear whether the stomatal density was high or low in comparison to leaf size.

A study by Hasan et al. (2016) have found that when H₂O₂ is applied at concentrations lower than 100 mg L⁻¹ the chlorophyll content of the leaves are improved. In this study the beans that were treated with 0.1 ml L⁻¹ of H₂O₂ weekly had a slightly higher Chl A, B and carotenoid content than the plants treated twice a week with the same concentration of H₂O₂. A plausible reason could be that the Chl A, B and carotenoid content of the plants in the former treatment had higher initial contents of the three pigments than those of the bean plants treated with the 0.1 ml L⁻¹ of H₂O₂ twice a week. All three pigments in the bean plants in all the

treatments, except those treated weekly with the low age H_2O_2 concentration, increased slightly in the first week of the study. Throughout the first week of chlorophyll content measurements the plants that received the 0.1 ml L^{-1} concentration H_2O_2 had the highest chlorophyll content, the plants that were treated weekly with the low concentration of H_2O_2 had the lowest chlorophyll content. In terms of the weekly changes in the chlorophyll A and B content, the plants treated with the higher concentration of H_2O_2 weekly, twice a week with the same concentration of H_2O_2 , those that received no treatment or the low H_2O_2 concentration treatments all increased. The carotenoid content of all the treatments increased, except the plants treated weekly with the high concentration of H_2O_2 . Thus a concentration of 0.05 ml L^{-1} and 0.1 ml L^{-1} H_2O_2 positively affected chlorophyll and carotenoid content.

Beetroot

Studies by Orabi et al. (2015) and Khandaker et al. (2012) found that the application of H_2O_2 in low concentrations have a significantly positive effect on plant growth and on the biochemical properties and physiological activities of plants as well. The beetroot plants in this study that received the 0.1 ml L^{-1} H_2O_2 concentrations weekly as well as those that were treated with the 0.05 ml L^{-1} concentration weekly did not differ significantly in height from the plants that received no treatment. The plant height of the previously mentioned treatments throughout the study had the tallest plants.

The beetroot plants that received the 0.05 ml L^{-1} concentration twice a week, grew in the same sub-sub block as the beans that flooded also had the smallest plants. The beetroot that received the high concentration of H_2O_2 twice a week had plants that were significantly shorter than the plants in any of the other treatments, except those that grew in the flooded area. The beetroot plants of the former treatment, however had a lot of insect activity (ant nests and white grubs) near the beets of the plants which could have affected their growth. A similar trend in the leaf area and leaf fresh mass of the beetroot plants were found than those of the plant heights. Numerous authors have stated the importance of plant roots for crop growth. They found that root growth directly affects both shoot growth and crop yield (Vamerali et al. 2003, Zhang et al. 2009, Wang et al. 2014, Ota et al. 2020). The accumulation of water in this part of the plot (even when irrigating) as well as the insect activity in the other block could have affected root growth, therefore shoot growth and leaf area as well. The beet widths of the beetroot plants were negatively affected by the water and insect activity, of the previously mentioned plots. The beet widths of the plants that were treated with the high concentration of H_2O_2 weekly resulted in the widest beets, however they were not significantly wider than those in the control group nor those treated twice a week with the low or high

concentrations of H_2O_2 . The weekly application of the high H_2O_2 concentrations had beet lengths that were significantly longer than the beets in any of the other treatments. The beet lengths were the shortest in the plants treated with the low H_2O_2 concentration weekly and twice a week and also the plants treated twice a week with the high H_2O_2 concentration. In terms of the fresh and dry mass of the beets, the plants that grew in the control group and those treated weekly with the high concentration of H_2O_2 had the heaviest beets and the beets in the other three treatments the lowest.

Throughout the first week of the study the stomatal density of the beetroot fluctuated from day to day and throughout the study. The stomatal densities of the plants that received no treatment and those that were treated twice a week with the high H_2O_2 concentration did not differ significantly from one another and were significantly higher than the other treatments for most of the study. The stomatal densities of the beetroot treated with the low concentration of H_2O_2 weekly and twice a week fluctuated daily but ultimately seemed to decrease in the first week of treatments. The stomatal density of the beetroot in the control group stayed relatively the same, whereas the plants treated weekly and twice a week with the high H_2O_2 concentration ultimately seemed to increase in the first week of treatments. When looking at the weekly stomatal changes, the stomatal densities of the low H_2O_2 treatments seemed to increase, while those in the control group and those of the high H_2O_2 treatments stayed relatively the same. A study by Salisbury (1928) found that the stomatal density tends to be greater in smaller leaves and less on larger leaves. In this study it was not clear whether the stomatal density was high or low in comparison to leaf size. A study by Peet et al. 2017 have found comparable results.

The Chl A, B and carotenoid content of the beetroot plants fluctuated daily in the first week of the study. Those treated weekly with 0.05 and 0.1 ml L^{-1} of H_2O_2 , however did show a decrease in their Chl A, B and carotenoid content at the end of the first week whereas in the other treatments it increased. The weekly changes in the content of Chl A, B and carotenoids in the plants in all the treatments showed an overall increase. The Swiss chard plants treated with twice a week with 0.1 ml L^{-1} H_2O_2 had the highest Chl A, B and carotenoid content. Hasan et al. (2016) found in their study that the exogenous application of H_2O_2 also increases the chlorophyll content of leaves.

Swiss chard

Studies by Orabi et al. (2015) and Khandaker et al. (2012) found that the application of H_2O_2 in low concentrations have a significantly positive effect on plant growth and on the

biochemical properties and physiological activities of plants as well. The Swiss chard plants in this study that received the 0.05 ml L⁻¹ H₂O₂ concentrations weekly had significantly taller plants than those in any of the other treatments. The plant height of the previously mentioned treatments throughout the study had the tallest plants.

The Swiss chard plants that received the 0.05 ml L⁻¹ H₂O₂ twice a week, also grew in the same sub-sub block as the beans that tended to flood and had the smallest plants initially. The Swiss chard plants treated with 0.05 ml L⁻¹ H₂O₂ twice a week and those treated with 0.1 ml L⁻¹ H₂O₂ weekly and twice a week did not differ significantly in height in the last month of the trial regardless of insect activity (ant nests and aphids) and pathogen infection (Powdery mildew, Black spot and *Sclerotium rolfsii*). Studies have determined that dry matter production (Guzel and Terzi 2013) and leaf growth (Deng et al. 2012a) is increased with the exogenous application of H₂O₂. Similar results were found in this study with the leaf fresh and dry mass of the Swiss chard plants treated weekly with 0.05 ml L⁻¹ H₂O₂, being significantly heavier than the plants in the control group and those in the other treatments. The fresh and dry mass of the plants that received the same concentration of H₂O₂ but twice a week and those treated with 0.1 ml L⁻¹ H₂O₂ weekly and twice a week did not differ significantly from the plants that received no treatment. Studies by Ahmad et al. (2014) and Hasan et al. (2016) found that when hydrogen peroxide is applied at concentrations lower than 100 mg L⁻¹ plant shoot weight can be increased and in this study it can be confirmed that when applying 0.01 ml L⁻¹ H₂O₂ as a foliar spray the shoot weight is not significantly increased. The application of the 0.05 ml L⁻¹ H₂O₂ twice a week had a similar effect than the application of 0.1 ml L⁻¹ H₂O₂. In terms of leaf area index, the Swiss chard treated with 0.05 ml L⁻¹ H₂O₂ weekly did not have a mean leaf area index significantly greater than the plants that received no treatment. The application of the 0.05 ml L⁻¹ H₂O₂ twice a week and those that received the 0.1 ml L⁻¹ H₂O₂ treatments had leaf area indexes significantly smaller than the control and weekly applied 0.05 ml L⁻¹ H₂O₂.

Throughout the first week of the study the stomatal density off the Swiss chard fluctuated daily with the plants treated with the 0.1 ml L⁻¹ of H₂O₂ had the highest stomatal density for most of the first week of the study. The weekly changes in the stomatal densities of all the treatments stayed relatively the same except the Swiss chard treated twice a week with 0.1 ml L⁻¹ of H₂O₂ which increased but for most of the study did not differ significantly from the stomatal density of the plants in the control group. Overall the foliar application of H₂O₂ in this study did not affect the stomatal density on the leaves of the Swiss chard plants.

The initial Chl A, B and carotenoid content started between 9-11 µg ml⁻¹, 3-4 µg ml⁻¹ and 690-800 µg ml⁻¹ respectively in the first week of the study and fluctuated daily. The plants treated twice a week with 0.1 ml L⁻¹ H₂O₂ had a steady increase in all three of the previously

mentioned pigments. All the treatments resulted in an increase in Chl A, B and carotenoid content of the Swiss chard plants. The weekly changes in the content of Chl A, B and carotenoids in the plants treated with 0.05 ml L⁻¹ H₂O₂ weekly showed an overall increase. Hasan et al. (2016) found in their study that the exogenous application of H₂O₂ also increases the chlorophyll content of leaves. The Chl A, B and carotenoid content of all the other treatments in this study, however, stayed relatively the same, except those treated twice a week with 0.05 ml L⁻¹ H₂O₂ and 0.1 ml L⁻¹ H₂O₂ weekly and twice a week that decreased.

From the field study it was clear that elevated H₂O₂ could positively effect plant growth and yield. It was also evident that the different vegetable types (root, leaf and fruiting) will react differently to the concentrations and application rates of H₂O₂, which could be exploited in intensifying vegetable production per unit area. More research is, therefore required with various vegetable species to make accurate predictions of how yield would be affected.

CHAPTER 4

Effect of airing hydroponically grown vegetables with pure oxygen and pure carbon dioxide.

4.1 Introduction

The land that is available for cultivation is decreasing as a result of both urbanisation and industrialization (Kopittke et al. 2019, Nuissl and Siedentop 2021). Conventional agricultural practices contribute to the former as these practices negatively impact the environment (Corwin and Scudiero 2019). It is essential to develop methods to sufficiently produce food in order to feed the growing global population in a sustainable manner. Moving away from soil as growth media for food production is an alternative approach in sustainable food production. By using hydroponics, plants are grown in a nutrient rich solution with or without the use of an inert media (gravel, peat moss, vermiculite etc.) for support is a technique known as hydroponics (Hochmuth and Hochmuth 2001, Bridgwood 2002). The word hydroponics plainly meaning “water work” comes from the Greek words “hydro” which means water and “ponos” which means labour. Professor William Gericke invented the word hydroponics in the early 1930’s. It is a description of plants grown with their roots floating in a water based nutrient solution.

Any technique where plants are produced without the use of soil as a rooting medium is known as a soilless plant culture (Savvas et al. 2013). Production systems without soil are advantageous as the media is sterile, nutrient availability and fertilizer applications are better controlled and more sustainable (Raviv and Lieth 2008). Plant production in a soilless culture is a very cost-effective system in comparison to soil-based production (Raviv and Lieth 2008, Nejad and Ismaili 2014). In general, soilless systems have both greater water and nutrient use efficiencies (Van Os 1999, Savvas 2009) and as a result thereof the use of these systems have progressively become more crucial over the last five decades (Schmilewski 2009). The utilization of this method of food production could result in the conservation of the already depleting land, existing water resources (Resh and Howard 2012, Sharma et al. 2019) and reduce the demand for more land as the plants can be grown in vertical towers. Through the efficient use of water, fertilizer and pesticides and protected cultivation it is possible to increase crop yield (Resh and Howard 2012). Very profitable crops varieties such as cereal, flowers, fodder, green leafy vegetables, peppers and tomatoes grow well in hydroponic systems (Arias et al. 2000, RIRDC 2001, Buchanan et al 2013, Koyama et al. 2013). Numerous high value crops can successfully be grown in hydroponic systems such as peppers, leafy greens, cucumbers, tomatoes, lettuce and various herbs (Arias et al. 2000, Buchanan et al 2013, Koyama et al. 2013, ARC 2022).

The nutrient solution in a hydroponic systems functions as not only the nutrient supply but also the water and oxygen supply. The oxygen that is dissolved in the nutrient solution of a hydroponic systems is essential for not only root formation but also growth (Soffer and Burger 1988). A study by Cherif et al. (1997) found that treatment of hydroponically grown tomatoes with high oxygen concentrations could increase plant growth. Suyantohadi et al. (2010) found that when supplying hydroponically grown lettuce with concentrated dissolved oxygen in nutrient solution (20-30 mg L⁻¹), it effectively improved the growth under low temperatures. Studies have shown the importance of aeration in hydroponic systems for plants growth. Could the application of pure O₂ and CO₂ therefore potentially increase plant growth and yield? It is however expected that different crops will respond differently to additional O₂ and CO₂, therefore a leafy, fruiting and rooting vegetable crop were studied. The aim of this study was to investigate the effect of pure O₂ and CO₂ on plant growth and yield when used as aeration in a hydroponic system. The objectives were to determine the reaction of greenhouse grown Swiss chard, beans and beetroot plants as influenced O₂ and CO₂ in terms of the stomatal density, chlorophyll content, leaf area, growth and yield. It was expected that Swiss chard and beetroot treated with pure O₂ will show an increase in leaf area, have a greater stomatal density, show an increase in chlorophyll content, exhibit increased growth and produce higher yields than the plants treated with pure CO₂. It is expected that the Swiss chard will have a greater increase in both root and shoot growth than the beetroot. Secondly, all the vegetables treated with the additional CO₂ will have a greater number of stomata, show an increase in chlorophyll content, exhibit increased shoot growth and produce higher yields than the plants that received no treatment. It is expected that the plants treated with the pure oxygen will have better root development than those treated with additional carbon dioxide and those receiving no treatment.

4.2 Methodology

4.2.1 Experimental location and design

This experimental study was conducted in a glasshouse at Innovation Africa @UP (Previously known as the Hatfield Experimental farm, University of Pretoria (25°45'06.4"S 28°15'33.5"E). It was equipped with a wet wall ventilation system and rotary tables. The trial consisted of 15 seedlings of Swiss chard (Fordhook Giant), beetroot (Crimson Globe) and bean (Star 2000) seedlings. This trial had three treatments each namely: A (Control, air), B (100% carbon dioxide) and C (100% oxygen) being pumped through the water. Fifty seedlings of each crop were cultivated in seedlings trays filled with a sand and coir mixture (6:1 ratio). After approximately two weeks the seedlings were transferred to the hydroponic system.

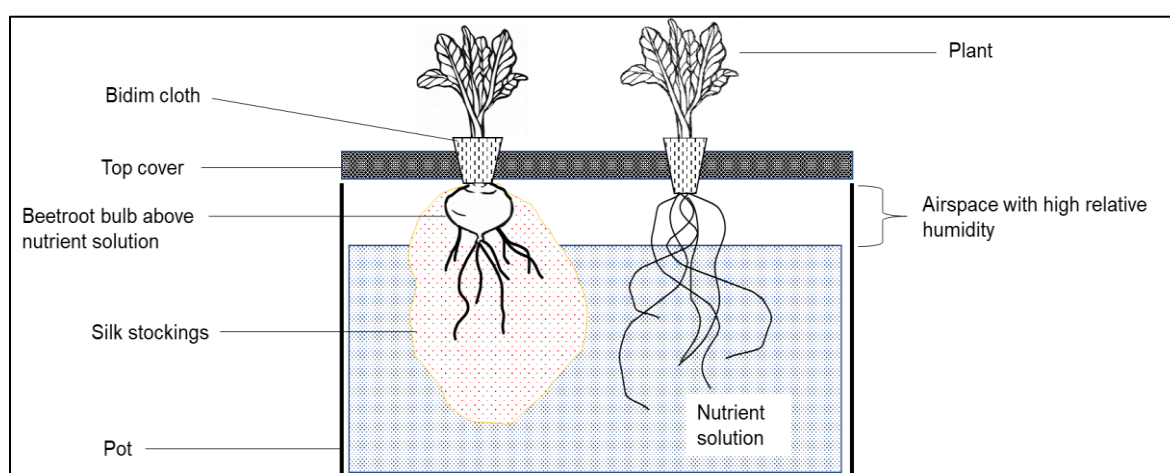


Figure 4.1: The suspended, non-circulating hydroponic pot system used in this study.

This system (Figure 4.1) is exceptionally efficient with water use. Many other hydroponic systems require electricity and pumps for aeration and circulation which adds to production costs and complicates the system, however this system does not require it. The core concepts of this hydroponic system (Kratky 2004) are that the upper parts of the plant roots have to be exposed to air with a high relative humidity so that the roots cannot dry out, the lower part of the plant root system takes up water and nutrients and lastly the nutrient solution level within the pot can stay the same or be lowered but not increased as the roots will 'drown'.

A total of fifteen pots were used with each containing one seedling of each crop (Figure 4.1). The beetroot seedlings were also placed inside a silk stocking, which would allow the beet to grow unhindered in the humid airspace (Figure 1) with the roots still in the nutrient solution. Each treatment, therefore consisted of 5 pots placed on a rotary table in a completely

randomised design. The water and nutrient solution were aerated through either a mechanical pump or a self-made system for the 100% O₂ and CO₂ treatments. A hydroponic fertilizer (1 g L⁻¹) and calcium nitrate (1 g L⁻¹) fertilizer were mixed with 10 litres of tap water. Eight litres of this solution was added to each of the 15 pots. Unfortunately the bean seedlings began to die in the first week of the study (the leaves became white in colour), the roots rotted and they were therefore terminated.

4.2.2 Oxygen and carbon dioxide pump systems

The treatments for the hydroponic trial were applied by mean of a self-made CO₂ and O₂ system (Figure 4.2). Each system consisted of two 2 litre Coke bottles (cleaned), airline tubing, a check valve, a sinker and an air stone diffuser.

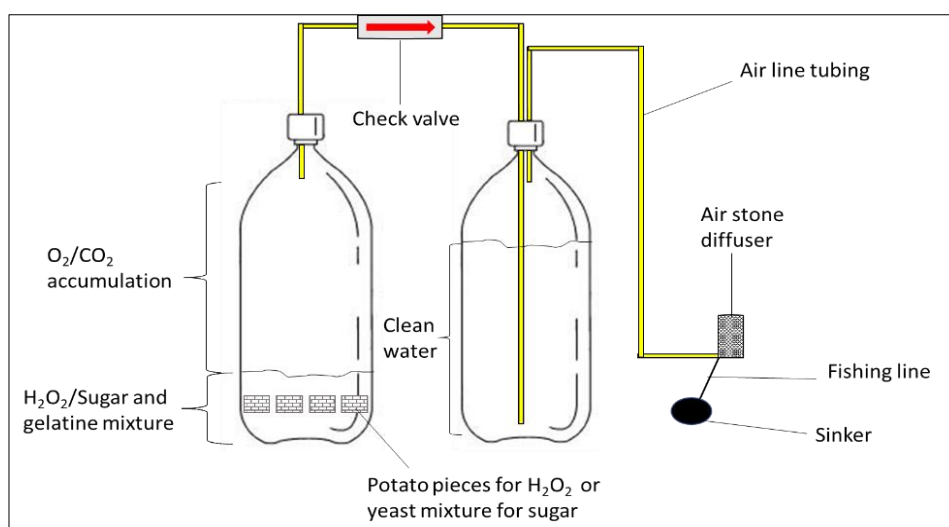


Figure 4.2: The oxygen and carbon dioxide pump system.

A soldering iron was used to make one hole in the cap of the first bottle and two holes in the cap of the second bottle. The holes were just big enough for the airline tubing to fit and the tubing was cut at an angle to ensure a tight fit. Only about 2-3 cm of the tubing had to be inside the first bottle. In the first hole of the second bottle the tubing was set approximately one centimetre from the bottom. The tubing in the remaining hole of the second bottle was also set 2-3 cm inside the bottle and long enough to reach the bottom of the hydroponic pot. The tubing in each hydroponic pot was fitted with an air stone diffuser and small sinker. The sinker was added to prevent the air stone from floating and ensuring that the bubbles were released near the bottom of the pot. The tubing between bottles one and two were cut in half and a check valve was inserted to prevent the backflow of the oxygen and/or carbon dioxide

into the first bottle. Hot glue was applied to the caps where the airline tubes went into the bottles to ensure that the system was airtight.

Carbon dioxide treatment

The CO₂ treatment consisted of a mixture of sugar and gelatine (Figure 4.2) and was added to the first bottle. For each of the five CO₂ systems/treatments 250 g of white sugar was added to 250 g of water in a pot. The pot was heated on the stove whilst three gelatine sheets were soaking in cold water. Once the sugar was dissolved, the soaked gelatine was added to the pot, stirred till combined and removed from the heat. The mixture was then left to cool down until room temperature before it was poured into the bottle. The bottles were placed overnight in the fridge for the gelatine to set. Five litres of a yeast-water mixture was then prepared by combining approximately 10 grams of yeast and five teaspoons of sugar to five litres of lukewarm water. One litre of this mixture was then added to each of the bottles containing the gelatine. The second bottle of the system was filled with tap water and cleaned the formed gas from any impurities that could have been present. The system takes about 24 hours to build enough pressure before the first bubbles can be seen. With this system, carbon dioxide production cannot be stopped once it started. Carbon dioxide will be produced throughout the entire day and night. This system is temperature sensitive therefore it will produce less carbon dioxide when temperatures are lower and more when temperatures are higher. In order to determine whether or not CO₂ was produced, shaking the bottle containing the yeast mixture would increase pressure as more carbon dioxide was released and would result in the first bubbles appearing in the hydroponic pot. The yeast sugar and gelatine bottle was replaced every 10 - 12 days, as the system is temperature dependant, in the glasshouse and due to the fact that during this process of carbon dioxide production, yeast also produces an alcohol (ethanol) that will inhibit their growth and cell viability.

Oxygen treatment

The oxygen treatment consisted of 500 ml of H₂O₂ in the first bottle of each of the five aeration systems (Figure 4.2). A quarter of a peeled medium sized potato, cut into blocks (small enough to fit the cap), was added to the hydrogen peroxide every second day. The potato pieces functioned as a catalyst for the hydrogen peroxide to release pure O₂. The second bottle of the system was filled with water and cleaned the formed gas from any impurities that could have been present. In bottle one, the H₂O₂ was replaced every 10 -14 days, depending on whether a slice of potato still caused a reaction to take place. The

temperature experienced in the glasshouse also had an effect on the speed of the reactions that took place in the oxygen treatments.

4.2.3 Measurements

Measurements such as general observations, plant height, root development, insect activity, solution electrical conductivity (EC) and pH were taken weekly with a pH/EC/TDS/Temperature meter. Final measurements included fresh and dry mass of the roots and biomass. During the first week frequent EC and pH readings were taken to determine how quickly the 100% CO₂ and O₂ treatments would affect pH and EC. These measurements indicated that the water nutrient solution should be replaced every 7-10 days.

Plant height

The plant heights were measured weekly. This variable was measured in centimetres (cm) using a ruler, placed vertically on the surface of the top cover (Figure 41). The measurement was taken at the highest stem meristem (not including the pods) for the beans, from the top cover to the tip of the longest leaf for Swiss chard and beetroot. The root length was also measured at the end of the trial with a ruler from the root-base to the tip of the longest root for Swiss chard and beetroot. Measuring the root length of the beetroot plants, it did not include the edible part (EP) of the root (Figure 4.3).

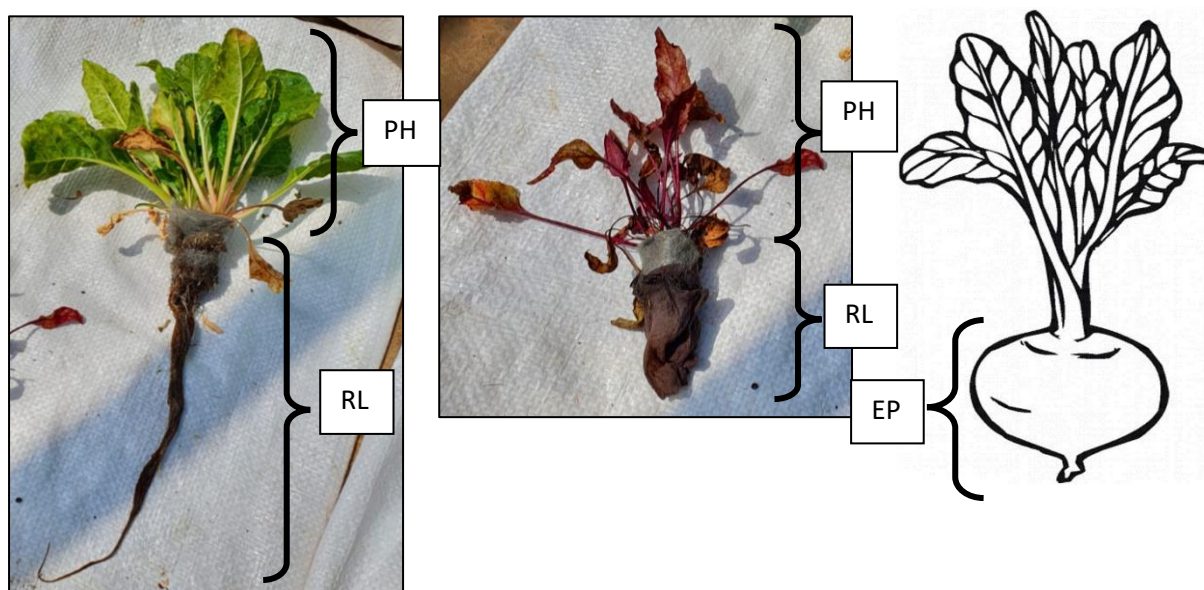


Figure 4.3: The root length (RL), plant height (PH) and edible part (EP) from Swiss chard (left) and beetroot plants (right).

Mass measurements

The fresh and dry mass of the biomass and root mass of the plants were weighed at the end of the study (at the end of 7 weeks). The plants were placed into labelled paper bags after the fresh weight and all other necessary measurements were taken and dried in an oven at 70°C for 2-3 days. After the samples have dried they were weighed again to measure the dry mass.

4.2.4 Statistical analysis

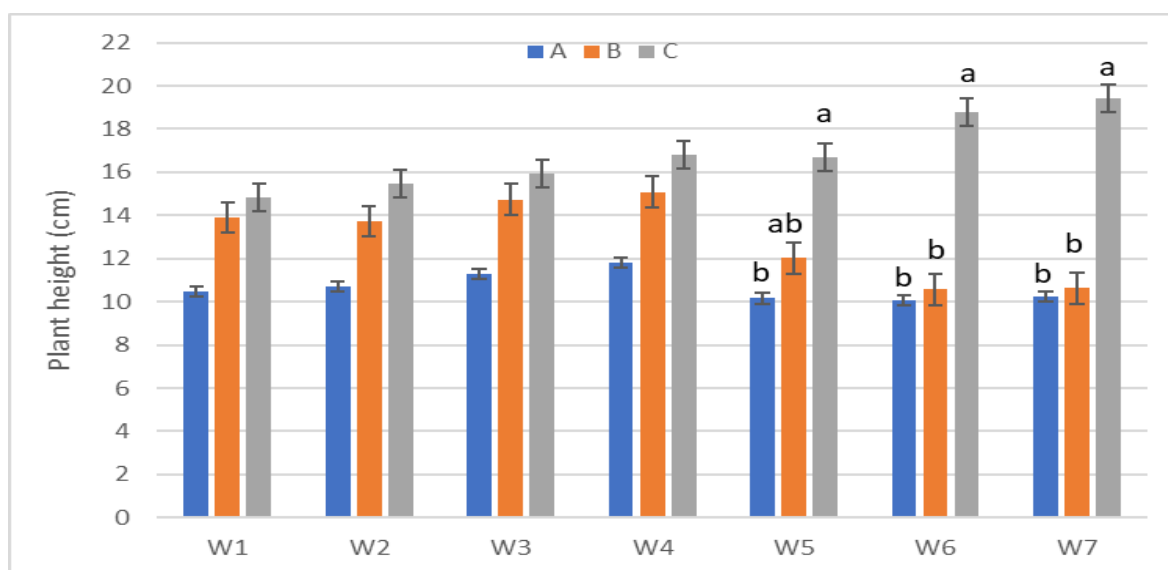
The data was analysed using the statistical package SAS (model 9.4), to assess if the treatment means were significantly ($P < 0.05$) affected by the treatments. The Tukey LSD test was used to determine if there was a significant difference by probability (5%). The ANOVA tables are included in the Appendix D.

4.3 Results

4.3.1 Plant height

Beetroot

The beetroot plants (Figure 4.4) that received no treatment (C) throughout the study had the tallest plants and the plants that was aerated with pure CO₂ (B) had the second tallest plants and those aerated with pure O₂ (A) were the shortest plants. From week one to four there was no significant difference in plant height between treatments. Throughout the trial the plants in treatment B was consistently, but not significantly, taller than those in treatment A. In the last three weeks of the trial, the plants in treatment A and B had a decrease in plant height. From figure 4.5 one can see that initially the plants were still healthy, but from week 5 to 7 the plants started to die off and leaf growth was very slow. Throughout the trial the plants were also infested with aphids and at one time powdery mildew as well (treated with Efecto: Aphid plus and Efecto Funginex respectively). The plants were treated once the insect or pathogen was identified and treated accordingly.



A to C represents the different treatments applied in the study and W1-W7 represents weeks one to seven. A = pure O₂ bubbled through the hydroponic pot; B = pure CO₂ bubbled through the hydroponic pot; C = no treatment (air) bubbled through the hydroponic pot. Bars of the same week with different letters indicates a significant difference ($P < 0.05$) between treatments and bars without letters indicated no significant difference.

Figure 4.4: Weekly effect of air, pure O₂ and pure CO₂ used as aeration on plant height of beetroot plants grown in a hydroponic pot system.

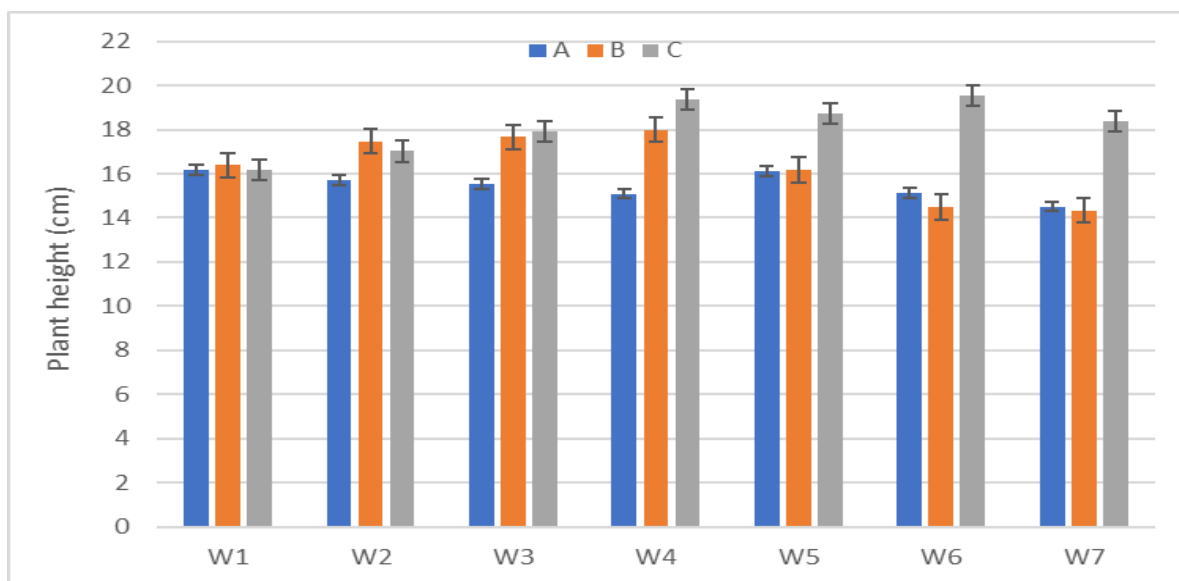


Figure 4.5: A beetroot plant in week three (left) and in week 6 (right) of the trial.

Swiss chard

During the first two weeks the plants that were aerated with pure CO₂ (B) resulted in plants taller than the plants aerated with pure O₂ (A) or the control (air, C) (Figure 4.6). From week 3 to the seventh week the plants of treatment C (control) were taller than the plants in any of the other treatments. The beetroot and Swiss chard plants that had no treatment (C) resulted in the tallest plants and the plants treated with the pure oxygen (A) the smallest plants.

From figure 4.7 it can be seen that initially the plants were still healthy with good leaf growth, but from week 5 the plants started to die off and leaf growth was very slow. Throughout the trial the plants were infested with not only aphids but red spider mites and at one time powdery mildew as well. The plants were treated once the insect or pathogen was identified and treated accordingly as plant grown would also have been further affected by the insects and/or pathogen.



A to C represents the different treatments applied in the study and W1-W7 represents weeks one to seven. A = pure O₂ bubbled through the hydroponic pot; B = pure CO₂ bubbled through the hydroponic pot; C = no treatment (air) bubbled through the hydroponic pot. Bars of the same week with no letters indicated no significant difference ($P > 0.05$) between treatments.

Figure 4.6: Weekly effect of air, pure O₂ and pure CO₂ used as aeration on plant height of Swiss chard plants grown in a hydroponic pot system.



Figure 4.7: A Swiss chard plant aerated with air in week three (left) and in week 6 (right) of the trial.

4.3.2 Plant roots

Beetroot root length

From figure 4.8 it is apparent that the beetroot plants did not have a lot or very noticeable root growth. Due to no roots being visible, no measurements of the roots were taken.



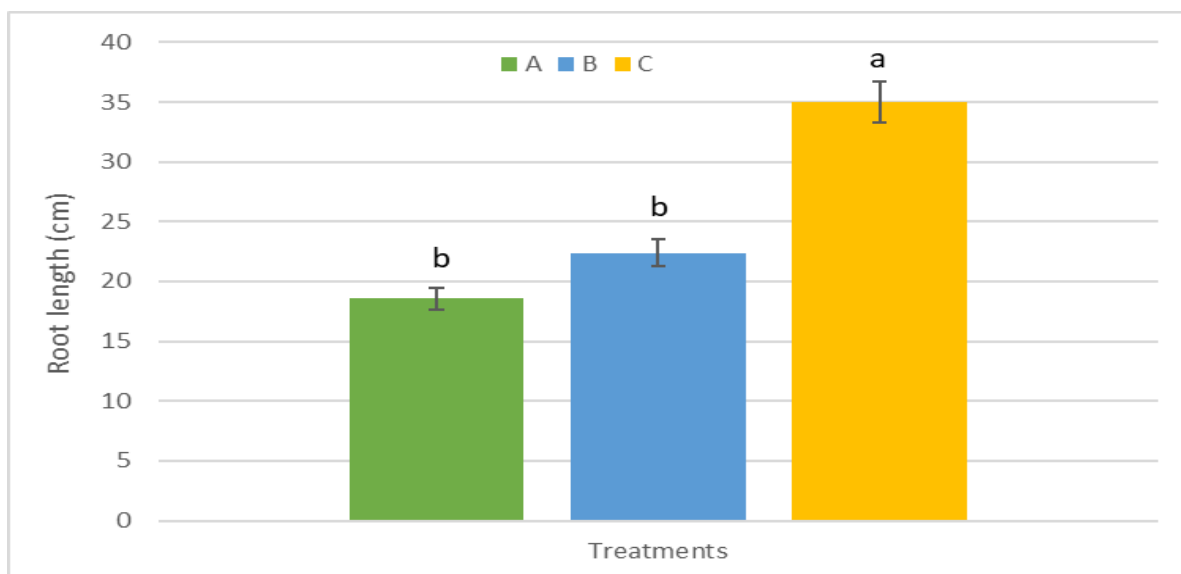
Figure 4.8: Roots at the harvest of the beetroot plants aerated with pure O₂ (left) and pure CO₂ (right).

Swiss chard root length

The Swiss chard plants that received no treatment (C) throughout the study had roots that were significantly longer than the roots in any of the other treatments (Figure 4.9). The plants that was aerated with pure CO₂ (B) had the second longest roots, however they were not significantly longer than the roots of the plants aerated with pure O₂ (A). The plants that were received no treatment (C) not only had the tallest plants but also the longest roots and those in treatment A had both the shortest plants and roots.

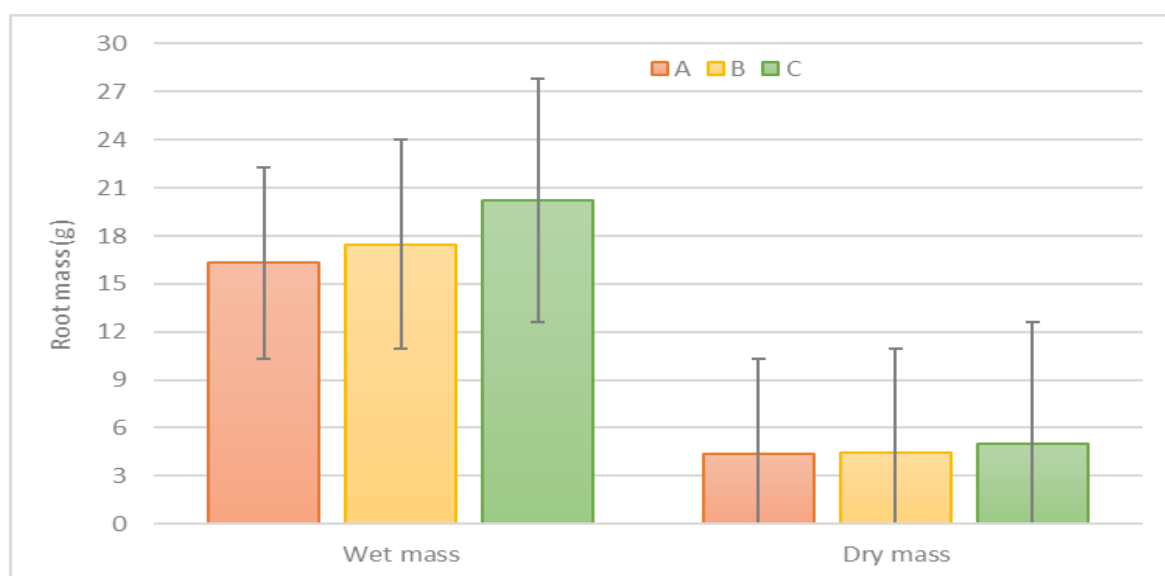
Swiss chard root mass

The root wet and dry mass of the Swiss chard plants followed a similar trend to the root length of the plants. Both the fresh and dry mass of the roots but it did not differ significantly between treatments (Figure 4.10). The beetroot plants did not produce roots that could be measured in terms of mass.



A to C represents the different treatments applied in the study. A = pure O₂ bubbled through the hydroponic pot; B = pure CO₂ bubbled through the hydroponic pot; C = no treatment (air) bubbled through the hydroponic pot. Bars with different letters indicated significant differences ($P < 0.05$) between treatments.

Figure 4.9: Effect of air, pure O₂ and pure CO₂ used as aeration on the root length of Swiss chard plants grown in a hydroponic pot system.



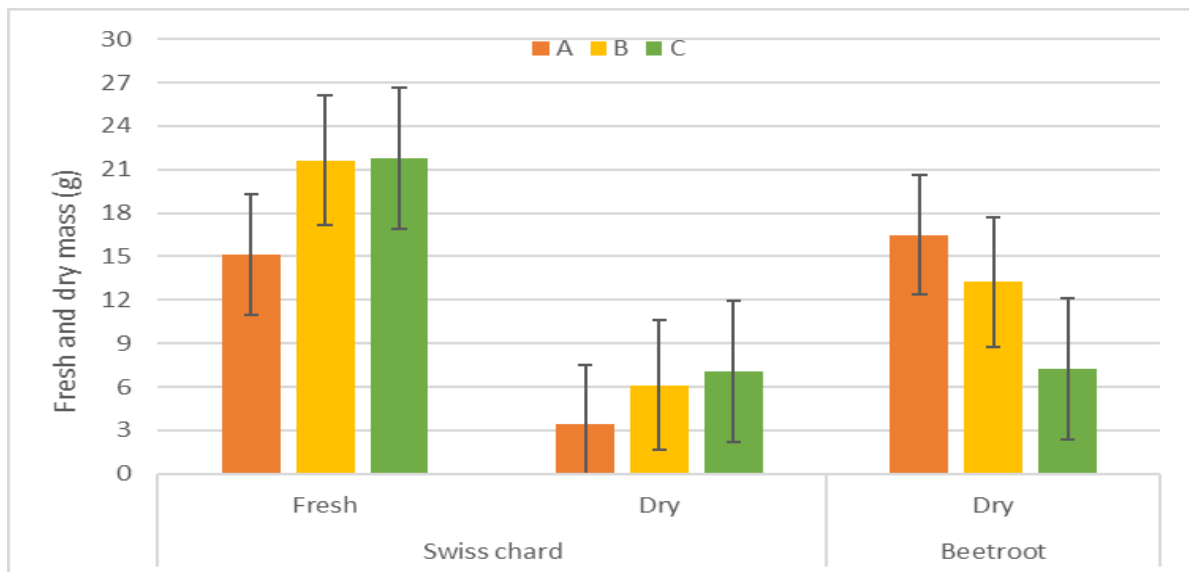
A to C represents the different treatments applied in the study. A = pure O₂ bubbled through the hydroponic pot; B = pure CO₂ bubbled through the hydroponic pot; C = no treatment (air) bubbled through the hydroponic pot. Bars without letters indicated no significant differences ($P > 0.05$) between treatments.

Figure 4.10: Effect of air, pure O₂ and pure CO₂ used as aeration on the root mass of Swiss chard plants grown in a hydroponic pot system.

4.3.3 Leaves

Fresh and dry mass

The leaf dry mass of the beetroot plants (Figure 4.11) that were aerated with pure O₂ (A) did not have leaves that were significantly heavier than those that were aerated with pure CO₂ (B) or those in the control group (C). The trend of the beetroot leaf dry mass had the opposite trend of the Swiss chard plants. The Swiss chard plants that received no treatment (C) had the heaviest leaves (fresh and dry) among the treatments, but it was not significantly heavier than those of treatment A or B (in either the fresh or dry mass).



A to C represents the different treatments applied in the study and W1-W7 represents weeks one to seven. A = pure O₂ bubbled through the hydroponic pot; B = pure CO₂ bubbled through the hydroponic pot; C = no treatment (air) bubbled through the hydroponic pot. Bars without letters indicated no significant differences ($P > 0.05$) between treatments.

Figure 4.11: Effect of pure O₂, pure CO₂ and air has on the leaf fresh and dry mass of Swiss chard and beetroot plants grown in a hydroponic pot.

4.4 Discussion and conclusions

The beetroot plants that had been aerated with air (21% O₂ and 78% N), had the tallest plants throughout the study. This treatment initially did not seem to affect plant height of beetroot as the plant's height did not differ significantly between treatments. Closer to the end of the trial, however, the beetroot plants receiving no treatment increased in height, whereas the plants in the other treatments reduced in height. Swiss chard plants that received air were significantly taller, but the root mass and length was also significantly better than the plants aerated with 100% O₂ and 100% CO₂. It had been reported that elevated CO₂ concentrations can improve plant growth (Viktor and Cramer 2003, He et al. 2004, He et al. 2013, Cramer et al. 2005) but negative effects of elevated CO₂ in the rootzone have also been found (Cramer 2002), particularly when the elevated CO₂ is accompanied with deficient O₂ (Boru et al. 2003). Throughout the trial the roots of the Swiss chard started to turn brown but new root growth was also present. Shavrukov et al. (2012) found that when aeration is inadequate the dissolved oxygen content will decrease and result in root browning and root rot. These symptoms were prevalent in the plants when it was close to the time for the O₂ producing bottles to be replaced. In a study by Baiyin et al. (2021) they found that aeration intensity affected plant growth due to the fact that low intensities resulted in increased plant and root growth. In this study the plants that had air (21% O₂ and 78% N) bubbled through the system throughout the day, whereas the aeration intensity of the other treatments were dependent on the rate of the reactions taking place in the bottles. The rate of the reaction were then also affected by temperature. On days that the temperature in the glasshouse were high the reactions occurred faster and during cooler temperatures (at night or early morning) the reactions took place slower. The aeration rate of the different treatments could be a factor that affected the plant growth. A study by Bateer et al. (2021) on different aeration intensity in hydroponics found that increasing aeration intensity did not result in plant growth to increase. They found that plant growth only increased when aeration intensity is low or until it reached a certain extent. The beetroot did not develop beets and could be attributed to not only the air temperature that was high but also the water-nutrient solution that increased in temperature.

It was also evident that the aeration of hydroponically grown beetroot and Swiss chard with pure oxygen and carbon dioxide will positively effect plant growth and yield, however the beans were not able to grow in this study. More research is therefore required with various crops as well as commercial grade O₂ and CO₂ sources to eliminate any negative side effects of the chemical procedures used to produce O₂ and CO₂.

CHAPTER 5

SUMMARY AND RECOMMENDATIONS

The population is currently almost 8 billion and predicted to reach 9.55 billion by the year 2050, which will cause a greater demand for food. Changes in the diets of people will also add to the food demand. An increased output of food will be required (60-110%) in order to meet the growing demand. Two methods such as intensification and clearing more land have already been developed to increase yield and to close the yield gap, but these do have negative problems associated with them. Genetic manipulation, management and production practices mainly focus on increasing yield. Other techniques such as the release of additional oxygen and carbon dioxide in close proximity to plants or the improvement of existing CO₂ and O₂ fertilization techniques can however be explored further. This could increase photosynthesis directly and thus crop growth and yield. The main aim of this study was to investigate the application of additional carbon dioxide and oxygen on plant growth and yield.

5.1 Summary

5.1.1 Pot trial

In the preliminary trial the application of additional CO₂ via CO₂ tablets and CaCO₃ initially showed promising results in terms of plant height, the concentrations of chlorophyll A and B and stomatal density. The direct application of additional CO₂ via CaCO₃ and CO₂ tablets on the soil resulted in damage to the plant roots and increased the soil EC to such an extent that it could not be measured. The plants ultimately died early on in the trial. A second trial was conducted to try to improve on the applications of this study.

The application of additional CO₂ through CaCO₃ in the second pot trial improved bean and beetroot plant growth. The plant growth parameters, bean pod parameters, beets parameters, beet yield and the analysis showed no significant differences between the control (ambient glasshouse) and the plants receiving the additional CO₂ through CaCO₃. This could be due to the fact that the plants grew in different glasshouses. Bean pods per plant and yield; plant growth and biomass of Swiss chard were negatively affected by the CaCO₃ treatments but not root length, root dry mass or root to shoot ratio of the lower CaCO₃ treatment. The plants in the elevated CO₂ chamber throughout the second trial improved the plant height of the beans but not the beets or Swiss chard, the root length in all the crops (except Swiss chard), the biomass of the beans and beetroot, shoot to root ratio in Swiss chard significantly, number of pods per plant significantly and yield in all the crops but Swiss chard.

In the laboratory analysis of the leaves of the three crops, an increase in the stomatal density of all three crops were observed in the elevated CO₂ chamber treatment but not those receiving additional CO₂ from the CaCO₃ treatments. The chlorophyll A and B content of the bean plants that received the higher CaCO₃ treatment were slightly higher than those in the elevated CO₂ chamber, ambient glasshouse and chamber. In terms of the total carotenoid concentration, there was not a difference between the treatments except those in the CO₂ chamber having a slightly higher concentration. The chl A and the total carotenoid concentration of the beetroot receiving additional CO₂ from the CaCO₃ treatments were higher than those in the ambient glasshouse and the elevated CO₂ chamber, but chl B in the elevated CO₂ chamber were higher than those receiving additional CO₂. The chl A, B and the total carotenoid content of the Swiss chard plants receiving additional CO₂ from the CaCO₃ were higher than those grown in the ambient CO₂ glasshouse but not those in the elevated CO₂ chamber.

The photosynthetic rates of all three crops grown in the elevated CO₂ chamber were significantly higher than any of the other treatments and those receiving additional CO₂ did not differ significantly from the plants in ambient CO₂ levels. In terms of stomatal conductance, the beans and beetroot receiving additional CO₂ and those in the elevated CO₂ chamber did not differ from one another but were significantly lower than those in the ambient glasshouse (and chamber for the beans). The Swiss chard plants receiving additional CO₂ had higher stomatal conductances than those in the elevated CO₂ chamber but not significantly different from those in the ambient glasshouse.

5.1.2 Field study

The foliar application of hydrogen peroxide were found to be affective in increasing plant height in all three crops. The beans treated with the 0.1 ml L⁻¹ concentration of H₂O₂, regardless of application rate had significantly taller plants than any of the other treatments. The beetroot, however, were found to have greater plant height when treated weekly with either 0.05 ml L⁻¹ or 0.1 ml L⁻¹ H₂O₂ concentration but plant height was not significantly greater than those in the control. The Swiss chard plants treated with 0.05 ml L⁻¹ H₂O₂ concentration weekly had significantly taller plants than any of the other treatments and a leaf area index significantly greater than all the treatments except the control.

The Swiss chard plants treated with the low concentration twice a week and the high concentrations, regardless of application rate, had a leaf area index significantly lower than those treated weekly with the low H₂O₂ concentration but not the control. The biomass and yield were significantly higher in the Swiss chard plants treated weekly with the low H₂O₂

concentration than any other treatment. The leaf area and biomass of the beetroot plants treated weekly with either the high or low H₂O₂ concentration were greater than those treated with the same concentrations of H₂O₂ but at a higher application rate. In terms of yield, the plants treated with the high concentration, regardless of application rate, resulted in yields significantly greater than those treated with the low H₂O₂ concentration but not those in the control.

The biomass of the beans treated weekly with the high H₂O₂ concentration were significantly greater than any of the other treatments but in terms of fresh biomass, only those treated weekly with the high H₂O₂ concentration were significantly greater than any other treatment. The pods of the bean plants treated with the high H₂O₂ concentration, regardless of application rate, positively affected pod length and number of seeds whereas those treated with the low H₂O₂ concentration did not differ significantly from the control. The yield of the beans treated with the low H₂O₂ concentration, regardless of application rate, were significantly lower than any of the other treatments and those treated with the high H₂O₂ concentrations did not differ significantly from the control. The beetroot treated with the high H₂O₂ concentration weekly resulted in the biggest, heaviest beets and yield among treatments whereas those treated with the low H₂O₂ concentrations and the high concentration twice a week were significantly smaller and lighter than those in the control.

In the laboratory analysis of the leaves of the three crops found that the stomatal densities were not significantly affected by any of the H₂O₂ treatments. The photosynthetic pigments of the bean plants overall increased in all the treatments. The beans treated weekly with the low and high H₂O₂ concentration resulted in higher concentrations than those treated with the same concentrations but at the higher application rate. In the beetroot plants the opposite was found than in the beans, the plants treated twice a week with the high H₂O₂ concentration had a greater concentration of photosynthetic pigments than any of the other treatments. The highest chlorophyll A, B and the total carotenoid concentrations were found in the beans treated with the low H₂O₂ concentration weekly followed by the plants treated with the high H₂O₂ concentrations, regardless of application rate. It was only in the concentration of chlorophyll B and the total carotenoids that decreased with the low H₂O₂ concentration applied twice a week and the control. The Swiss chard plants treated weekly with the low H₂O₂ concentration and the high H₂O₂ concentration, regardless of application resulted in the highest chlorophyll A and total carotenoid concentrations, respectively.

5.1.3 Hydroponic trial

The plants aerated with air throughout the trial resulted in the tallest beetroot and Swiss chard plants followed by those aerated with CO₂. In terms of root length and mass the Swiss chard plants aerated with air also did the best. The aeration with O₂ negatively affected not only the growth parameters (plant height and root length) but also the leaf and root mass of the Swiss chard plants. The beetroot plants on the other hand had the highest biomass when aerated with pure O₂ followed by those aerated with pure CO₂. In this trial the bean plants died off soon after the treatments were applied and were clearly not adapted to growing in this system.

5.2 Recommendations

The study on the application of additional CO₂ and O₂ via calcium carbonate and hydrogen peroxide to beans, beetroot and Swiss chard grown in glasshouses, an open field and a hydroponic system should be seen as a first step in determining whether CO₂ or O₂ fertilization techniques could improve growth and yield. The results in these studies should be seen as techniques that could enhance plant growth and yield, but should be studied further by including other aspects such as transpiration, respiration, temperature, soil type, leaf area, application rates, crop type etc. The application of additional CO₂ and O₂ in pot and field trials shows promising results in terms of plant growth, but in future studies an existing commercial CO₂ fertilizer such as AGROSOL could be included as another treatment. Due to COVID19 and import permits we were unable to obtain the latter fertilizer for the glasshouse and open field studies. Aerating a hydroponic solution with pure O₂ or CO₂ initially showed promising results in the growth of beetroot and Swiss chard, however further studies where 1: O₂ and CO₂ from a commercial source should be used, and 2: pure O₂ and CO₂ are applied simultaneously is recommended.

REFERENCES

- Abass S, Mohamed H. 2011. Alleviation of adverse effects of drought stress on common bean (*Phaseolus vulgaris* L.) by exogenous application of hydrogen peroxide. *Bangladesh Journal of Botany*, 40: 75-83..
- Adeyemi O, Keshavarz-Afshar R, Jahanzad E, Battaglia ML, Luo Y, Sadeghpour A. 2020. Effect of wheat cover crop and split nitrogen application on corn yield and nitrogen use efficiency. *Agronomy*, 10: 1081.
- Adom D. 2018. The role of good post-harvest management practices in the development of the agriculture sector in Ghana. p. 169-188.
- Ahmad I, Maqsood S, Basra A, Wahid A. 2014. Exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide improves the productivity of hybrid maize at low temperature stress. *International Journal of Agriculture and Biology*, 16: 1560-8530.
- Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist*, 165: 351-372.
- Ainsworth EA, Long SP. 2021. 30 years of free-air carbon dioxide enrichment (FACE): What have we learned about future crop productivity and its potential for adaptation? *Global Change Biology*, 27: 27-49.
- Ainsworth EA, Rogers A. 2007. The response of photosynthesis and stomatal conductance to rising [CO₂]: Mechanisms and environmental interactions. *Plant, Cell and Environment*, 30: 258-270.
- Ajetomobi J. 2016. Effects of weather extremes on crop yields in Nigeria. *African Journal of Food, Agriculture, Nutrition and Development*, 16: 11168-11184.
- Alakhdar HH, Shoala T. 2021. Exogenous application of hydrogen peroxide in different resistant bean cultivars of *Phaseolus vulgaris* to *Tetranychus urticae* (Acari: Tetranychidae). *Arthropod-Plant Interactions*, 15: 439-445.
- Alcock DJ, Harrison MT, Rawnsley RP, Eckard RJ. 2015. Can animal genetics and flock management be used to reduce greenhouse gas emissions but also maintain productivity of wool-producing enterprises? *Agricultural Systems*, 132: 25-34.
- Alexandratos N, Bruinsma J. 2012. World agriculture towards 2030/2050: The 2012 revision. *ESA Working paper No. 12-03*. Rome, FAO.
- Ali A. 2018. Role of hydrogen peroxide in management of root rot and wilt disease of thyme plant. *Journal of Phytopathology and Pest Management*, 5: 1-13.

- Amthor JS. 2001. Effects of atmospheric CO₂ concentration on wheat yield: Review of results from experiments using various approaches to control CO₂ concentration. *Field Crops Research*, 73: 1-34.
- Andrea M, Peres T, Luchini L, Pettinelli Jr A. 2000. Impact of long-term pesticide applications on some soil biological parameters. *Journal of Environmental Science and Health Part B*, 35: 297-307.
- Anon. 2019. Chapter 26 - Intercropping, multicropping, and rotations. In: Campanhola C, Pandey S editors. *Sustainable Food and Agriculture*: Academic Press. p. 243-248.
- Appiah S, Mbatchou V, Adom D, Ayisi F. 2018. Pesticidal effects of extracts from *hyptis suaveolens* and *hyptis spicigera* on cowpea weevils. *International Journal of Environment, Agriculture and Biotechnology*, 3: 1691-1699.
- ARC. 2022. Hydroponic Vegetable Production Available at <https://www.arc.agric.za/arc-vopi/Pages/Crop%20Science/Hydroponic-Vegetable-Production.aspx#:~:text=%E2%80%8BWhich%20crops%20can%20be,herbs%20in%20gravel%20flow%20systems>. [accessed 10 December 2022].
- Arencibia AD, Bernal A, Zayas C, Carmona E, Cordero C, González G, García R, Santana I. 2012. Hydrogen peroxide induced phenylpropanoids pathway eliciting a defensive response in plants micro propagated in Temporary Immersion Bioreactors (TIBs). *Plant Science*, 195: 71-79.
- Arias R, Lee TC, Specca D, Janes H. 2000. Quality comparison of hydroponic tomatoes (*Lycopersicon esculentum*) ripened on and off vine. *Journal of food science*, 65: 545-548.
- Arias-Estévez M, López-Periago E, Martínez-Carballo E, Simal-Gándara J, Mejuto JC, García-Río L. 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agriculture, Ecosystems and Environment*, 123: 247-260.
- Ashfaq F, Khan MIR, Khan NA. 2014. Exogenously applied H₂O₂ promotes proline accumulation, water relations, photosynthetic efficiency and growth of wheat (*Triticum aestivum* L.) under salt stress. *Annual Research and Review in Biology*: 105-120.
- Asumadu-Sarkodie S, Owusu PA. 2016a. Feasibility of biomass heating system in Middle East Technical University, Northern Cyprus Campus. *Cogent Engineering*, 3: 1134304.
- Baas R, Gislerød H, Warmenhoven M. 1997. Effect of aeration on rooting and growth of roses. *Acta Horticulturae*, 450: 113.
- Badenes-Pérez FR. 2018. Trap crops and insectary plants in the order *Brassicales*. *Annals of the Entomological Society of America*, 112: 318-329.

- Baiyin B, Tagawa K, Yamada M, Wang X, Yamada S, Yamamoto S, Ibaraki Y. 2021. Study on plant growth and nutrient uptake under different aeration intensity in hydroponics with the application of particle image velocimetry. *Agriculture*, 11: 1140.
- Ball BC, Bingham I, Rees RM, Watson CA, Litterick A. 2005. The role of crop rotations in determining soil structure and crop growth conditions. *Canadian Journal of Soil Science*, 85: 557-577.
- Balmford A, Green RE, Scharlemann JPW. 2005. Sparing land for nature: Exploring the potential impact of changes in agricultural yield on the area needed for crop production. *Global Change Biology*, 11: 1594-1605.
- Bateer B, Tagawa K, Yamada M, Wang X, Yamada S, Yamamoto S, Ibaraki Y. 2021. Study on plant growth and nutrient uptake under different aeration intensity in hydroponics with the application of particle image velocimetry. *Agriculture*, 11: 1140.
- Bates BC, Walker K, Beare S, Page S. 2010. Incorporating climate change in water allocation planning. *Waterlines Report Series*.
- Bedos C, Cellier P, Calvet R, Barriuso E, Gabrielle B. 2002. Mass transfer of pesticides into the atmosphere by volatilization from soils and plants: overview. *Agronomy*, 22: 21-33.
- Ben Haj Koubaier H, Snoussi A, Essaidi I, Chaabouni MM, Thonart P, Bouzouita N. 2014. Betalain and phenolic compositions, antioxidant activity of Tunisian red beet (*Beta vulgaris* L. conditiva) roots and stems extracts. *International Journal of Food Properties*, 17: 1934-1945.
- Bhargava A, Srivastava S. 2019. *Participatory plant breeding: Concept and applications*. Springer. p. 29-68.
- Bhattarai SP, Huber S, Midmore DJ. 2004. Aerated subsurface irrigation water gives growth and yield benefits to zucchini, vegetable soybean and cotton in heavy clay soils. *Annals of applied biology*, 144: 285-298.
- Bisbis MB, Gruda N, Blanke M. 2018. Potential impacts of climate change on vegetable production and product quality – A review. *Journal of Cleaner Production*, 170: 1602-1620.
- Björkman O. 1981. Responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, Ziegler H editors. *Physiological Plant Ecology I: Responses to the Physical Environment*. Berlin, Heidelberg: Springer Berlin Heidelberg. p. 57-107.
- Blum J, Fridovich I. 1983. Superoxide, hydrogen peroxide, and oxygen toxicity in two free-living nematode species. *Archives of Biochemistry and Biophysics*, 222: 35-43.
- Boincean B. 2014. Fifty years of field experiments with crop rotations and continuous cultures at the Selectia research institute for field crops. p. 175-199.

- Bonfig KB, Schreiber U, Gabler A, Roitsch T, Berger S. 2006. Infection with virulent and avirulent *P. syringae* strains differentially affects photosynthesis and sink metabolism in Arabidopsis leaves. *Planta*, 225: 1-12.
- Boru G, Vantoai T, Alves J, Hua D, Knee M. 2003. Responses of soybean to oxygen deficiency and elevated root-zone carbon dioxide concentration. *Annals of Botany*, 91: 447-453.
- Bouchenak M, Lamri-Senhadj M. 2013. Nutritional quality of legumes, and their role in cardiometabolic risk prevention: A review. *Journal of Medicinal Food*, 16: 185-198.
- Branca G, McCarthy N, Lipper L, Jolejole MC. 2011. Climate-smart agriculture: A synthesis of empirical evidence of food security and mitigation benefits from improved cropland management. *Mitigation of Climate Change in Agriculture Series*, 3: 1-42.
- Bridgwood L. 2002. *Hydroponics: Soilless Gardening Explained*. Crowood Press.
- Broberg MC, Högy P, Feng Z, Pleijel H. 2019. Effects of elevated CO₂ on wheat yield: Non-linear response and relation to site productivity. *Agronomy*, 9: 243.
- Buchanan D, Omaye S. 2013. Comparative study of ascorbic acid and tocopherol concentrations in hydroponic- and soil-grown lettuces. *Food and Nutrition Sciences*, 04: 1047-1053.
- Caixeta V, Mata A, Curvelo C, Tavares W, Ferreira L, Pereira AI. 2018. Hydrogen peroxide for insect and algae control in a lettuce hydroponic environment, *Journal of Agricultural Science* 10: 221.
- Campbell BM, Thornton P, Zougmore R, van Asten P, Lipper L. 2014. Sustainable intensification: What is its role in climate smart agriculture? *Current Opinion in Environmental Sustainability*, 8: 39-43.
- Carol RJ, Dolan L. 2006. The role of reactive oxygen species in cell growth: Lessons from root hairs. *Journal of Experimental Botany*, 57: 1829-1834.
- Cassman KG. 1999. Ecological intensification of cereal production systems: Yield potential, soil quality, and precision agriculture. *Proceedings of the National Academy of Sciences*, 96: 5952-5959
- Cassman KG, Dobermann A, Walters DT, Yang H. 2003. Meeting cereal demand while protecting natural resources and improving environmental quality. *Annual Review of Environment and Resources*, 28: 315-358.
- Ceclu L, Oana-Viorela N. 2020. Red beetroot: Composition and health effects-A Review. *Journal of Nutritional Medicine and Diet Care*, 5.
- Chagvardieff P, d'Aletto T, André M. 1994. Specific effects of irradiance and CO₂ concentration doublings on productivity and mineral content in lettuce. *Advances in Space Research*, 14: 269-275.

- Chen YE, Cui JM, Su YQ, Yuan S, Yuan M, Zhang HY. 2015. Influence of stripe rust infection on the photosynthetic characteristics and antioxidant system of susceptible and resistant wheat cultivars at the adult plant stage. *Frontiers in Plant Science*, 6: 779.
- Chérif M, Tirilly Y, Bélanger RR. 1997. Effect of oxygen concentration on plant growth, lipidperoxidation, and receptivity of tomato roots to *Pythium* F. under hydroponic conditions. *European Journal of Plant Pathology*, 103: 255-264.
- Chhikara N, Kushwaha K, Sharma P, Gat Y, Panghal A. 2019. Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry*, 272: 192-200.
- Ching TM. 1959. Activation of germination in Douglas fir seed by hydrogen peroxide. *Plant Physiology*, 34: 557-563.
- Choudhary A, Sultana R, Pratap A, Nadarajan N, Jha UC. 2011. Breeding for abiotic stresses in pigeon pea. *Journal of Food Legumes*, 24: 165-174.
- Cirera X, Masset E. 2010. Income distribution trends and future food demand. *Philosophical transactions of the Royal Society B: Biological sciences*, 365: 2821-2834.
- Clifford T, Howatson G, West DJ, Stevenson EJ. 2015. The potential benefits of red beetroot supplementation in health and disease. *Nutrients*, 7: 2801-2822.
- Cortés J, Mahecha MD, Reichstein M, Myneni RB, Chen C, Brenning A. 2021. Where are global vegetation greening and browning trends significant? *Geophysical Research Letters*, 48: e2020GL091496.
- Corwin DL, Scudiero E. 2019. Chapter One - Review of soil salinity assessment for agriculture across multiple scales using proximal and/or remote sensors. In: Sparks DL editor. *Advances in Agronomy*: Academic Press. p. 1-130.
- Cramer MD. 2002. Inorganic carbon utilization by root systems. *Plant Roots*: CRC Press. p. 1094-1119.
- Creamer N, Baldwin K. 2019. Summer cover crops in: University NCS editor. NC State Extension Publications.
- Dat J, Vandenabeele S, Vranova E, Van Montagu M, Van Breusegem F. 2000. Dual action of the active oxygen species during plant stress responses. *Cellular and Molecular Life Sciences CMLS*, 57: 779-795.
- De Datta SK. 1981. Principles and practices of rice production. Wiley-Interscience Productions, New York, USA. *Environmental Change*, 63: 102100.

- De Graaf MA, Van Groenigen KJ, Six J, Hungate B, van Kessel C. 2006. Interactions between plant growth and soil nutrient cycling under elevated CO₂: A meta-analysis. *Global Change Biology*, 12: 2077-2091.
- Deng J, Harrison MT, Liu K, Ye J, Xiong X, Fahad S, Huang L, Tian X, Zhang Y. 2022. Integrated crop management practices improve grain yield and resource use efficiency of super hybrid rice. *Frontiers in Plant Science*, 13.
- Deng XP, Cheng YJ, Wu XB, Kwak SS, Chen W, Eneji AE. 2012a. Exogenous hydrogen peroxide positively influences root growth and metabolism in leaves of sweet potato seedlings. *Australian Journal of Crop Science*, 6: 1572-1578.
- Deng XP, Cheng YJ, Wu XB, Kwak SS, Chen W, Eneji AE. 2012b. Exogenous hydrogen peroxide positively influences root growth and exogenous hydrogen peroxide positively influences root growth and metabolism in leaves of sweet potato seedlings. *Australian Journal of Crop Science*, 6: 1572.
- Devlet A. 2021. Crop production and yield limiting factors. *MAS Journal of Applied Sciences*, 7.
- Diatta AA, Thomason WE, Abaye O, Thompson TL, Battaglia ML, Vaughan LJ, Lo M. 2020. Assessment of nitrogen fixation by mungbean genotypes in different soil textures using 15N natural abundance method. *Journal of Soil Science and Plant Nutrition*, 20: 2230-2240.
- Dilworth LL, Riley CK, Stennett DK. 2017. Chapter 5 - Plant constituents: Carbohydrates, oils, resins, balsams, and plant hormones. In: Badal S, Delgoda R editors. *Pharmacognosy*. Boston: Academic Press. p. 61-80.
- Dong J, Gruda N, Lam SK, Li X, Duan Z. 2018. Effects of elevated CO₂ on nutritional quality of vegetables: a review. *Frontiers in Plant Science*, 9: 924.
- Dóra S, Mónika M. 2022. Red beetroot (*Beta vulgaris* L.). In: Dr. Prashant K editor. *Root Vegetables*. Rijeka: IntechOpen. p. Ch. 1.
- Dorais M. 2003. The use of supplemental lighting for vegetable crop production: Light intensity, crop response, nutrition, crop management, cultural practices.
- Edesi L, Järvan M, Noormets M, Lauringson E, Adamson A, Akk E. 2012. The importance of solid cattle manure application on soil microorganisms in organic and conventional cultivation. *Acta Agriculturae Scandinavica, Section B — Soil and Plant Science*, 62: 583-594.
- Eliazer Nelson ARL, Ravichandran K, Antony U. 2019. The impact of the green revolution on indigenous crops of India. *Journal of Ethnic Foods*, 6: 8.
- Esmeijer M (ed). 1999. *CO₂ in greenhouse horticulture*. Applied Plant Research.

- Evans LT. 1993. *Crop evolution, adaptation, and yield*. Cambridge; Cambridge University Press.
- Evenson RE, Gollin D. 2003. Assessing the impact of the green evolution, 1960 to 2000. *Science*, 300: 758-762.
- Faber M, Van Jaarsveld PJ, Laubscher R. 2007. The contribution of dark-green leafy vegetables to total micronutrient intake of two-to five-year-old children in a rural setting. *Water SA*, 33: 407-412.
- Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S. 2017. Crop production under drought and heat stress: Plant responses and management options. *Frontiers in Plant Science*: 1147.
- FAO 2020. Land use in agriculture by the numbers. FAO Statistics Division website.
- FAO and DWFI. 2015. Yield gap analysis of field crops – Methods and case studies, by Sadras VO, Cassman KGG, Grassini P, Hall AJ, Bastiaanssen WGM, Laborte AG, Milne AE, Sileshi G, Steduto P. FAO Water Reports No. 41, Rome, Italy
- FAO, 2018. FAOSTAT statistics database, Crops
- Farquhar GD, Sharkey TD. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, 33: 317-345.
- Farquhar GD, Von Caemmerer Sv, Berry JA. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta*, 149: 78-90.
- Fitzgerald T, Kuwayama Y, Olmstead S, Thompson A. 2020. Dynamic impacts of U.S. energy development on agricultural land use. *Energy Policy*, 137: 111163.; FAO 2021.
- Flexas J. 2016. Genetic improvement of leaf photosynthesis and intrinsic water use efficiency in C₃ plants: Why so much little success? *Plant science*, 251: 155-161.
- Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, Mueller ND, O'Connell C, Ray DK, West PC. 2011. Solutions for a cultivated planet. *Nature*, 478: 337-342.
- Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD *et al.* 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature*, 422: 442-446.
- Gao J, Han X, Seneweera S, Ping L, Zong Y, Dong Q, Lin E, Hao X. 2015. Leaf photosynthesis and yield components of mung bean under fully open-air elevated [CO₂]. *Journal of Integrative Agriculture*, 14: 977-983.
- Gao Y, Guo YK, Lin SH, Fang YY, Bai JG. 2010. Hydrogen peroxide pre-treatment alters the activity of antioxidant enzymes and protects chloroplast ultrastructure in heat-stressed cucumber leaves. *Scientia Horticulturae*, 126: 20-26.

- Gao ZJ, Han XH, Xiao XG. 2009. Purification and characterisation of polyphenol oxidase from red Swiss chard (*Beta vulgaris subspecies cicla*) leaves. *Food Chemistry*, 117: 342-348.
- Gaudin AC, McClymont SA, Holmes BM, Lyons E, Raizada MN. 2011. Novel temporal, fine-scale and growth variation phenotypes in roots of adult-stage maize (*Zea mays* L.) in response to low nitrogen stress. *Plant, cell and environment*, 34: 2122-2137.
- Ge XM, Cai HL, Lei X, Zhou X, Yue M, He JM. 2015. Heterotrimeric G protein mediates ethylene-induced stomatal closure via hydrogen peroxide synthesis in *Arabidopsis*. *The Plant Journal*, 82: 138-150.
- Gechev TS, Hille J. 2005. Hydrogen peroxide as a signal controlling plant programmed cell death. *Journal of Cell Biology*, 168: 17-20.
- Gibbs HK, Ruesch AS, Achard F, Clayton MK, Holmgren P, Ramankutty N, Foley JA. 2010. Tropical forests were the primary sources of new agricultural land in the 1980s and 1990s. *Proceedings of the National Academy of Sciences*, 107: 16732-16737.
- Gil PM, Ferreyra R, Barrera C, Zúñiga C, Gurovich L. 2009. Effect of injecting hydrogen peroxide into heavy clay loam soil on plant water status, net CO₂ assimilation, biomass, and vascular anatomy of avocado trees. *Chilean Journal of Agricultural Research*, 69: 97-106.
- Gill HK, Garg H. 2014. Pesticide: Environmental impacts and management strategies. *Pesticides-toxic aspects*, 8: 187.
- Gislerød HR, Nelson P. 1989. The interaction of relative air humidity and carbon dioxide enrichment in the growth of *Chrysanthemum× morifolium* Ramat. *Scientia Horticulturae*, 38: 305-313.
- Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C. 2010. Food security: The challenge of feeding 9 billion people. *Science*, 327: 812-818.
- Gosling SN, Arnell NW. 2016. A global assessment of the impact of climate change on water scarcity. *Climatic Change*, 134: 371-385.
- Grafiadellis I, Mattas K, Maloupa E, Tzouramani I, Galanopoulos K. 2000. An economic analysis of soilless culture in gerbera production. *HortScience*, 35: 300-303.
- Green RE, Cornell SJ, Scharlemann JP, Balmford A. 2005. Farming and the fate of wild nature. *Science*, 307: 550-555.
- Gross K, Rosenheim JA. 2011. Quantifying secondary pest outbreaks in cotton and their monetary cost with causal-inference statistics. *Ecological Applications*, 21: 2770-2780.

- Gruda NS, Bisbis M, Tanny J. 2019a. Impacts of protected vegetable cultivation on climate change and adaptation strategies for cleaner production – A review. *Journal of Cleaner Production*.
- Gruda NS, Bisbis M, Tanny J. 2019b. Influence of climate change on protected cultivation: Impacts and sustainable adaptation strategies - A review. *Journal of Cleaner Production*, 225: 481-495.
- Guldiken B, Toydemir G, Nur Memis K, Okur S, Boyacioglu D, Capanoglu E. 2016. Home-processed red beetroot (*Beta vulgaris* L.) products: Changes in antioxidant properties and bioaccessibility. *International Journal of Molecular Sciences*, 17: 858.
- Guzel S, Terzi R. 2013. Exogenous hydrogen peroxide increases dry matter production, mineral content and level of osmotic solutes in young maize leaves and alleviates deleterious effects of copper stress. *Botanical Studies*, 54: 26.
- Harrison MT, Cullen BR, Mayberry DE, Cowie AL, Bilotto F, Badgery WB, Liu K, Davison T, Christie KM, Muleke A. 2021. Carbon myopia: The urgent need for integrated social, economic and environmental action in the livestock sector. *Global Change Biology*, 27: 5726-5761.
- Harrison MT, Evans JR, Dove H, Moore AD. 2011. Recovery dynamics of rainfed winter wheat after livestock grazing 2. Light interception, radiation-use efficiency and dry-matter partitioning. *Crop and Pasture Science*, 62: 960-971.
- Harrison MT, Evans JR, Moore AD. 2012a. Using a mathematical framework to examine physiological changes in winter wheat after livestock grazing: 1. Model derivation and coefficient calibration. *Field Crops Research*, 136: 116-126.
- Harrison MT, Evans JR, Moore AD. 2012b. Using a mathematical framework to examine physiological changes in winter wheat after livestock grazing: 2. Model validation and effects of grazing management. *Field Crops Research*, 136: 127-137.
- Hasan SA, Irfan M, Masrahi YS, Khalaf MA, Hayat S. 2016. Growth, photosynthesis, and antioxidant responses of *Vigna unguiculata* L. treated with hydrogen peroxide. *Cogent Food and Agriculture*, 2: 1155331.
- Hasegawa T, Sakai H, Tokida T, Nakamura H, Zhu C, Usui Y, Yoshimoto M, Fukuoka M, Wakatsuki H, Katayanagi N. 2013. Rice cultivar responses to elevated CO₂ at two free-air CO₂ enrichment (FACE) sites in Japan. *Functional Plant Biology*, 40: 148-159.
- He J, Austin P, Nichols M, Lee SK. 2004. Effect of root-zone (RZ) CO₂ on productivity and photosynthesis in aeroponically grown lettuce plants, *Acta Horticulturae*, 648: 39–45.

- He J, Austin PT, Nichols MA, Lee SK. 2007. Elevated root-zone CO₂ protects lettuce plants from midday depression of photosynthesis. *Environmental and Experimental Botany*, 61: 94-101.
- He J, Qin L, Lee SK. Effects of Elevated Root-Zone CO₂ and Root-Zone Temperature on Productivity and Photosynthesis of Aeroponically Grown Lettuce Plants. In: Kuang T, Lu C, Zhang L (eds), 2013// 2013, Berlin, Heidelberg: Springer Berlin Heidelberg. pp. 707-711.
- He L, Gao Z. 2009. Pre-treatment of seed with H₂O₂ enhances drought tolerance of wheat (*Triticum aestivum* L.) seedlings. *African Journal of Biotechnology*, 8: 6151-6157.
- Hernández-Barrera A, Velarde-Buendía A, Zepeda I, Sanchez F, Quinto C, Sánchez-Lopez R, Cheung AY, Wu HM, Cardenas L. 2015. Hyper, a hydrogen peroxide sensor, indicates the sensitivity of the Arabidopsis root elongation zone to aluminium treatment. *Sensors*, 15: 855-867.
- Hochmuth GJ, Hochmuth RC. 2001. Nutrient solution formulation for hydroponic (perlite, rockwool, NFT) tomatoes in Florida. *HS796. University of Florida* 1-10.
- Hocking P, Meyer C. 1991. Effects of CO₂ enrichment and nitrogen stress on growth, and partitioning of dry matter and nitrogen in wheat and maize. *Functional Plant Biology*, 18: 339-356.
- Hossain MA, Bhattacharjee S, Armin SM, Qian P, Xin W, Li HY, Burritt DJ, Fujita M, Tran LSP. 2015. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: Insights from ROS detoxification and scavenging. *Frontiers in Plant Science*, 6.
- Hounsborne N, Hounsborne B, Tomos D, Edwards-Jones G. 2008. Plant metabolites and nutritional quality of vegetables. *Journal of Food Science*, 73: R48-R65.
- Hu Y, Ge Y, Zhang C, Ju T, Cheng W. 2009. Cadmium toxicity and translocation in rice seedlings are reduced by hydrogen peroxide pre-treatment. *Plant Growth Regulation*, 59: 51-61.
- Hu Y, Zhong S, Zhang M, Liang Y, Gong G, Chang X, Tan F, Yang H, Qiu X, Luo L. 2020. Potential role of photosynthesis in the regulation of reactive oxygen species and defence responses to *Blumeria graminis* f. sp. *tritici* in wheat. *International Journal of Molecular Sciences*, 21: 5767.
- Huang B, Gamm U, Jonas S, Khokha M, Choma M. 2015. Quantitative optical coherence tomography imaging of intermediate flow defect phenotypes in ciliary physiology and pathophysiology. *Journal of Biomedical Optics*, 20: 030502.

- Hulme MF, Vickery JA, Green RE, Phalan B, Chamberlain DE, Pomeroy DE, Nalwanga D, Mushabe D, Katebaka R, Bolwig S. 2013. Conserving the birds of Uganda's banana-coffee arc: Land sparing and land sharing compared. *PLOS One*, 8: e54597.
- Huss CP, Holmes KD, Blubaugh CK. 2022. Benefits and risks of intercropping for crop resilience and pest management. *Journal of Economic Entomology*, 115: 1350-1362.
- Imai K, Coleman, Yanagisawa. 1985. Increase in atmospheric partial pressure of carbon dioxide and growth and yield of rice (*Oryza Sativa* L.). *Japanese Journal of Crop Science*, 54: 413-418.
- Imai K, Murata Y. 1979. Effect of carbon dioxide concentration on growth and dry matter production of crop plants: VII. Influence of light intensity and temperature on the effect of carbon dioxide-enrichment in some C3-and C4-species. *Japanese Journal of Crop Science*, 48: 409-417.
- IPCC. 2014. Mitigation of climate change. Contribution of working group III to the fifth assessment report of the intergovernmental panel on climate change.
- Irmak S. 2008. Plant growth and yield as affected by wet soil conditions due to flooding or over-irrigation. *Institute of Agriculture and Natural Resources, Nebraska Extension Publications*
- Ishibashi Y, Yamaguchi H, Yuasa T, Iwaya-Inoue M, Arima S, Zheng SH. 2011. Hydrogen peroxide spraying alleviates drought stress in soybean plants. *Journal of Plant Physiology*, 168: 1562-1567.
- Jablonski LM, Wang X, Curtis PS. 2002. Plant reproduction under elevated CO₂ conditions: A meta-analysis of reports on 79 crop and wild species. *New Phytologist*, 156: 9-26.
- Jackson MB.1979. Aeration in the nutrient film technique of glasshouse crop production and the importance of oxygen, ethylene and carbon dioxide. *S Horticulturae*, 98: 61-78.
- Jiang YP, Cheng F, Zhou YH, Xia XJ, Mao WH, Shi K, Chen ZX, Yu JQ. 2012. Hydrogen peroxide functions as a secondary messenger for brassinosteroids-induced CO₂ assimilation and carbohydrate metabolism in *Cucumis sativus*. *Journal of Zhejiang University Science B*, 13: 811-823.
- Johnston M, Licker R, Foley J, Holloway T, Mueller N, Barford C, Kucharik C. 2011. Closing the gap: Global potential for increasing biofuel production through agricultural intensification. *Environmental Research Letters*, 6: 034028.

- Kaiser E, Morales A, Harbinson J, Kromdijk J, Heuvelink E, Marcelis LF. 2015. Dynamic photosynthesis in different environmental conditions. *Journal of Experimental Botany*, 66: 2415-2426.
- Kant S, Seneweera S, Rodin J, Materne M, Burch D, Rothstein S, Spangenberg G. 2012. Improving yield potential in crops under elevated CO₂: Integrating the photosynthetic and nitrogen utilization efficiencies. *Frontiers in Plant Science*, 3.
- Kao CH. 2014. Role of hydrogen peroxide in rice plants. *Crop, Environment and Bioinformatics*, 11: 1-10.
- Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P. 1999. Systemic signalling and acclimation in response to excess excitation energy in Arabidopsis. *Science*, 284: 654-657.
- Khandaker MM, Boyce AN, Osman N. 2012. The influence of hydrogen peroxide on the growth, development and quality of wax apple (*Syzygium samarangense*, [Blume] Merrill and LM Perry var. *jambu madu*) fruits. *Plant Physiology and Biochemistry*, 53: 101-110.
- Kiaya V. 2014. Post-harvest losses and strategies to reduce them. *Technical Paper on Postharvest Losses, Action Contre la Faim (ACF)*, 25: 1-25.
- Kopittke PM, Menzies NW, Wang P, McKenna BA, Lombi E. 2019. Soil and the intensification of agriculture for global food security. *Environment International*, 132: 105078.
- Koyama M, Nakamura C, Nakamura K. 2013. Changes in phenols contents from buckwheat sprouts during growth stage. *Journal of Food Science and Technology*, 50: 86-93.
- Kratky BA. 2004. A suspended pot, non-circulating hydroponic method: International Society for Horticultural Science (ISHS), Leuven, Belgium. pp. 83-89.
- Kretschmer M, Damoo D, Djamei A, Kronstad J. 2019. Chloroplasts and plant immunity: Where are the fungal effectors? *Pathogens*, 9: 19.
- Kumar M, Sirhindi G, Bhardwaj R, Kumar S, Jain G. 2010. Effect of exogenous H₂O₂ on antioxidant enzymes of *Brassica juncea* L. seedlings in relation to 24-epibrassinolide under chilling stress. *Indian Journal of Biochemistry and Biophysics*, 47: 378-382.
- Kumar RR, Goswami S, Singh K, Rai GK, Rai RD. 2013. Modulation of redox signal transduction in plant system through induction of free radical/ROS scavenging redox-sensitive enzymes and metabolites. *Australian Journal of Crop Science*, 7: 1744-1751.
- Lawson T, Blatt MR. 2014. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant physiology*, 164: 1556-1570.

- LeBlanc BD, Sheffield RE, Kruse J, Karen EN. 2011. Environmental best management practices in agronomic crops (soybeans, cotton, wheat, corn and feed grains). LSU AgCenter Communications, Louisiana State University Agricultural Center.
- Lee RD, Gaskin JW, Schomberg H, Hawkins GL, Harris GH, Barentine RM, Bellows B. 2016. Success with cover crops. *The University of Georgia, College of Agricultural and Environmental Sciences, Family and Consumer Sciences*.
- Li J, Jia H. 2013. Hydrogen peroxide is involved in cGMP modulating the lateral root development of *Arabidopsis thaliana*. *Plant Signalling and Behaviour*, 8.
- Li J, Qiu Z, Zhang XW, Wang L. 2011. Exogenous hydrogen peroxide can enhance tolerance of wheat seedlings to salt stress. *Acta Physiologiae Plantarum*, 33: 835-842.
- Liao W, Xiao H, Zhang M. 2009. Role and relationship of nitric oxide and hydrogen peroxide in adventitious root development of marigold. *Acta Physiologiae Plantarum*, 31: 1279-1289.
- Liao WB, Huang GB, Yu JH, Zhang ML. 2012. Nitric oxide and hydrogen peroxide alleviate drought stress in marigold explants and promote its adventitious root development. *Plant Physiology and Biochemistry*, 58: 6-15.
- Licker R, Johnston M, Foley JA, Barford C, Kucharik CJ, Monfreda C, Ramankutty N. 2010. Mind the gap: How do climate and agricultural management explain the 'yield gap' of croplands around the world? *Global ecology and biogeography*, 19: 769-782.
- Lin CC, Kao CH. 2001. Cell wall peroxidase activity, hydrogen peroxide level and NaCl-inhibited root growth of rice seedlings. *Plant and Soil*, 230: 135-143.
- Lin J, Jach M, Ceulemans R. 2001. Stomatal density and needle anatomy of Scots pine (*Pinus sylvestris*) are affected by elevated CO₂. *New Phytologist*, 150: 665-674.
- Liu K, Harrison MT, Hunt J, Angessa TT, Meinke H, Li C, Tian X, Zhou M. 2020a. Identifying optimal sowing and flowering periods for barley in Australia: A modelling approach. *Agricultural and Forest Meteorology*, 282: 107871.
- Liu Y, Wan Q, Wu R, Wang X, Wang H, Wang Z, Shi C, Bi Y. 2012. Role of hydrogen peroxide in regulating glucose-6-phosphate dehydrogenase activity under salt stress. *Biologia plantarum*, 56: 313-320.
- Long SP, Ainsworth EA, Leakey AD, Morgan PB. 2005. Global food insecurity. Treatment of major food crops with elevated carbon dioxide or ozone under large-scale fully open-air conditions suggests recent models may have overestimated future yields. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360: 2011-2020.

- Long SP, Ainsworth EA, Leakey ADB, Nösberger J, Ort DR. 2006a. Food for thought: Lower-than-expected crop yield stimulation with rising CO₂ concentrations. *Science*, 312: 1918-1921.
- Long SP, Ainsworth EA, Rogers A, Ort DR. 2004. Rising atmospheric carbon dioxide: Plants FACE the future. *Annual Review of Plant Biology*, 55: 591-628.
- Long SP, Zhu X, Naildu SL, ORT DR. 2006b. Can improvement in photosynthesis increase crop yields? *Plant, Cell and Environment*, 29: 315-330.
- Luo Y, Hui D, Zhang D. 2006. Elevated CO₂ stimulates net accumulations of carbon and nitrogen in land ecosystems: A meta-analysis. *Ecology*, 87: 53-63.
- Ma F, Wang L, Li J, Samma MK, Xie Y, Wang R, Wang J, Zhang J, Shen W. 2014. Interaction between HY1 and H₂O₂ in auxin-induced lateral root formation in *Arabidopsis*. *Plant Molecular Biology*, 85: 49-61.
- Małolepsza U, Różalska S. 2005. Nitric oxide and hydrogen peroxide in tomato resistance: Nitric oxide modulates hydrogen peroxide level in o-hydroxyethylrutin-induced resistance to *Botrytis cinerea* in tomato. *Plant Physiology and Biochemistry*, 43: 623-635.
- Massee I. 1913. The sterilisation of seed. *Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew)*, 1913: 183-187.
- Maswada H, Abd El-Rahman LA. 2014. Inducing salinity tolerance in wheat plants by hydrogen peroxide and lithovit" a nano-CaCO₃ fertilizer. *Journal of Agricultural Research. Kafr El-Sheikh Univ*, 40: 696-719.
- Matson PA, Vitousek PM. 2006. Agricultural intensification: Will land spared from farming be land spared for nature? *Conservation Biology*, 20: 709-710.
- Medlyn BE, Barton C, Broadmeadow M, Ceulemans R, De Angelis P, Forstreuter M, Freeman M, Jackson S, Kellomäki S, Laitat E. 2001. Stomatal conductance of forest species after long-term exposure to elevated CO₂ concentration: A synthesis. *New Phytologist*, 149: 247-264.
- Menalled F, Bass T, Buschena D, Cash D, Malone M, Maxwell B, McVay K, Miller P, Soto R, Weaver D. 2008. An introduction to the principles and practices of sustainable farming. *Montana State University*: 1-4.
- Mittler R. 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science*, 11: 15-19.
- Morimoto T, Masuda T, Nonami H. 1989. Oxygen enrichment in deep hydroponic culture improves growth of spinach. *Environment Control in Biology*, 27: 97-102.
- Mortensen LM, Gislørød HR. 1990. Effects of air humidity and supplementary lighting on foliage plants. *Scientia Horticulturae*, 44: 301-308.

- Mortensen LM. 1994. Effects of elevated CO₂ concentrations on growth and yield of eight vegetable species in a cool climate. *Scientia Horticulturae*, 58: 177-185.
- Mousavi SR, Eskandari H. 2011. A general overview on intercropping and its advantages in sustainable agriculture. *Journal of Applied Environmental and Biological Sciences*, 1: 482-486.
- Mueller ND, Gerber JS, Johnston M, Ray DK, Ramankutty N, Foley JA. 2012. Closing yield gaps through nutrient and water management. *Nature*, 490: 254-257.
- Murphy-Bokern D, Stoddard FL, Watson CA. 2017. *Legumes in cropping systems*. CABI.
- Nazir F, Fariduddin Q, Khan TA. 2020. Hydrogen peroxide as a signalling molecule in plants and its crosstalk with other plant growth regulators under heavy metal stress. *Chemosphere*, 252: 126486.
- Nazir F, Hussain A, Fariduddin Q. 2019. Hydrogen peroxide modulate photosynthesis and antioxidant systems in tomato (*Solanum lycopersicum* L.) plants under copper stress. *Chemosphere*, 230: 544-558.
- Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT. 2002. Hydrogen peroxide and nitric oxide as signalling molecules in plants. *Journal of Experimental Botany*, 53: 1237-1247.
- Nejad RA, Ismaili A. 2014. Changes in growth, essential oil yield and composition of geranium (*Pelargonium graveolens* L.) as affected by growing media. *Journal of the Science of Food and Agriculture*, 94: 905-910.
- Nellemann C. 2009. *The environmental food crisis: The environment's role in averting future food crises: A UNEP rapid response assessment*. UNEP/Earthprint.
- Nie M, Lu M, Bell J, Raut S, Pendall E. 2013. Altered root traits due to elevated CO₂: A meta-analysis. *Global Ecology and Biogeography*, 22: 1095-1105.
- Niu L, Liao W. 2016. Hydrogen peroxide signalling in plant development and abiotic responses: Crosstalk with nitric oxide and calcium. *Frontiers in Plant Science*, 7.
- NOAA. 2022. Carbon dioxide now more than 50% higher than pre-industrial levels. Available at <https://www.noaa.gov/news-release/carbon-dioxide-now-more-than-50-higher-than-pre-industrial-levels>.
- Norby RJ, Gunderson CA, Wullschlegel SD, O'Neill EG, McCracken MK. 1992. Productivity and compensatory responses of yellow-poplar trees in elevated CO₂. *Nature*, 357: 322-324.
- Noya I, González-García S, Bacenetti J, Fiala M, Moreira MT. 2018. Environmental impacts of the cultivation-phase associated with agricultural crops for feed production. *Journal of Cleaner Production*, 172: 3721-3733.

- Nuißl H, Siedentop S. 2021. Urbanisation and land use change. In: Weith T, Barkmann T, Gaasch N, Rogga S, Strauß C, Zscheischler J editors. *Sustainable Land Management in a European Context: A Co-Design Approach*. Cham: Springer International Publishing. p. 75-99.
- OECD (Organisation for Economic Co-operation and Development) and FAO. 2021. OECD-FAO agricultural outlook 2021-2030. Paris, OECD Publishing. <https://doi.org/10.1787/19428846-en>
- Oldfield EE, Bradford MA, Wood SA. 2019. Global meta-analysis of the relationship between soil organic matter and crop yields. *Soil*, 5: 15-32.
- Oquist K, Strock JS, Mulla D. 2007. Influence of alternative and conventional farming practices on subsurface drainage and water quality. *Journal of Environmental Quality*, 36: 1194-1204.
- Orabi SA, Dawood MG, Salman S. 2015. Comparative study between the physiological role of hydrogen peroxide and salicylic acid in alleviating the harmful effect of low temperature on tomato plants grown under sand-ponic culture. *Journal of Scientific Agriculture*, 9: 49-59.
- Orozco-Cárdenas ML, Narváez-Vásquez J, Ryan CA. 2001. Hydrogen peroxide acts as a second messenger for the induction of defence genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *The Plant Cell*, 13: 179-191.
- Osman K, Al-Humaid A, Al-Rehiyani S, Al-Redhaiman K. 2010. Monitoring of pesticide residues in vegetables marketed in Al-Qassim region, Saudi Arabia. *Ecotoxicology and Environmental Safety*, 73: 1433-1439.
- Ota R, Ohkubo Y, Yamashita Y, Ogawa-Ohnishi M, Matsubayashi Y. 2020. Shoot-to-root mobile CEPD-like 2 integrates shoot nitrogen status to systemically regulate nitrate uptake in Arabidopsis. *Nature communications*, 11: 1-9.
- Owusu PA, Asumadu-Sarkodie S. 2016. A review of renewable energy sources, sustainability issues and climate change mitigation. *Cogent Engineering*, 3: 1167990.
- PAN Europe. 2013. Contribution on research needs for 2014-2020: Crop rotation – The forgotten practice in European Agriculture. Position paper.
- Parajuli R, Thoma G, Matlock MD. 2019. Environmental sustainability of fruit and vegetable production supply chains in the face of climate change: A review. *Science of The Total Environment*, 650: 2863-2879.
- Pardey PG, Beddow JM, Hurley TM, Beatty TKM, Eidman VR. (2014). A bounds analysis of world food futures: Global agriculture through to 2050. *Australian Journal of Agricultural and Resource Economics* 58, 571–589.

- Parry MAJ, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu XG, Price GD, Condon AG, Furbank RT. 2010. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. *Journal of experimental botany*, 62: 453-467.
- Paul MJ. 2021. Improving photosynthetic metabolism for crop yields: What is going to work? *Frontiers in Plant Science*, 12.
- Peel JR, Mandujano Sánchez MC, López Portillo J, Golubov J. 2017. Stomatal density, leaf area and plant size variation of *Rhizophora mangle* (Malpighiales: Rhizophoraceae) along a salinity gradient in the Mexican Caribbean. *Revista de Biología Tropical*, 65: 701-712.
- Pellegrini P, Fernández RJ. 2018. Crop intensification, land use, and on-farm energy-use efficiency during the worldwide spread of the green revolution. *Proceedings of the National Academy of Sciences*, 115: 2335-2340.
- Pfannschmidt T, Yang C. 2012. The hidden function of photosynthesis: a sensing system for environmental conditions that regulates plant acclimation responses. *Protoplasma*, 249: 125-136.
- Phalan B, Balmford A, Green RE, Scharlemann JP. 2011a. Minimising the harm to biodiversity of producing more food globally. *Food Policy*, 36: S62-S71.
- Phalan B, Onial M, Balmford A, Green RE. 2011b. Reconciling food production and biodiversity conservation: Land sharing and land sparing compared. *Science*, 333: 1289-1291.
- Phelan DC, Harrison MT, Kemmerer EP, Parsons D. 2015. Management opportunities for boosting productivity of cool-temperate dairy farms under climate change. *Agricultural Systems*, 138: 46-54.
- Poorter H, Böhler J, van Dusschoten D, Climent J, Postma JA. 2012. Pot size matters: A meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology*, 39: 839-850.
- Prokopy LS, Floress K, Klotthor-Weinkauff D, Baumgart-Getz A. 2008. Determinants of agricultural best management practice adoption: Evidence from the literature. *Journal of Soil and Water Conservation*, 63: 300-311.
- Pyo YH, Lee TC, Logendra L, Rosen RT. 2004. Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris subspecies cicla*) extracts. *Food Chemistry*, 85: 19-26.
- Qaim M. 2017. Globalisation of agrifood systems and sustainable nutrition. *Proceedings of the Nutrition Society*, 76: 12-21.

- Quan LJ, Zhang B, Shi WW, Li HY. 2008. Hydrogen peroxide in plants: A versatile molecule of the reactive oxygen species network. *Journal of Integrative Plant Biology*, 50: 2-18.
- Rackham RL, McNeilan RA. 1999. *Cover crops for home gardens*. Oregon State University Extension Service.
- Rajashekar C, Baek KH. 2014. Hydrogen peroxide alleviates hypoxia during imbibition and germination of bean seeds (*Phaseolus vulgaris* L.). *American Journal of Plant Sciences*, 5: 3572.
- Rao R, Li Y. 2003. Management of flooding effects on growth of vegetable and selected field crops. *HortTechnology*, 13: 610-616.
- Raudales RE, Parke JL, Guy CL, Fisher PR. 2014. Control of waterborne microbes in irrigation: A review. *Agricultural Water Management*, 143: 9-28.
- Raviv M, Lieth JH, Raviv M. 2008. Significance of soilless culture in agriculture. *Soilless culture theory and practice*: 1-11.
- Razem FA. 2008. An overview of hydrogen peroxide production and cellular determination in plants. *Hebron University Res. J*, 3: 84-96.
- Reed ST, D'Ambrosio FR, Li Y, Rao R. 2005. Flooding influences on growth and development of bush bean under greenhouse conditions. *Journal of vegetable science*, 11: 43-56.
- Renard D, Tilman D. 2019. National food production stabilized by crop diversity. *Nature*, 571: 257-260.
- Resh HM and Howard M .2012. *Hydroponic food production: A definitive guidebook for the advanced home gardener and the commercial hydroponic grower*. CRC Press, Santa Barbara, California.
- Reyes-Fox M, Steltzer H, Trlica MJ, McMaster GS, Andales AA, LeCain DR, Morgan JA. 2014. Elevated CO₂ further lengthens growing season under warming conditions. *Nature*, 510: 259-262.
- Riedell W, Pikul J, Carpenter-Boggs L. 2022. Crop rotation improves soil fertility and plant mineral nutrition. *Soil/Water Research, Progress Report*.
- RIRDC .2001. *Hydroponics as an agricultural production system*, Publication No 01/141, Project No HAS-9A, Rural Industries Research and Development Corporation (RIRDC), Australian Government, Kingston, PO Box 4776, ACT2604.
- Rogers H, Dahlman R. 1993. Crop responses to CO₂ enrichment. *Plant Ecology*, 104-105: 117-131.

- Rogers HH, Bingham GE, Cure JD, Smith JM, Surano KA. 1983. Responses of selected plant species to elevated carbon dioxide in the field. *Journal of Environmental Quality*, 12: 569-574.
- Rogers HH, Runion GB, Krupa SV, Prior SA. 1997. Plant responses to atmospheric carbon dioxide enrichment: Implications in root—soil—microbe interactions. *Advances in carbon dioxide effects research*, 61: 1-34.
- Rogers HH, Runion GB, Krupa SV. 1994. Plant responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution*, 83: 155-189.
- Salisbury EJ. 1928. I. On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. *Philosophical Transactions of the Royal Society of London. Series B, Containing Papers of a Biological Character*, 216: 1-65.
- Salsman KJ, Jordan DN, Smith SD, Neuman DS. 1999. Effect of atmospheric CO₂ enrichment on root growth and carbohydrate allocation of *Phaseolus* spp. *International Journal of Plant Sciences*, 160: 1075-1081.
- Savvas D, Gianquinto G, Tuzel Y, Gruda N. 2013. Soilless culture. *FAO plant production and protection paper*, 217: 303-354.
- Savvas D, Patakioutas G, Ntatsi G, Karras G. 2009. Application of some systemic pesticides via the root system in substrate grown crops under conditions of complete nutrient solution recycling. *Acta Horticulturae*: 451-458.
- Scharfe J, Schön H, Weis E. 2005. Photosynthesis and carbohydrate metabolism in tobacco leaves during an incompatible interaction with *Phytophthora nicotianae*. *Plant, Cell and Environment*, 28: 1421-1435.
- Schmidhuber J, Tubiello FN. 2007. Global food security under climate change. *Proceedings of the National Academy of Sciences*, 104: 19703-19708.
- Schmilewski G. 2007. Growing medium constituents used in the EU. pp. 33-46.
- Schonbeck M. 2017. Soil health and organic farming. *Organic Farming Research Foundation: Santa Cruz, CA, USA*.
- Sharkey T, Laporte M, Kruger E. 2000. Will increased photosynthetic efficiency lead to increased yield in rice? *Studies in Plant Science*: Elsevier. p. 73-86.
- Sharma N, Acharya S, Kumar K, Singh N, Chaurasia O. 2019. Hydroponics as an advanced technique for vegetable production: An overview. *Journal of soil and water conservation*, 17: 364-371.
- Sharma P, Singh A, Kahlon C, Brar A, Grover K, Dia M, Steiner R. 2018. The role of cover crops towards sustainable soil health and agriculture - A review paper. *American Journal of Plant Sciences*, 09: 1935-1951.

- Shavrukov Y, Genc Y, Hayes J. 2012. The use of hydroponics in abiotic stress tolerance research. *Hydroponics—a standard methodology for plant biological research. Croatia: InTech*: 39-66.
- Shelton A, Badenes-Pérez F. 2006. Concepts and applications of trap cropping in pest management. *Annual Review of Entomology*, 51: 285-308.
- Shetty NP, Mehrabi R, Lütken H, Haldrup A, Kema GHJ, Collinge DB, Jørgensen HJL. 2007. Role of hydrogen peroxide during the interaction between the hemi-biotrophic fungal pathogen *Septoria tritici* and wheat. *New Phytologist*, 174: 637-647.
- Silver WL, Miya RK. 2001. Global patterns in root decomposition: Comparisons of climate and litter quality effects. *Oecologia*, 129: 407-419.
- Slavin JL, Lloyd B. 2012. Health benefits of fruits and vegetables. *Advances in Nutrition*, 3: 506-516.
- Soffer H, Burger DW. 1988. Effects of dissolved oxygen concentrations in aero-hydroponics on the formation and growth of adventitious roots. *Journal of the American Society for Horticultural Science*, 113: 218-221.
- Suh S, Johnson JA, Tambjerg L, Sim S, Broeckx-Smith S, Reyes W, Chaplin-Kramer R. 2020. Closing yield gap is crucial to avoid potential surge in global carbon emissions. *Global Environmental Change*, 63: 102100.
- Sun Y, Wang H, Liu S, Peng X. 2016. Exogenous application of hydrogen peroxide alleviates drought stress in cucumber seedlings. *South African Journal of Botany*, 106: 23-28.
- Suyantohadi A, Kyoren T, Hariadi M, Purnomo MH, Morimoto T. 2010. Effect of high concentrated dissolved oxygen on the plant growth in a deep hydroponic culture under a low temperature. *IFAC Proceedings Volumes*, 43: 251-255.
- Swarbrick PJ, Schulze-Lefert P, Scholes JD. 2006. Metabolic consequences of susceptibility and resistance (race-specific and broad-spectrum) in barley leaves challenged with powdery mildew. *Plant, cell and environment*, 29: 1061-1076.
- Teng N, Jin B, Wang Q, Hao H, Ceulemans R, Kuang T, Lin J. 2009. No detectable maternal effects of elevated CO₂ on *Arabidopsis thaliana* over 15 generations. *PLOS One*, 4: e6035.
- Terzi R, Kadioglu A, Kalaycioglu E, Saglam A. 2014. Hydrogen peroxide pre-treatment induces osmotic stress tolerance by influencing osmolyte and abscisic acid levels in maize leaves. *Journal of Plant Interactions*, 9: 559-565.
- Thompson SL, Govindasamy B, Mirin A, Caldeira K, Delire C, Milovich J, Wickett M, Erickson D. 2004. Quantifying the effects of CO₂-fertilized vegetation on future global climate and carbon dynamics. *Geophysical Research Letters*, 31.

- Thornton PK, Gerber PJ. 2010. Climate change and the growth of the livestock sector in developing countries. *Mitigation and Adaptation Strategies for Global Change*, 15: 169-184.
- Tian Q, Chen F, Liu J, Zhang F, Mi G. 2008. Inhibition of maize root growth by high nitrate supply is correlated with reduced IAA levels in roots. *Journal of plant physiology*, 165: 942-951.
- Tilman D, Balzer C, Hill J, Befort BL. 2011. Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences*, 108: 20260-20264.
- Tricker PJ, Trewin H, Kull O, Clarkson GJ, Eensalu E, Tallis MJ, Colella A, Doncaster CP, Sabatti M, Taylor G. 2005. Stomatal conductance and not stomatal density determines the long-term reduction in leaf transpiration of poplar in elevated CO₂. *Oecologia*, 143: 652-660.
- Tscharntke T, Clough Y, Wanger TC, Jackson L, Motzke I, Perfecto I, Vandermeer J, Whitbread A. 2012. Global food security, biodiversity conservation and the future of agricultural intensification. *Biological conservation*, 151: 53-59.
- United Nations Department of Economic and Social Affairs (UN DESA), Population Division. 2022. World Population Prospects 2022: Summary of Results. UN DESA/POP/2022/TR/NO. 3.
- United Nations Environment Programme (UNEP). 1998. *Best management practices for agricultural non-point sources of pollution - CEP Technical Report 41*. <https://wedocs.unep.org/20.500.11822/28673>
- USDA 2019. Beans, snap, green, raw. FoodData Central. <https://fdc.nal.usda.gov/fdc-app.html#/food-details/169961/measure>
- Václavík T, Lautenbach S, Kuemmerle T, Seppelt R. 2013. Mapping global land system archetypes. *Global Environmental Change*, 23: 1637-1647.
- Valin H, Sands RD, van der Mensbrugghe D, Nelson GC, Ahammad H, Blanc E, Bodirsky B, Fujimori S, Hasegawa T, Havlik P et al. 2014. The future of food demand: Understanding differences in global economic models. *Agricultural Economics*, 45: 51-67.
- Vamerali T, Ganis A, Bona S, Mosca G. 2003. Roots: the dynamic interface between plants and the earth. *Netherlands: Springer*: 169-177.
- Van der Kooi CJ, Reich M, Löw M, De Kok LJ, Tausz M. 2016. Growth and yield stimulation under elevated CO₂ and drought: A meta-analysis on crops. *Environmental and Experimental Botany*, 122: 150-157.

- Van Ittersum MK, Cassman KG, Grassini P, Wolf J, Titttonell P, Hochman Z. 2013. Yield gap analysis with local to global relevance: A review. *Field Crops Research*, 143: 4-17.
- Van Ittersum MK, Rabbinge R. 1997. Concepts in production ecology for analysis and quantification of agricultural input-output combinations. *Field Crops Research*, 52: 197-208.
- Van Loon MP, Hijbeek R, Ten Berge HF, De Sy V, Ten Broeke GA, Solomon D, van Ittersum MK. 2019. Impacts of intensifying or expanding cereal cropping in sub-Saharan Africa on greenhouse gas emissions and food security. *Global Change Biology*, 25: 3720-3730.
- Van Os EA. 1999. Closed soilless growing systems: A sustainable solution for Dutch greenhouse horticulture. *Water Science and Technology*, 39: 105-112.
- Vanuytrecht E, Raes D, Willems P, Geerts S. 2012. Quantifying field-scale effects of elevated carbon dioxide concentration on crops. *Climate Research*, 54: 35-47.
- Veal EA, Day AM, Morgan BA. 2007. Hydrogen peroxide sensing and signalling. *Molecular Cell*, 26: 1-14.
- Viktor A, Cramer MD. 2003. Variation in root-zone CO₂ concentration modifies isotopic fractionation of carbon and nitrogen in tomato seedlings. *New Phytologist*, 157: 45-54.
- Walters DR, McRoberts N, Fitt BD. 2008. Are green islands red herrings? Significance of green islands in plant interactions with pathogens and pests. *Biological Reviews*, 83: 79-102.
- Wang C, Liu W, Li Q, Ma D, Lu H, Feng W, Xie Y, Zhu Y, Guo T. 2014. Effects of different irrigation and nitrogen regimes on root growth and its correlation with above-ground plant parts in high-yielding wheat under field conditions. *Field Crops Research*, 165: 138-149.
- Wang CH. 2014. Farming methods effects on the soil fertility and crop production under a rice-vegetables cropping sequences. *Journal of Plant Nutrition*, 37: 1498-1513.
- Ware GW. 1980. Effects of pesticides on nontarget organisms. *Residue reviews*: 173-201.
- West TO, Marland G, King AW, Post WM, Jain AK, Andrasko K. 2004. Carbon management response curves: Estimates of temporal soil carbon dynamics. *Environmental Management*, 33: 507-518.
- Wheeler T, von Braun J. 2013. Climate change impacts on global food security. *Science*, 341: 508-513.
- White PA. 2014. The growing business of cover crops. Natural Wildlife Federation.

- Widuri L, Lakitan B, Sodikin E, Hasmeda M, Meihana M, Kartika K, Siaga E. 2018. Shoot and root growth in common bean (*Phaseolus vulgaris* L.) Exposed to Gradual Drought Stress. *AGRIVITA Journal of Agricultural Science*, 40.
- Winjum J, Brown S, Schlamadinger B. 1998. Forest harvests and wood products: Sources and sinks of atmospheric carbon dioxide. *Forest Science*, 44: 272-284.
- Wittwer S. 1978. Carbon dioxide fertilization of crop plants. *Problems in Crop Physiology*. Ed. US Gupta. Haryana Agric. Univ., Hissar, India: 310-333.
- Wittwer SH, Robb WM. 1964. Carbon dioxide enrichment of greenhouse atmospheres for food crop production. *Economic Botany*, 18: 34-56.
- Woodfine A. 2009. The potential of sustainable land management practices for climate change mitigation and adaptation in sub-Saharan Africa. *Rome, Food and Agriculture Organization of the United Nations*.
- Woodward FI. 1987. Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels. *Nature*, 327: 617-618.
- Xiong J, Yang Y, Fu G, Tao L. 2015. Novel roles of hydrogen peroxide (H₂O₂) in regulating pectin synthesis and demethylesterification in the cell wall of rice (*Oryza sativa*) root tips. *New Phytologist*, 206: 118-126.
- Xu Z, Jiang Y, Jia B, Zhou G. 2016. Elevated-CO₂ response of stomata and its dependence on environmental factors. *Frontiers in Plant Science*, 7.
- Zeidler C, Schubert D. 2014. From bioregenerative life support systems for space to vertical farming on earth – The 100% spin-off. Paper presented at the Life in Space for Life on Earth Symposium, Waterloo, Canada.
- Zelinová V, Bočová B, Huttová J, Mistrík I, Tamás L. 2013. Impact of cadmium and hydrogen peroxide on ascorbate-glutathione recycling enzymes in barley root. *Plant, Soil and Environment*, 59: 62-67.
- Zhang X, Chen S, Sun H, Wang Y, Shao L. 2009. Root size, distribution and soil water depletion as affected by cultivars and environmental factors. *Field Crops Research*, 114: 75-83.
- Zhou J, Li B, Xia L, Fan C, Xiong Z. 2019. Organic-substitute strategies reduced carbon and reactive nitrogen footprints and gained net ecosystem economic benefit for intensive vegetable production. *Journal of Cleaner Production*, 225: 984-994.
- Zhou J, Zhao L, Dong Y, Xu J, Bian W, Mao Y, Zhang X. 2010. Nitrogen and transplanting density interactions on the rice yield and N use rate. *Plant Nutrition and Fertilizer Science*, 16: 274-281.
- Zhu XG, Long SP, Ort DR. 2010. Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology*, 61: 235-261.

- Zhu Z, Piao S, Myneni RB, Huang M, Zeng Z, Canadell JG, Ciais P, Sitch S, Friedlingstein P, Arneth A. 2016. Greening of the Earth and its drivers. *Nature Climate Change*, 6: 791-795.

APPENDIX A
Preliminary pot trial

Tables 1 to 7: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the height of beans.

Table 1: The dependent variable of plant height of bean plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	21.1597500	4.2319500	1.06	0.3970
Error	34	135.1600000	3.9752941		
Corrected Total	39	156.3197500			

Table 2: The dependent variable of plant height of bean plants in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	41.0440000	8.2088000	1.70	0.1606
Error	34	163.8920000	4.8203529		
Corrected Total	39	204.9360000			

Table 3: The dependent variable of plant height of bean plants in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	37.8030000	7.5606000	1.28	0.2935
Error	34	200.1770000	5.8875588		
Corrected Total	39	237.9800000			

Table 4: The dependent variable of plant height of bean plants in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	94.2150000	18.8430000	3.55	0.0110
Error	34	180.7090000	5.3149706		
Corrected Total	39	274.9240000			

Table 5: The dependent variable of plant height of bean plants in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	36.99200000	36.99200000	20.33	0.0003
Error	18	32.74800000	1.81933333		
Corrected Total	19	69.74000000			

Table 6: The dependent variable of plant height of bean plants in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	31.50050000	31.50050000	24.33	0.0001
Error	18	23.30500000	1.29472222		
Corrected Total	19	54.80550000			

Table 7: The dependent variable of plant height of bean plants in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	31.00050000	31.00050000	17.26	0.0006
Error	18	32.32900000	1.79605556		
Corrected Total	19	63.32950000			

Tables 8 to 12: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the stomatal density in bean leaves.

Table 8: The dependent variable of the stomatal density in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	104.0000000	20.8000000	4.20	0.0069
Error	24	118.8000000	4.9500000		
Corrected Total	29	222.8000000			

Table 9: The dependent variable of the stomatal density in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	27.8666667	5.5733333	1.53	0.2189
Error	24	87.6000000	3.6500000		
Corrected Total	29	115.4666667			

Table 10: The dependent variable of the stomatal density in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	48.6666667	9.7333333	1.40	0.2596
Error	24	166.8000000	6.9500000		
Corrected Total	29	215.4666667			

Table 11: The dependent variable of the stomatal density in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	33.0666667	6.6133333	1.79	0.1536
Error	24	88.8000000	3.7000000		
Corrected Total	29	121.8666667			

Table 12: The dependent variable of the stomatal density in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3.60000000	3.60000000	1.11	0.3233
Error	8	26.00000000	3.25000000		
Corrected Total	9	29.60000000			

Table 13: The ANOVA table that follow shows whether additional carbon dioxide had an effect on the overall stomatal density.

Table 13: The dependent variable of the overall stomatal density of bean plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	24.17015385	4.83403077	3.07	0.0325
Error	20	31.51600000	1.57580000		
Corrected Total	25	55.68615385			

Table 14: The ANOVA table that follow shows whether additional carbon dioxide had an effect on the root length of beans.

Table 14: The dependent variable of the root length of bean plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	6539.492000	1307.898400	25.56	<.0001
Error	34	1739.672000	51.166824		
Corrected Total	39	8279.164000			

Table 15 to 17: The ANOVA table that follow shows whether additional carbon dioxide had an effect on the bean pods.

Table 15: The dependent variable of bean pod length.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.23680476	0.23680476	0.33	0.5926
Error	5	3.63016667	0.72603333		
Corrected Total	6	3.86697143			

Table 16: The dependent variable of the number of seeds in the bean pods.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.42857143	0.42857143	2.14	0.2031
Error	5	1.00000000	0.20000000		
Corrected Total	6	1.42857143			

Table 17: The dependent variable of bean pod width.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00428571	0.00428571	0.71	0.4366
Error	5	0.03000000	0.00600000		
Corrected Total	6	0.03428571			

Additional tables regarding data per harvest

Table 18 to 21: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on pod length per harvest.

Table 18: The dependent variable of pod lengths in harvest one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3.47700224	3.47700224	5.30	0.0384
Error	13	8.52256925	0.65558225		
Corrected Total	14	11.99957149			

Table 19: The dependent variable of pod lengths in harvest two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1.46300015	1.46300015	0.91	0.3546
Error	16	25.75321235	1.60957577		
Corrected Total	17	27.21621250			

Table 20: The dependent variable of pod lengths in harvest three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.16851152	2.16851152	1.00	0.3372
Error	12	26.04369786	2.17030816		
Corrected Total	13	28.21220938			

Table 21: The dependent variable of pod lengths in harvest four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	0	0.00000000	.	.	.
Error	7	25.18428572	3.59775510		
Corrected Total	7	25.18428572			

Table 22 to 25: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on pod width per harvest.

Table 22: The dependent variable of pod widths in harvest one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.10810484	0.02702621	0.79	0.5432
Error	26	0.89125000	0.03427885		
Corrected Total	30	0.99935484			

Table 23: The dependent variable of pod widths in harvest two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.01666126	0.00416531	2.20	0.0949
Error	28	0.05303571	0.00189413		
Corrected Total	32	0.06969697			

Table 24: The dependent variable of pod widths in harvest three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.02452778	0.00613194	1.76	0.1667
Error	27	0.09422222	0.00348971		
Corrected Total	31	0.11875000			

Table 25: The dependent variable of pod widths in harvest four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.20833333	0.20833333	16.82	0.0012
Error	13	0.16100000	0.01238462		
Corrected Total	14	0.36933333			

Table 26 to 29: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the number of seeds in the bean pods.

Table 26: The dependent variable of the number of seeds in harvest one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.10000000	2.10000000	2.87	0.1138
Error	13	9.50000000	0.73076923		
Corrected Total	14	11.60000000			

Table 27: The dependent variable of the number of seeds in harvest two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.88888889	0.88888889	2.06	0.1700
Error	16	6.88888889	0.43055556		
Corrected Total	17	7.77777778			

Table 28: The dependent variable of the number of seeds in harvest three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.38095238	2.38095238	3.23	0.0973
Error	12	8.83333333	0.73611111		
Corrected Total	13	11.21428571			

Table 29: The dependent variable of the number of seeds in harvest four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	0	0.00000000	.	.	.
Error	6	2.00000000	0.33333333		
Corrected Total	6	2.00000000			

APPENDIX B
Second pot trial

Table 1-to 11 The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the height of beans.

Table 1: The dependent variable of plant height of bean plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	43.0943900	10.7735975	6.99	0.0002
Error	44	67.8562222	1.5421869		
Corrected Total	48	110.9506122			

Table 2: The dependent variable of plant height of bean plants in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	23.6062948	5.9015737	2.05	0.1040
Error	44	126.7728889	2.8812020		
Corrected Total	48	150.3791837			

Table 3: The dependent variable of plant height of bean plants in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	27.1762268	6.7940567	1.82	0.1411
Error	44	163.8262222	3.7233232		
Corrected Total	48	191.0024490			

Table 4: The dependent variable of plant height of bean plants in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	138.6518220	34.6629555	11.35	<.0001
Error	44	134.3420556	3.0532285		
Corrected Total	48	272.9938776			

Table 5: The dependent variable of plant height of bean plants in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	202.0451111	50.5112778	14.78	<.0001
Error	44	150.3948889	3.4180657		
Corrected Total	48	352.4400000			

Table 6: The dependent variable of plant height of bean plants in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	89.0526077	22.2631519	4.86	0.0025
Error	44	201.4355556	4.5780808		
Corrected Total	48	290.4881633			

Table 7: The dependent variable of plant height of bean plants in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	159.8862982	39.9715745	8.54	<.0001
Error	44	205.8647222	4.6787437		
Corrected Total	48	365.7510204			

Table 8: The dependent variable of plant height of bean plants in week eight.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	238.9062642	59.7265660	15.20	<.0001
Error	44	172.9463889	3.9305997		
Corrected Total	48	411.8526531			

Table 9: The dependent variable of plant height of bean plants in week nine.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	207.9312959	51.9828240	14.09	<.0001
Error	44	162.3585000	3.6899659		
Corrected Total	48	370.2897959			

Table 10: The dependent variable of plant height of bean plants in week ten.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	271.8330102	67.9582526	12.09	<.0001
Error	44	247.4225000	5.6232386		
Corrected Total	48	519.2555102			

Table 11: The dependent variable of plant height of bean plants in week eleven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	271.8330102	67.9582526	12.09	<.0001
Error	44	247.4225000	5.6232386		
Corrected Total	48	519.2555102			

Tables 12 to 21: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the height of beetroot.

Table 12: The dependent variable of plant height of beetroot plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	7.98740517	2.66246839	2.26	0.1067
Error	25	29.51225000	1.18049000		
Corrected Total	28	37.49965517			

Table 13: The dependent variable of plant height of beetroot plants in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	9.04200766	3.01400255	1.60	0.2147
Error	25	47.12988889	1.88519556		
Corrected Total	28	56.17189655			

Table 14: The dependent variable of plant height of beetroot plants in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	12.54085345	4.18028448	3.15	0.0427
Error	25	33.20225000	1.32809000		
Corrected Total	28	45.74310345			

Table 15: The dependent variable of plant height of beetroot plants in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	12.12292720	4.04097573	4.54	0.0114
Error	25	22.27155556	0.89086222		
Corrected Total	28	34.39448276			

Table 16: The dependent variable of plant height of beetroot plants in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	15.95837069	5.31945690	5.47	0.0050
Error	25	24.31025000	0.97241000		
Corrected Total	28	40.26862069			

Table 17: The dependent variable of plant height of beetroot plants in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	66.9621034	22.3207011	10.30	0.0001
Error	25	54.1510000	2.1660400		
Corrected Total	28	121.1131034			

Table 18: The dependent variable of plant height of beetroot plants in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	80.0088534	26.6696178	14.73	<.0001
Error	25	45.2592500	1.8103700		
Corrected Total	28	125.2681034			

Table 19: The dependent variable of plant height of beetroot plants in week eight.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	131.0144741	43.6714914	36.87	<.0001
Error	25	29.6122500	1.1844900		
Corrected Total	28	160.6267241			

Table 20: The dependent variable of plant height of beetroot plants in week nine.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	114.6305738	38.2101913	38.10	<.0001
Error	25	25.0708056	1.0028322		
Corrected Total	28	139.7013793			

Table 21: The dependent variable of plant height of beetroot plants in week ten.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	114.9739301	38.3246434	27.72	<.0001
Error	25	34.5631389	1.3825256		
Corrected Total	28	149.5370690			

Table 22 to 31: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the height of Swiss chard.

Table 22: The dependent variable of plant height of Swiss chard plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	23.72837931	7.90945977	6.06	0.0030
Error	25	32.63300000	1.30532000		
Corrected Total	28	56.36137931			

Table 23: The dependent variable of plant height of Swiss chard plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	32.90065517	10.96688506	4.72	0.0096
Error	25	58.04900000	2.32196000		
Corrected Total	28	90.94965517			

Table 24: The dependent variable of plant height of Swiss chard plants in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	84.0673870	28.0224623	6.95	0.0015
Error	25	100.7808889	4.0312356		
Corrected Total	28	184.8482759			

Table 25: The dependent variable of plant height of Swiss chard plants in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	94.9362605	31.6454202	6.49	0.0021
Error	25	121.9782222	4.8791289		
Corrected Total	28	216.9144828			

Table 26: The dependent variable of plant height of Swiss chard plants in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	183.2580651	61.0860217	10.75	0.0001
Error	25	142.0805556	5.6832222		
Corrected Total	28	325.3386207			

Table 27: The dependent variable of plant height of Swiss chard plants in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	295.0884904	98.3628301	9.63	0.0002
Error	25	255.2728889	10.2109156		
Corrected Total	28	550.3613793			

Table 28: The dependent variable of plant height of Swiss chard plants in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	323.1111686	107.7037229	9.15	0.0003
Error	25	294.1205556	11.7648222		
Corrected Total	28	617.2317241			

Table 29: The dependent variable of plant height of Swiss chard plants in week eight.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	386.7050421	128.9016807	10.73	0.0001
Error	25	300.1928889	12.0077156		
Corrected Total	28	686.8979310			

Table 30: The dependent variable of plant height of Swiss chard plants in week nine.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	351.4921686	117.1640562	11.39	<.0001
Error	25	257.2395556	10.2895822		
Corrected Total	28	608.7317241			

Table 31: The dependent variable of plant height of Swiss chard plants in week ten.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	348.9781916	116.3260639	10.62	0.0001
Error	25	273.9142222	10.9565689		
Corrected Total	28	622.8924138			

Tables 32 to: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the stomatal density on bean leaves.

Table 32: The dependent variable of stomatal density in bean plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	47.6666667	11.9166667	2.03	0.1203
Error	25	146.5000000	5.8600000		
Corrected Total	29	194.1666667			

Table 33: The dependent variable of stomatal density in bean plants in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	201.8666667	50.4666667	11.26	<.0001
Error	25	112.0000000	4.4800000		
Corrected Total	29	313.8666667			

Table 34: The dependent variable of stomatal density in bean plants in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	118.4666667	29.6166667	4.10	0.0108
Error	25	180.5000000	7.2200000		
Corrected Total	29	298.9666667			

Table 35: The dependent variable of stomatal density in bean plants in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	28.2000000	7.0500000	1.37	0.2739
Error	25	129.0000000	5.1600000		
Corrected Total	29	157.2000000			

Table 36: The dependent variable of stomatal density in bean plants in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	178.5333333	44.6333333	9.77	<.0001
Error	25	114.1666667	4.5666667		
Corrected Total	29	292.7000000			

Table 37: The dependent variable of stomatal density in bean plants in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	618.2000000	154.5500000	45.10	<.0001
Error	25	85.6666667	3.4266667		
Corrected Total	29	703.8666667			

Table 38: The dependent variable of stomatal density in bean plants in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	476.4666667	119.1166667	42.24	<.0001
Error	25	70.5000000	2.8200000		
Corrected Total	29	546.9666667			

Table 39: The dependent variable of stomatal density in bean plants in week eight.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	101.8666667	25.4666667	7.28	0.0005
Error	25	87.5000000	3.5000000		
Corrected Total	29	189.3666667			

Table 40: The dependent variable of stomatal density in bean plants in week nine.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	75.8666667	18.9666667	4.26	0.0092
Error	25	111.3333333	4.4533333		
Corrected Total	29	187.2000000			

Tables 41 to 47: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the stomatal density on beetroot leaves.

Table 41: The dependent variable of stomatal density in beetroot plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	60.7916667	20.2638889	6.32	0.0034
Error	20	64.1666667	3.2083333		
Corrected Total	23	124.9583333			

Table 42: The dependent variable of stomatal density in beetroot plants in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	295.1250000	98.3750000	14.92	<.0001
Error	20	131.8333333	6.5916667		
Corrected Total	23	426.9583333			

Table 43: The dependent variable of stomatal density in beetroot plants in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1070.791667	356.930556	39.48	<.0001
Error	20	180.833333	9.041667		
Corrected Total	23	1251.625000			

Table 44: The dependent variable of stomatal density in beetroot plants in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4306.000000	1435.333333	483.82	<.0001
Error	20	59.333333	2.966667		
Corrected Total	23	4365.333333			

Table 45: The dependent variable of stomatal density in beetroot plants in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	135.4583333	45.1527778	7.45	0.0015
Error	20	121.1666667	6.0583333		
Corrected Total	23	256.6250000			

Table 46: The dependent variable of stomatal density in beetroot plants in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	144.3333333	48.1111111	11.06	0.0002
Error	20	87.0000000	4.3500000		
Corrected Total	23	231.3333333			

Table 47: The dependent variable of stomatal density in beetroot plants in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	166.1250000	55.3750000	15.86	<.0001
Error	20	69.8333333	3.4916667		
Corrected Total	23	235.9583333			

Tables 48 to 54: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the stomatal density on Swiss chard leaves.

Table 48: The dependent variable of stomatal density in Swiss chard plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	124.3333333	41.4444444	6.81	0.0024
Error	20	121.6666667	6.0833333		
Corrected Total	23	246.0000000			

Table 49: The dependent variable of stomatal density in Swiss chard plants in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	341.4583333	113.8194444	16.09	<.0001
Error	20	141.5000000	7.0750000		
Corrected Total	23	482.9583333			

Table 50: The dependent variable of stomatal density in Swiss chard plants in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	511.5000000	170.5000000	26.16	<.0001
Error	20	130.3333333	6.5166667		
Corrected Total	23	641.8333333			

Table 51: The dependent variable of stomatal density in Swiss chard plants in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	284.3333333	94.7777778	17.72	<.0001
Error	20	107.0000000	5.3500000		
Corrected Total	23	391.3333333			

Table 52: The dependent variable of stomatal density in Swiss chard plants in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3000.833333	1000.277778	86.35	<.0001
Error	20	231.666667	11.583333		
Corrected Total	23	3232.500000			

Table 53: The dependent variable of stomatal density in Swiss chard plants in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3307.791667	1102.597222	200.17	<.0001
Error	20	110.166667	5.508333		
Corrected Total	23	3417.958333			

Table 54: The dependent variable of stomatal density in Swiss chard plants in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2542.833333	847.611111	101.51	<.0001
Error	20	167.000000	8.350000		
Corrected Total	23	2709.833333			

Tables 55 to 57: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the overall stomatal density of beans, beetroot and Swiss chard.

Table 55: The dependent variable of the overall stomatal density of beans.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	129.5339410	32.3834853	4.74	0.0032
Error	40	273.5176569	6.8379414		
Corrected Total	44	403.0515979			

Table 56: The dependent variable of the overall stomatal density of beetroot.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	437.678383	145.892794	2.51	0.0832
Error	24	1397.346482	58.222770		
Corrected Total	27	1835.024865			

Table 57: The dependent variable of the overall stomatal density of Swiss chard.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1124.178857	374.726286	12.37	<.0001
Error	24	726.992002	30.291333		
Corrected Total	27	1851.170859			

Table 58 to 66: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the photosynthetic rate of beans.

Table 58: The dependent variable of the photosynthetic rate of beans in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	758.2594228	189.5648557	17.57	<.0001
Error	19	204.9558891	10.7871521		
Corrected Total	23	963.2153119			

Table 59: The dependent variable of the photosynthetic rate of beans in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1162.467595	290.616899	121.58	<.0001
Error	16	38.245497	2.390344		
Corrected Total	20	1200.713091			

Table 60: The dependent variable of the photosynthetic rate of beans in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	759.661257	189.915314	14.62	<.0001
Error	20	259.867779	12.993389		
Corrected Total	24	1019.529036			

Table 61: The dependent variable of the photosynthetic rate of beans in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	194.2236481	48.5559120	3.98	0.0156
Error	20	244.1459057	12.2072953		
Corrected Total	24	438.3695537			

Table 62: The dependent variable of the photosynthetic rate of beans in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	413.0098812	103.2524703	8.44	0.0004
Error	20	244.6107740	12.2305387		
Corrected Total	24	657.6206553			

Table 63: The dependent variable of the photosynthetic rate of beans in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	536.2725681	134.0681420	16.92	<.0001
Error	18	142.6073284	7.9226294		
Corrected Total	22	678.8798965			

Table 64: The dependent variable of the photosynthetic rate of beans in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	258.9153253	64.7288313	4.25	0.0119
Error	20	304.8072618	15.2403631		
Corrected Total	24	563.7225871			

Table 65: The dependent variable of the photosynthetic rate of beans in week eight.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	244.5642126	61.1410532	6.00	0.0027
Error	19	193.5220738	10.1853723		
Corrected Total	23	438.0862865			

Table 66: The dependent variable of the photosynthetic rate of beans in week nine.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	686.5528470	171.6382117	62.95	<.0001
Error	19	51.8054518	2.7266027		
Corrected Total	23	738.3582988			

Table 67 to 72: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the photosynthetic rate of beetroot.

Table 67: The dependent variable of the photosynthetic rate of beetroot in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	61.7837825	20.5945942	0.87	0.4808
Error	13	307.1825896	23.6294300		
Corrected Total	16	368.9663721			

Table 68: The dependent variable of the photosynthetic rate of beetroot in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	467.7857897	155.9285966	31.83	<.0001
Error	15	73.4931495	4.8995433		
Corrected Total	18	541.2789392			

Table 69: The dependent variable of the photosynthetic rate of beetroot in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	366.1657772	122.0552591	22.49	<.0001
Error	16	86.8422852	5.4276428		
Corrected Total	19	453.0080624			

Table 70: The dependent variable of the photosynthetic rate of beetroot in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	54.7261274	18.2420425	3.20	0.0536
Error	15	85.4059160	5.6937277		
Corrected Total	18	140.1320433			

Table 71: The dependent variable of the photosynthetic rate of beetroot in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	185.8052574	92.9026287	10.23	0.0026
Error	12	108.9676176	9.0806348		
Corrected Total	14	294.7728750			

Table 72: The dependent variable of the photosynthetic rate of beetroot in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	271.9858942	90.6619647	19.32	<.0001
Error	16	75.0846633	4.6927915		
Corrected Total	19	347.0705575			

Tables 73 to 78: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the photosynthetic rate of Swiss chard.

Table 73: The dependent variable of the photosynthetic rate of Swiss chard in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	326.5521352	108.8507117	16.68	<.0001
Error	14	91.3375061	6.5241076		
Corrected Total	17	417.8896413			

Table 74: The dependent variable of the photosynthetic rate of Swiss chard in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	344.8586022	114.9528674	13.65	0.0002
Error	14	117.9395533	8.4242538		
Corrected Total	17	462.7981555			

Table 75: The dependent variable of the photosynthetic rate of Swiss chard in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	343.9616658	114.6538886	19.96	<.0001
Error	15	86.1571520	5.7438101		
Corrected Total	18	430.1188178			

Table 76: The dependent variable of the photosynthetic rate of Swiss chard in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	49.7128083	16.5709361	0.70	0.5665
Error	14	330.6136818	23.6152630		
Corrected Total	17	380.3264901			

Table 77: The dependent variable of the photosynthetic rate of Swiss chard in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	475.4739622	158.4913207	15.74	0.0003
Error	11	110.7947848	10.0722532		
Corrected Total	14	586.2687471			

Table 78: The dependent variable of the photosynthetic rate of Swiss chard in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	93.6458616	31.2152872	2.60	0.0906
Error	15	180.1329092	12.0088606		
Corrected Total	18	273.7787708			

Tables 79 to 87: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the stomatal conductance of beans.

Table 79: The dependent variable of the stomatal conductance of beans in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.05205429	0.01301357	1.49	0.2442
Error	19	0.16573240	0.00872276		
Corrected Total	23	0.21778669			

Table 80: The dependent variable of the stomatal conductance of beans in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.07717435	0.01929359	15.36	<.0001
Error	16	0.02009391	0.00125587		
Corrected Total	20	0.09726827			

Table 81: The dependent variable of the stomatal conductance of beans in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.08780734	0.02195183	3.54	0.0243
Error	20	0.12395949	0.00619797		
Corrected Total	24	0.21176682			

Table 82: The dependent variable of the stomatal conductance of beans in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.08839863	0.02209966	2.53	0.0727
Error	20	0.17476398	0.00873820		
Corrected Total	24	0.26316262			

Table 83: The dependent variable of the stomatal conductance of beans in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.21148678	0.05287170	32.90	<.0001
Error	20	0.03213893	0.00160695		
Corrected Total	24	0.24362571			

Table 84: The dependent variable of the stomatal conductance of beans in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00889345	0.00222336	0.67	0.6232
Error	18	0.06000987	0.00333388		
Corrected Total	22	0.06890332			

Table 85: The dependent variable of the stomatal conductance of beans in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.08962421	0.02240605	3.48	0.0259
Error	20	0.12870625	0.00643531		
Corrected Total	24	0.21833046			

Table 86: The dependent variable of the stomatal conductance of beans in week eight.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.13741374	0.03435343	15.17	<.0001
Error	19	0.04302481	0.00226446		
Corrected Total	23	0.18043855			

Table 87: The dependent variable of the stomatal conductance of beans in week nine.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.17181182	0.04295295	35.14	<.0001
Error	19	0.02322190	0.00122221		
Corrected Total	23	0.19503372			

Tables 88 to 93: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the stomatal conductance of beetroot.

Table 88: The dependent variable of the stomatal conductance of beetroot in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.00813233	0.00271078	0.94	0.4488
Error	13	0.03742345	0.00287873		
Corrected Total	16	0.04555578			

Table 89: The dependent variable of the stomatal conductance of beetroot in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.00385474	0.00128491	0.76	0.5335
Error	15	0.02534121	0.00168941		
Corrected Total	18	0.02919595			

Table 90: The dependent variable of the stomatal conductance of beetroot in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.02998454	0.00999485	4.69	0.0156
Error	16	0.03411239	0.00213202		
Corrected Total	19	0.06409694			

Table 91: The dependent variable of the stomatal conductance of beetroot in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.03450984	0.01150328	10.45	0.0006
Error	15	0.01650639	0.00110043		
Corrected Total	18	0.05101623			

Table 92: The dependent variable of the stomatal conductance of beetroot in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.02388238	0.01194119	8.20	0.0057
Error	12	0.01746530	0.00145544		
Corrected Total	14	0.04134768			

Table 93: The dependent variable of the stomatal conductance of beetroot in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.03450284	0.01150095	5.45	0.0089
Error	16	0.03376010	0.00211001		
Corrected Total	19	0.06826294			

Tables 94 to 99: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the stomatal conductance of Swiss chard.

Table 94: The dependent variable of the stomatal conductance of Swiss chard in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.00281971	0.00093990	0.20	0.8968
Error	14	0.06685898	0.00477564		
Corrected Total	17	0.06967869			

Table 95: The dependent variable of the stomatal conductance of Swiss chard in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.03261751	0.01087250	2.79	0.0792
Error	14	0.05454686	0.00389620		
Corrected Total	17	0.08716437			

Table 96: The dependent variable of the stomatal conductance of Swiss chard in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.08483096	0.02827699	4.16	0.0249
Error	15	0.10199190	0.00679946		
Corrected Total	18	0.18682285			

Table 97: The dependent variable of the stomatal conductance of Swiss chard in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.07129099	0.02376366	3.20	0.0562
Error	14	0.10396578	0.00742613		
Corrected Total	17	0.17525677			

Table 98: The dependent variable of the stomatal conductance of Swiss chard in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.12791543	0.04263848	8.70	0.0030
Error	11	0.05391267	0.00490115		
Corrected Total	14	0.18182810			

Table 99: The dependent variable of the stomatal conductance of Swiss chard in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.05409272	0.01803091	1.59	0.2323
Error	15	0.16957842	0.01130523		
Corrected Total	18	0.22367113			

Table 100 to 102: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the root length of beans, beetroot and Swiss chard.

Table 100: The dependent variable of the root length of beans.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	148.261200	37.065300	0.84	0.5056
Error	44	1935.106556	43.979694		
Corrected Total	48	2083.367755			

Table 101: The dependent variable of the root length of beetroot.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1036.487847	345.495949	2.64	0.0718
Error	25	3276.354222	131.054169		
Corrected Total	28	4312.842069			

Table 102: The dependent variable of the root length of Swiss chard.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2486.308621	828.769540	5.17	0.0064
Error	25	4003.909200	160.156368		
Corrected Total	28	6490.217821			

Table 103 to 105: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the dry mass of the roots of beans, beetroot and Swiss chard.

Table 103: The dependent variable of the root dry mass of beans.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2855.017247	713.754312	12.55	<.0001
Error	44	2502.510680	56.875243		
Corrected Total	48	5357.527927			

Table 104: The dependent variable of the root dry mass of Swiss chard.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2907.574935	969.191645	52.20	<.0001
Error	25	464.199410	18.567976		
Corrected Total	28	3371.774345			

Table 105: The dependent variable of the root dry mass of beetroot.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4.48924824	1.49641608	0.89	0.4593
Error	25	41.97176556	1.67887062		
Corrected Total	28	46.46101379			

Table 106 to 108: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the dry mass of the roots of beans, beetroot and Swiss chard.

Table 106: The dependent variable of the leaf dry mass of beans.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	181.0741262	45.2685316	34.89	<.0001
Error	44	57.0964289	1.2976461		
Corrected Total	48	238.1705551			

Table 107: The dependent variable of the leaf dry mass of Swiss chard.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	19.86377594	6.62125865	6.12	0.0029
Error	25	27.03636889	1.08145476		
Corrected Total	28	46.90014483			

Table 108: The dependent variable of the leaf dry mass of beetroot.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4.48924824	1.49641608	0.89	0.4593
Error	25	41.97176556	1.67887062		
Corrected Total	28	46.46101379			

Table 109: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the average number of pods per plant.

Table 109: The dependent variable of the number of pods per bean plant.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	103.8547009	25.9636752	27.68	<.0001
Error	34	31.8888889	0.9379085		
Corrected Total	38	135.7435897			

Table 110 to 112: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the overall bean pod length, width and number of seeds.

Table 110: The dependent variable of the overall bean pod length.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.46382353	1.36595588	1.20	0.3603
Error	12	13.65500000	1.13791667		
Corrected Total	16	19.11882353			

Table 111: The dependent variable of the overall bean pod width.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.02882353	0.00720588	0.67	0.6282
Error	12	0.13000000	0.01083333		
Corrected Total	16	0.15882353			

Table 112: The dependent variable of the overall number of seeds in the bean pods.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.85784314	0.46446078	0.79	0.5554
Error	12	7.08333333	0.59027778		
Corrected Total	16	8.94117647			

Table 113 to 116: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the bean pod lengths.

Table 113: The dependent variable of the pod length in the first harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.3375000	1.8343750	0.36	0.8352
Error	26	132.7225000	5.1047115		
Corrected Total	30	140.0600000			

Table 114: The dependent variable of the pod length in the second harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.53123701	1.88280925	2.51	0.0641
Error	28	20.97603571	0.74914413		
Corrected Total	32	28.50727273			

Table 115: The dependent variable of the pod length in the third harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6.56327778	1.64081944	0.79	0.5438
Error	27	56.29672222	2.08506379		
Corrected Total	31	62.86000000			

Table 116: The dependent variable of the pod length in the fourth harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	17.32800000	17.32800000	4.33	0.0578
Error	13	52.01600000	4.00123077		
Corrected Total	14	69.34400000			

Tables 117 to 120: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the bean pod widths.

Table 117: The dependent variable of the pod width in the first harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.10810484	0.02702621	0.79	0.5432
Error	26	0.89125000	0.03427885		
Corrected Total	30	0.99935484			

Table 118: The dependent variable of the pod width in the second harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.01666126	0.00416531	2.20	0.0949
Error	28	0.05303571	0.00189413		
Corrected Total	32	0.06969697			

Table 119: The dependent variable of the pod width in the third harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	11.4367153	2.8591788	0.72	0.5887
Error	27	107.9029722	3.9964064		
Corrected Total	31	119.3396875			

Table 120: The dependent variable of the pod width in the fourth harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	8.00833333	8.00833333	1.58	0.2314
Error	13	66.04100000	5.08007692		
Corrected Total	14	74.04933333			

Tables 121 to 124: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the number of seeds in the pods.

Table 121: The dependent variable of the number of seeds in the pods in the first harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.91102151	0.47775538	0.45	0.7703
Error	26	27.50833333	1.05801282		
Corrected Total	30	29.41935484			

Table 122: The dependent variable of the number of seeds in the pods in the second harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.08658009	3.52164502	6.39	0.0009
Error	28	15.42857143	0.55102041		
Corrected Total	32	29.51515152			

Table 123: The dependent variable of the number of seeds in the pods in the third harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	12.57152778	3.14288194	4.16	0.0094
Error	27	20.39722222	0.75545267		
Corrected Total	31	32.96875000			

Table 124: The dependent variable of the number of seeds in the pods in the fourth harvest.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00000000	0.00000000	0.00	1.0000
Error	13	13.60000000	1.04615385		
Corrected Total	14	13.60000000			

Tables 125 to 126: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the beets of beetroot.

Table 125: The dependent variable of the beetroot beet length.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.22067433	0.07355811	0.57	0.6377
Error	25	3.20622222	0.12824889		
Corrected Total	28	3.42689655			

Table 126: The dependent variable of the beetroot beet width.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.45452490	0.15150830	0.90	0.4550
Error	25	4.20788889	0.16831556		
Corrected Total	28	4.66241379			

Tables 127: The ANOVA tables that follow shows whether additional carbon dioxide had an effect on the dry mass of beetroot beets.

Table 127: The dependent variable of the beetroot beet dry mass.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1.85132345	0.61710782	1.51	0.2359
Error	25	10.20618000	0.40824720		
Corrected Total	28	12.05750345			

APPENDIX C

Field trial

Tables 1 to 8: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the height of bean plants over a period of eight weeks.

Table 1: The dependent variable of plant height of bean plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1524.856469	381.214117	28.07	<.0001
Error	153	2077.940050	13.581308		
Corrected Total	157	3602.796519			

Table 2: The dependent variable of plant height of bean plants in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1188.373407	297.093352	12.56	<.0001
Error	152	3596.182262	23.659094		
Corrected Total	156	4784.555669			

Table 3: The dependent variable of plant height of bean plants in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1430.398577	357.599644	12.33	<.0001
Error	152	4408.315117	29.002073		
Corrected Total	156	5838.713694			

Table 4: The dependent variable of plant height of bean plants in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2878.153036	719.538259	20.89	<.0001
Error	152	5236.451391	34.450338		
Corrected Total	156	8114.604427			

Table 5: The dependent variable of plant height of bean plants in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4112.59635	1028.14909	25.97	<.0001
Error	152	6018.75033	39.59704		
Corrected Total	156	10131.34669			

Table 6: The dependent variable of plant height of bean plants in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5344.23064	1336.05766	28.56	<.0001
Error	149	6969.93918	46.77812		
Corrected Total	153	12314.16982			

Table 7: The dependent variable of plant height of bean plants in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6674.38207	1668.59552	28.19	<.0001
Error	148	8759.82351	59.18800		
Corrected Total	152	15434.20559			

Table 8: The dependent variable of plant height of bean plants in week eight.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7901.16876	1975.29219	27.83	<.0001
Error	147	10434.05223	70.97995		
Corrected Total	151	18335.22099			

Tables 9 to 16: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the height of beetroot plants over a period of eight weeks.

Table 9: The dependent variable of plant height of beetroot plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	549.326875	137.331719	8.75	<.0001
Error	155	2434.014062	15.703317		
Corrected Total	159	2983.340937			

Table 10: The dependent variable of plant height of beetroot plants in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	549.326875	137.331719	8.75	<.0001
Error	155	2434.014062	15.703317		
Corrected Total	159	2983.340937			

Table 11: The dependent variable of plant height of beetroot plants in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	314.951500	78.737875	4.60	0.0015
Error	155	2651.010937	17.103296		
Corrected Total	159	2965.962437			

Table 12: The dependent variable of plant height of beetroot plants in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	456.942651	114.235663	6.10	0.0001
Error	154	2883.430776	18.723576		
Corrected Total	158	3340.373428			

Table 13: The dependent variable of plant height of beetroot plants in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	631.079956	157.769989	9.56	<.0001
Error	153	2525.356563	16.505598		
Corrected Total	157	3156.436519			

Table 14: The dependent variable of plant height of beetroot plants in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	803.684385	200.921096	11.69	<.0001
Error	150	2577.613453	17.184090		
Corrected Total	154	3381.297839			

Table 15: The dependent variable of plant height of beetroot plants in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	639.733430	159.933357	7.15	<.0001
Error	135	3021.349499	22.380367		
Corrected Total	139	3661.082929			

Tables 16 to 23: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the height of Swiss chard plants over a period of eight weeks.

Table 16: The dependent variable of plant height of Swiss chard plants in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1023.426813	255.856703	9.94	<.0001
Error	155	3988.431172	25.731814		
Corrected Total	159	5011.857984			

Table 17: The dependent variable of plant height of Swiss chard plants in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1465.111625	366.277906	11.58	<.0001
Error	155	4902.224375	31.627254		
Corrected Total	159	6367.336000			

Table 18: The dependent variable of plant height of Swiss chard plants in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2068.797875	517.199469	15.37	<.0001
Error	155	5214.414063	33.641381		
Corrected Total	159	7283.211938			

Table 19: The dependent variable of plant height of Swiss chard plants in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2507.101250	626.775312	17.71	<.0001
Error	155	5484.064609	35.381062		
Corrected Total	159	7991.165859			

Table 20: The dependent variable of plant height of Swiss chard plants in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2312.694375	578.173594	14.60	<.0001
Error	155	6139.720625	39.611101		
Corrected Total	159	8452.415000			

Table 21: The dependent variable of plant height of Swiss chard plants in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2369.75156	592.43789	10.02	<.0001
Error	151	8931.29510	59.14765		
Corrected Total	155	11301.04667			

Table 22: The dependent variable of plant height of Swiss chard plants in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2720.22467	680.05617	8.84	<.0001
Error	150	11543.01075	76.95341		
Corrected Total	154	14263.23542			

Table 23: The dependent variable of plant height of Swiss chard plants in week eight.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2382.21016	595.55254	6.27	0.0001
Error	150	14251.16219	95.00775		
Corrected Total	154	16633.37235			

Tables 24 to 25: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the leaf area of beetroot and Swiss chard plants over a period of eight weeks.

Table 24: The dependent variable of the leaf area index of beetroot plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.33668705	0.08417176	1.86	0.1284
Error	66	2.99089680	0.04531662		
Corrected Total	70	3.32758385			

Table 25: The dependent variable of the leaf area index of Swiss chard plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	54.4322750	13.6080687	2.47	0.0629
Error	35	193.1492625	5.5185504		
Corrected Total	39	247.5815375			

Tables 26 to 32: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the stomatal density in beans over a period of 168 hours.

Table 26: The dependent variable of the stomatal density of beans on day zero (pre-treatment).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	33.53333333	8.38333333	1.48	0.2388
Error	25	141.8333333	5.67333333		
Corrected Total	29	175.3666667			

Table 27: The dependent variable of the stomatal density of beans on day one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	63.13333333	15.78333333	1.31	0.2921
Error	25	300.3333333	12.01333333		
Corrected Total	29	363.4666667			

Table 27: The dependent variable of the stomatal density of beans on day two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	33.6666667	8.4166667	2.11	0.1098
Error	25	99.83333333	3.99333333		
Corrected Total	29	133.5000000			

Table 28: The dependent variable of the stomatal density of beans on day three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	75.4666667	18.8666667	4.11	0.0108
Error	25	114.8333333	4.59333333		
Corrected Total	29	190.3000000			

Table 29: The dependent variable of the stomatal density of beans on day four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	297.5333333	74.38333333	41.17	<.0001

Error	25	45.1666667	1.8066667		
Corrected Total	29	342.7000000			

Table 30: The dependent variable of the stomatal density of beans on day five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	86.8666667	21.7166667	1.42	0.2558
Error	25	381.8333333	15.2733333		
Corrected Total	29	468.7000000			

Table 31: The dependent variable of the stomatal density of beans on day six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	128.8666667	32.2166667	3.97	0.0126
Error	25	203.0000000	8.1200000		
Corrected Total	29	331.8666667			

Table 32 The dependent variable of the stomatal density of beans on day seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	106.3333333	26.5833333	1.99	0.1266
Error	25	333.6666667	13.3466667		
Corrected Total	29	440.0000000			

Tables 33 to 40: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the stomatal density in beetroot over a period of 168 hours.

Table 33: The dependent variable of the stomatal density of beetroot on day zero (pre-treatment).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	97.2000000	24.3000000	2.94	0.0404
Error	25	206.6666667	8.2666667		
Corrected Total	29	303.8666667			

Table 34: The dependent variable of the stomatal density of beetroot on day one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	161.4666667	40.3666667	5.31	0.0031
Error	25	190.0000000	7.6000000		
Corrected Total	29	351.4666667			

Table 35: The dependent variable of the stomatal density of beetroot on day two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	69.8000000	17.4500000	3.05	0.0356
Error	25	143.1666667	5.7266667		
Corrected Total	29	212.9666667			

Table 36: The dependent variable of the stomatal density of beetroot on day three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	104.3333333	26.0833333	3.84	0.0144
Error	25	169.6666667	6.7866667		
Corrected Total	29	274.0000000			

Table 37: The dependent variable of the stomatal density of beetroot on day four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	118.8666667	29.7166667	8.54	0.0002
Error	25	87.0000000	3.4800000		
Corrected Total	29	205.8666667			

Table 38: The dependent variable of the stomatal density of beetroot on day five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	41.0000000	10.2500000	1.87	0.1475
Error	25	137.1666667	5.4866667		
Corrected Total	29	178.1666667			

Table 39: The dependent variable of the stomatal density of beetroot on day six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	203.8666667	50.9666667	3.39	0.0240
Error	25	376.0000000	15.0400000		
Corrected Total	29	579.8666667			

Table 40: The dependent variable of the stomatal density of beetroot on day seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	144.3333333	36.0833333	4.51	0.0070
Error	25	199.8333333	7.9933333		
Corrected Total	29	344.1666667			

Tables 41 to 48: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the stomatal density in Swiss chard over a period of 168 hours.

Table 41: The dependent variable of the stomatal density of Swiss chard on day zero (pre-treatment).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	184.8000000	46.2000000	5.28	0.0032
Error	25	218.6666667	8.7466667		
Corrected Total	29	403.4666667			

Table 42: The dependent variable of the stomatal density of Swiss chard on day one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	530.3333333	132.5833333	8.05	0.0003
Error	25	411.8333333	16.4733333		
Corrected Total	29	942.1666667			

Table 43: The dependent variable of the stomatal density of Swiss chard on day two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	977.8000000	244.4500000	16.46	<.0001
Error	25	371.1666667	14.8466667		
Corrected Total	29	1348.966667			

Table 44: The dependent variable of the stomatal density of Swiss chard on day three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	482.466667	120.616667	5.28	0.0032
Error	25	571.000000	22.840000		
Corrected Total	29	1053.466667			

Table 45: The dependent variable of the stomatal density of Swiss chard on day four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	436.5333333	109.1333333	9.63	<.0001
Error	25	283.3333333	11.3333333		
Corrected Total	29	719.8666667			

Table 46: The dependent variable of the stomatal density of Swiss chard on day five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1339.866667	334.966667	60.10	<.0001
Error	25	139.333333	5.573333		
Corrected Total	29	1479.200000			

Table 47: The dependent variable of the stomatal density of Swiss chard on day six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	101.8000000	25.4500000	2.14	0.1059
Error	25	297.6666667	11.9066667		
Corrected Total	29	399.4666667			

Table 48: The dependent variable of the stomatal density of Swiss chard on day seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	690.1333333	172.5333333	16.01	<.0001
Error	25	269.3333333	10.7733333		
Corrected Total	29	959.4666667			

Tables 49 to 53: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the stomatal density in beans over a period of five weeks.

Table 49: The dependent variable of the stomatal density of beans within week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	122.5333333	30.63333333	2.28	0.0892
Error	25	336.1666667	13.44666667		
Corrected Total	29	458.7000000			

Table 50: The dependent variable of the stomatal density of beans within week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	138.8000000	34.70000000	3.10	0.0335
Error	25	280.0000000	11.20000000		
Corrected Total	29	418.8000000			

Table 51: The dependent variable of the stomatal density of beans within week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	82.66666667	20.66666667	5.09	0.0039
Error	25	101.5000000	4.06000000		
Corrected Total	29	184.1666667			

Table 52: The dependent variable of the stomatal density of beans within week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	48.00000000	12.00000000	2.03	0.1202
Error	25	147.5000000	5.90000000		
Corrected Total	29	195.5000000			

Table 53: The dependent variable of the stomatal density of beans within week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	75.1333333	18.7833333	7.85	0.0003
Error	25	59.8333333	2.3933333		
Corrected Total	29	134.9666667			

Tables 54 to 58: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the stomatal density in beetroot over a period of five weeks.

Table 54: The dependent variable of the stomatal density of beetroot within week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	144.3333333	36.0833333	4.51	0.0070
Error	25	199.8333333	7.9933333		
Corrected Total	29	344.1666667			

Table 55: The dependent variable of the stomatal density of beetroot within week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	109.8000000	27.4500000	2.27	0.0895
Error	25	301.6666667	12.0666667		
Corrected Total	29	411.4666667			

Table 56: The dependent variable of the stomatal density of beetroot within week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	111.2000000	27.8000000	6.43	0.0011
Error	25	108.1666667	4.3266667		
Corrected Total	29	219.3666667			

Table 57: The dependent variable of the stomatal density of beetroot within week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	65.1333333	16.2833333	2.07	0.1145
Error	25	196.3333333	7.8533333		
Corrected Total	29	261.4666667			

Tables 58 to 62: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the stomatal density in Swiss chard over a period of five weeks.

Table 58: The dependent variable of the stomatal density of Swiss chard within week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	690.1333333	172.5333333	16.01	<.0001
Error	25	269.3333333	10.7733333		
Corrected Total	29	959.4666667			

Table 59: The dependent variable of the stomatal density of Swiss chard within week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	410.333333	102.583333	1.71	0.1784
Error	25	1496.333333	59.853333		
Corrected Total	29	1906.666667			

Table 60: The dependent variable of the stomatal density of Swiss chard within week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	471.800000	117.950000	4.99	0.0043
Error	25	591.000000	23.640000		
Corrected Total	29	1062.800000			

Table 61: The dependent variable of the stomatal density of Swiss chard within week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	49.466667	12.366667	0.28	0.8893
Error	25	1111.500000	44.460000		
Corrected Total	29	1160.966667			

Table 62: The dependent variable of the stomatal density of Swiss chard within week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	266.2000000	66.5500000	3.52	0.0206
Error	25	472.5000000	18.9000000		
Corrected Total	29	738.7000000			

Tables 63 to 65: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the stomatal density in beans, beetroot and Swiss chard.

Table 63: The dependent variable of the overall stomatal density of Swiss chard.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	38.2603350	9.5650837	0.42	0.7928
Error	20	456.3392144	22.8169607		
Corrected Total	24	494.5995494			

Table 64: The dependent variable of the overall stomatal density of beans.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3.6000000	0.9000000	0.07	0.9895
Error	20	246.4000000	12.3200000		
Corrected Total	24	250.0000000			

Table 65: The dependent variable of the overall stomatal density of beetroot.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	16.5000000	4.1250000	0.52	0.7240
Error	15	119.5000000	7.9666667		
Corrected Total	19	136.0000000			

Tables 66 to 71: The ANOVA tables that follow shows whether the foliar application of hydrogen peroxide had an effect on the dry and fresh mass of beans, beetroot and Swiss chard.

Table 66: The dependent variable of the leaf dry mass of beans.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	16397.89580	4099.47395	18.82	<.0001
Error	147	32019.17959	217.81755		
Corrected Total	151	48417.07539			

Table 67: The dependent variable of the leaf fresh mass of beans.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	265100.5986	66275.1497	16.05	<.0001
Error	147	606925.5450	4128.7452		
Corrected Total	151	872026.1436			

Table 68: The dependent variable of the leaf dry mass of beetroot.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	488.804592	122.201148	1.91	0.1131
Error	135	8656.906530	64.125234		
Corrected Total	139	9145.711122			

Table 69: The dependent variable of the leaf fresh mass of beetroot.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	13626.7708	3406.6927	2.79	0.0287
Error	135	164626.5687	1219.4561		
Corrected Total	139	178253.3395			

Table 70: The dependent variable of the leaf dry mass of Swiss chard.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	15638.2549	3909.5637	2.64	0.0363
Error	150	222516.0309	1483.4402		
Corrected Total	154	238154.2858			

Table 71: The dependent variable of the leaf fresh mass of Swiss chard.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2068315.38	517078.84	5.65	0.0003
Error	150	13726047.83	91506.99		
Corrected Total	154	15794363.21			

Tables 72 to 74: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the overall pod lengths, widths and number of seeds of bean pods.

Table 72: The dependent variable of the overall pod length of bean pods.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	18.49063000	4.62265750	7.40	0.0017
Error	15	9.37582500	0.62505500		
Corrected Total	19	27.86645500			

Table 73: The dependent variable of the overall number of seeds in the bean pods.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4.70000000	1.17500000	2.71	0.0701
Error	15	6.50000000	0.43333333		
Corrected Total	19	11.20000000			

Table 74: The dependent variable of the overall pod width of bean pods.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.01315470	0.00328867	0.53	0.7159
Error	15	0.09314750	0.00620983		
Corrected Total	19	0.10630220			

Tables 75 to 78: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the pod lengths of bean harvest over a period of eight weeks.

Table 75: The dependent variable of pod length of beans of harvest number one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	74.0769333	18.5192333	11.24	<.0001
Error	145	238.9017500	1.6475983		
Corrected Total	149	312.9786833			

Table 76: The dependent variable of pod length of beans of harvest number two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	398.5686917	99.6421729	41.72	<.0001
Error	145	346.2762917	2.3881124		
Corrected Total	149	744.8449833			

Table 77: The dependent variable of pod length of beans of harvest number three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	66.5903333	16.6475833	15.36	<.0001
Error	145	157.1084167	1.0835063		
Corrected Total	149	223.6987500			

Table 78: The dependent variable of pod length of beans of harvest number four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	253.4689333	63.3672333	29.41	<.0001
Error	145	312.4466667	2.1548046		
Corrected Total	149	565.9156000			

Tables 79 to 82: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the pod widths of beans of each harvest over a period of eight weeks.

Table 79: The dependent variable of pod width of beans of harvest number one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.21506667	0.05376667	7.98	<.0001
Error	145	0.97666667	0.00673563		
Corrected Total	149	1.19173333			

Table 80: The dependent variable of pod length of beans of harvest number two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.99426667	0.24856667	40.02	<.0001
Error	145	0.90066667	0.00621149		
Corrected Total	149	1.89493333			

Table 81: The dependent variable of pod length of beans of harvest number three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.30493333	0.07623333	8.74	<.0001
Error	145	1.26500000	0.00872414		
Corrected Total	149	1.56993333			

Table 82: The dependent variable of pod length of beans of harvest number four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.11400000	0.02850000	1.93	0.1085
Error	145	2.14100000	0.01476552		
Corrected Total	149	2.25500000			

Tables 83 to 86: The ANOVA tables that follow show whether the foliar applications of hydrogen peroxide had an effect on the number of seeds within the pods of the beans of each harvest over a period of eight weeks.

Table 83: The dependent variable of the number of seeds within the beans of harvest number one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	39.6266667	9.9066667	14.46	<.0001
Error	145	99.3666667	0.6852874		
Corrected Total	149	138.9933333			

Table 84: The dependent variable of the number of seeds within the beans of harvest number two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	39.6266667	9.9066667	14.46	<.0001
Error	145	99.3666667	0.6852874		
Corrected Total	149	138.9933333			

Table 85: The dependent variable of the number of seeds within the beans of harvest number three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	28.7066667	7.1766667	11.94	<.0001
Error	145	87.1666667	0.6011494		
Corrected Total	149	115.8733333			

Table 86: The dependent variable of the number of seeds within the beans of harvest number four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	125.8933333	31.4733333	29.99	<.0001
Error	145	152.1666667	1.0494253		
Corrected Total	149	278.0600000			

Tables 87 to 88: The ANOVA tables that follow show whether the foliar application of hydrogen peroxide had an effect on the length and width of beetroot beets.

Table 87: The dependent variable of the length of beetroot beets.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	94.3978390	23.5994598	7.22	<.0001
Error	136	444.6279411	3.2693231		
Corrected Total	140	539.0257801			

Table 88: The dependent variable of the width of beetroot beets.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	74.0277342	18.5069336	8.07	<.0001
Error	136	311.6998544	2.2919107		
Corrected Total	140	385.7275887			

Tables 89 to 90: The ANOVA tables that follow show whether the foliar application of hydrogen peroxide had an effect on the fresh and dry mass of beetroot beets and bean pods.

Table 89: The dependent variable of the dry mass of beetroot beets.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3724.79781	931.19945	9.18	<.0001
Error	136	13795.66140	101.43869		
Corrected Total	140	17520.45921			

Table 90: The dependent variable of the fresh mass of beetroot beets.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	400617.941	100154.485	10.05	<.0001
Error	135	1345554.138	9967.068		
Corrected Total	139	1746172.079			

APPENDIX D

Tables 1 to 7: The ANOVA tables that follow shows whether aeration with pure CO₂ or O₂ had an effect on the height of beetroot.

Table 1: The dependent variable of the plant height in beetroot in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	52.2973333	26.1486667	1.47	0.2680
Error	12	213.0960000	17.7580000		
Corrected Total	14	265.3933333			

Table 2: The dependent variable of the plant height in beetroot in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	58.5293333	29.2646667	1.51	0.2603
Error	12	232.7200000	19.3933333		
Corrected Total	14	291.2493333			

Table 3: The dependent variable of the plant height in beetroot in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	58.0053333	29.0026667	1.31	0.3049
Error	12	264.9440000	22.0786667		
Corrected Total	14	322.9493333			

Table 4: The dependent variable of the plant height in beetroot in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	64.4253333	32.2126667	1.49	0.2647
Error	12	259.7840000	21.6486667		
Corrected Total	14	324.2093333			

Table 5: The dependent variable of the plant height in beetroot in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	97.2637692	48.6318846	4.23	0.0467
Error	10	115.0070000	11.5007000		
Corrected Total	12	212.2707692			

Table 6: The dependent variable of the plant height in beetroot in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	158.7364848	79.3682424	13.39	0.0028
Error	8	47.4253333	5.9281667		
Corrected Total	10	206.1618182			

Table 7: The dependent variable of the plant height in beetroot in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	159.7963889	79.8981944	21.42	0.0019
Error	6	22.3791667	3.7298611		
Corrected Total	8	182.1755556			

Tables 8 to 14: The ANOVA tables that follow shows whether aeration with pure CO₂ or O₂ had an effect on the height of Swiss chard.

Table 8: The dependent variable of the plant height in Swiss chard in week one.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.1920000	0.0960000	0.00	0.9981
Error	12	607.0840000	50.5903333		
Corrected Total	14	607.2760000			

Table 9: The dependent variable of the plant height in Swiss chard in week two.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	8.3320000	4.1660000	0.09	0.9132
Error	12	546.1640000	45.5136667		
Corrected Total	14	554.4960000			

Table 10: The dependent variable of the plant height in Swiss chard in week three.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	17.1693333	8.5846667	0.25	0.7861
Error	12	419.4400000	34.9533333		
Corrected Total	14	436.6093333			

Table 11: The dependent variable of the plant height in Swiss chard in week four.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	47.7213333	23.8606667	0.66	0.5357
Error	12	435.2680000	36.2723333		
Corrected Total	14	482.9893333			

Table 12: The dependent variable of the plant height in Swiss chard in week five.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	22.7160000	11.3580000	0.42	0.6672
Error	12	325.5280000	27.1273333		
Corrected Total	14	348.2440000			

Table 13: The dependent variable of the plant height in Swiss chard in week six.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	73.7920000	36.8960000	1.21	0.3330
Error	12	366.8880000	30.5740000		
Corrected Total	14	440.6800000			

Table 14: The dependent variable of the plant height in Swiss chard in week seven.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	52.0893333	26.0446667	0.66	0.5326
Error	12	470.5080000	39.2090000		
Corrected Total	14	522.5973333			

Tables 15 to 17: The ANOVA tables that follow shows whether aeration with pure CO₂ or O₂ had an effect on the length, fresh and dry mass of Swiss chard roots.

Table 15: The dependent variable of the root length in Swiss chard plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	600.099231	300.049615	6.56	0.0151
Error	10	457.430000	45.743000		
Corrected Total	12	1057.529231			

Table 16: The dependent variable of the root fresh mass in Swiss chard plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	39.5613333	19.7806667	0.35	0.7140
Error	12	685.0888000	57.0907333		
Corrected Total	14	724.6501333			

Table 17: The dependent variable of the root dry mass in Swiss chard plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.07527500	0.53763750	0.52	0.6100
Error	11	11.43327500	1.03938864		
Corrected Total	13	12.50855000			

Tables 18 to 20: The ANOVA tables that follow shows whether aeration with pure CO₂ or O₂ had an effect on the leaf fresh and dry mass of Swiss chard and beetroot plants.

Table 18: The dependent variable of the leaf wet mass in Swiss chard plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	143.810920	71.905460	0.29	0.7521
Error	12	2957.998680	246.499890		
Corrected Total	14	3101.809600			

Table 19: The dependent variable of the leaf dry mass in Swiss chard plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	35.9392933	17.9696467	0.85	0.4524
Error	12	254.3110800	21.1925900		
Corrected Total	14	290.2503733			

Table 20: The dependent variable of the leaf dry mass in beetroot plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	7.04166667	7.04166667	56.33	0.0843
Error	1	0.12500000	0.12500000		
Corrected Total	2	7.16666667			