The effect of enriched CO_2 concentrations on hydroponically grown Lettuce (*Lactuca sativa*)

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The effect of enriched CO_2 concentrations on hydroponically grown Lettuce (*Lactuca sativa*)

A research to determine the effect of enriched CO_2 concentrations on plant characteristics of four lettuce varieties grown in a hydroponic vertical farm.

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Preface

Before you lies the graduation research thesis about 'The effect of enriched CO_2 concentrations on hydroponically grown Lettuce (*Lactuca sativa*)' that was written during my graduation internship that was conducted at Own Greens in Burgh-Haamstede, the Netherlands.

By writing this graduation thesis I will finish my study Applied Biology at Aeres university of Applied Sciences in Almere. Both the internship and my research were very educational. The goals of this research have ultimately been reached and has given me a lot of motivation and ideas for future research within Vertical Farming.

Things that were altered after feedback in the Introduction are: the way statistics was done on the data, it was changed from an Anova to a Paired T-Test, and the order of sub-questions has changed, where the first three sub-questions are now production related.

I want to thank both Saskia Mol of Own Greens and Wieneke van der Heide of Aeres University of Applied Sciences for the excellent guidance and supervision during the writing of this thesis and the freedom that was given to me by doing independent research, setting up experiments, and sometimes giving me a push into the right direction that helped me develop new skills. I also want to thank my fellow students Friso Termeer and Ilse Hagoort for giving me some feedback along the way.

I hope you enjoy your reading,

Richard Steenvoorden

Leiden, 10th of June 2019.

Contents

Samenvatting 4

Summary 5

1. Introduction 6

1.1 Historical carbon dioxide levels on earth 6

1.2 Photosynthesis 7

1.3 C3, C4 and CAM-plants 7

1.4 Limitations in crop growth 8

1.5 Urban farming: Hydroponics 9

1.6 Knowledge gap 9

2. Material and methods 11

2.1 Two experiments 11

2.2 Method 11

2.2.1 Sowing 12

2.2.2 Planting seedlings 14

2.2.3 Harvesting 15

3. Results 17

3.1 Yield 18

3.2 Height 19

3.3 Stem length 21

3.4 Water-use efficiency 22

3.5 Taste 23

4. Discussion 24

4.1 Yield 24

4.2 Height 24

4.3 Stem length 25

4.4 Water-use efficiency 25

4.5 Taste 25

4.6 Reflection 26

5. Conclusion 28

5.1 Sub questions 28

5.2 Main question 29

5.3 Recommendation 29

Bibliography 30

Appendix I – Plant nutrients 33

Appendix II – Seed sprouting 34

Appendix III – Experiment 1 35

Appendix IV – Experiment 2 41

Appendix V – SPSS statistics 48

Appendix VI – Checklist schriftelijk rapporteren 50

Samenvatting

Dit afstudeerwerkstuk is geschreven naar aanleiding van de opdracht binnen het bedrijf Own Greens om een protocol te schrijven voor het gebruik van verhoogde concentraties CO_2 voor de productie van hun gewassen. Naar aanleiding van dat protocol is er binnen de verticale landbouw en hydroponic teelt onderzocht wat de effecten zijn van deze verhoogde concentraties CO_2 op de productie, het watergebruik en de smaak van sla.

Uit literatuuronderzoek bleek dat er over het algemeen wel bekend is dat een verschil in de concentratie van CO_2 effect heeft op de ontwikkeling van de plant, maar dat dit per variëteit binnen een soort kan verschillen door de sensitiviteit voor CO_2. Naar aanleiding van deze informatie is de volgende hoofdvraag opgesteld:

“Wat is het effect van verhoogde CO_2-concentraties op de productie, smaak en het watergebruik op verschillende variëteiten van sla (Lactuca sativa)?“

Het doel van het onderzoek was om meer kennis te verkrijgen over de effecten van extra CO_2 op de karaktereigenschappen van sla op hydroponic teeltsystemen. Hiermee wordt bekeken of het nut heeft voor bedrijven die aan hydroponic vertical farming doen om te gaan telen met verhoogde concentraties van CO_2, welke concentratie het meest geschikt is en wat de gevolgen dit heeft op het versgewicht, de hoogte, de stamlengte, het watergebruik en de smaak van sla.

Om hier antwoord op te krijgen zijn vier slavariëteiten in een hydroponic teelsysteem getest op 600, 1000 en 1500 ppm CO_2. Dit onderzoek was verdeeld over twee experimenten, die elk vijf weken liepen. Het eerste experiment had drie variëteiten sla: Ilema, Red Span en Cristabel met een 600 ppm tegenover 1500 ppm CO_2 . Het tweede experiment had ook drie variëteiten sla, waarbij Ilema werd vervangen door Tough Red. Hier werd een controle van 600 ppm tegenover 1000 ppm CO_2 geplaatst.

Uit de resultaten bleek dat er variatie in CO_2-sensitiviteit is. Gekeken naar alle eigenschappen levert telen op 1000 ppm CO_2 het meeste significante verschil op diverse karakteristieken bij de huidige geteste slavariëteiten.

Aanbevolen wordt bij elke variëteit die in de toekomst mogelijk geteeld gaat worden eerst op verschillende CO_2 concentraties te testen om te onderzoeken wat de meest optimale teeltomstandigheden zijn. Om het huidige productieproces te optimaliseren voor de best geteste variëteiten Cristabel en Red Span, wordt voor nu 1000 ppm CO_2 aangeraden.

Summary

This graduation research thesis is written following the assignment conducted at the Own Greens company to write a protocol for the use of increased concentrations of CO_2 for the production of their crops. As a result of writing that protocol, it was researched what these effects of increased CO_2 concentrations are on production, water-use efficiency and the taste of lettuce within vertical farming and hydroponic cultivation.

Literature research showed that it is generally known that a difference in concentration of CO_2 has an effect on the development of the plant, but that this can even differ greatly per variety within a species due to the difference in sensitivity to CO_2. Based on this information, the following main question has been prepared:

"What is the effect of enriched CO_2 concentrations on the production, taste and water-use efficiency Lettuce (Lactuca sativa) varieties?"

The goal was to gain more knowledge about the effects of enriched CO_2 concentrations on the characteristics of lettuce varieties in practice, cultivated under hydroponic well-balanced indoor conditions and thereby improving and optimizing the growth of crops in hydroponic systems, and give an advice on the dose of CO_2 per variety of crop and what the consequences are on the yield, height, stem length, water-use efficiency and taste of those varieties.

For answering this, four lettuce varieties were tested on an hydroponic cultivation system at 600, 1000 and 1500 ppm CO_2. This research was divided into two experiments, each of which ran for five weeks. The first experiment had three varieties of lettuce: Ilema, Red Span and Cristabel with a 600 ppm compared to 1500 ppm CO_2. The second experiment also had three varieties of lettuce, with Ilema being replaced by Tough Red. A 600 ppm CO_2 concentration was here compared to 1000 ppm CO_2.

The results showed that there is indeed a lot of variation in CO_2 sensitivity. Concluding that growing at 1000 ppm CO_2 yields the most significant difference on current examined lettuce varieties.

It is recommended that for every variety that may be cultivated in the future, it must first be tested on different CO_2 concentrations to conclude what the optimum growing conditions are. To optimize the current production process, with the chosen best varieties Cristabel and Red Span, 1000 ppm CO_2 as a general CO_2 concentration is recommended.

1. Introduction

Carbon dioxide (CO_2) is one of the most important molecules for life on earth. Currently there are around 412 parts per million (ppm) CO_2 molecules in the air (ProOxygen, 2019). Before the industrial revolution (around 1750) it was approximately 260-280 ppm (Wigley, 1983), which has stayed almost constant for the last 10.000 years (Eggleton, 2013). In the history of the earth there was a lot of fluctuation in CO_2 concentrations. This is for example measured through the fossil stomatal index, because plant stomata react on atmospheric pressure and CO_2 and this can be seen in their fossils (figure 1) (Mills, et al., 2019).

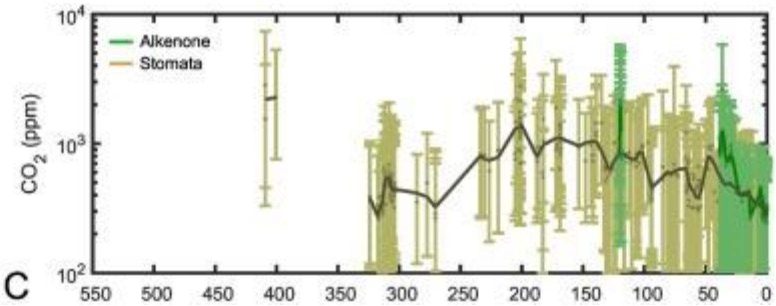


Figure 1: Estimates for atmospheric CO2 concentrations the past 400 million years from alkenone and stomata isotopes (Mills, et al., 2019).

1.1 Historical carbon dioxide levels on earth

The first terrestrial plants evolved around 700 million years ago (Ma) in the late Precambrian according to molecular evidence and corresponding to first fossil evidence around 480-460 (Ma) (Heckman, et al., 2001). This evolution of land plant species contributed to the greening of the earth which in effect caused the plummeting of global atmospheric CO_2 levels, partly seen in figure 1 where 400 Ma the atmospheric CO_2 concentration was a lot higher than 325 Ma. Around this time period, from the Early tot the Middle Devonian period, the global CO_2 levels dropped from 6300 to 3950 ppm and in the Late Devonian ultimately to 1800 ppm (Le Hir, et al., 2011). This was the period when rooted vascular plants spread around the globe, which had an important effect on global weather processes by enhancing CO_2 uptake out of the atmosphere (Berner, 1997).

In the Devonian the first primitive plants evolved, and the first forests developed (Smith, 2007). The mean CO_2 level in the late Devonian was approximately around 2100 ppm (Le Hir, et al., 2011). The further greening of the world acted as a carbon sink, and the plummeting atmospheric CO_2 levels may have been one of the reasons that led to a mass extinction event by cooling the earth (Algeo, 1998).

Flowering plants evolved later, around 125 to 110 Ma. The oldest flowering plant fossil was found in China and was dated 125 Ma (Weiss, 2002). CO_2 levels in the Cretaceous period (145 to 66 million years ago) were approximately between 1400 and 1000 ppm (Nordt, et al., 2003). These levels, if we go fast-forward in time, slowly decreased to the pre-industrial concentration of 280 ppm as measured by Wigley (1983). The current rising CO_2 concentrations in the atmosphere are at level with concentrations in the mid-Pliocene, that was around 2-4 Ma (Keeling, 2013).

From winter to summer the earth is seasonal ‘breathing’. Global CO_2 levels show a cyclic variation of 5 ppm during one year according to measurements on Mauna Loa (Tans & Thoning, 2018). This corresponds with seasonal uptake of CO_2 by the global flora during photosynthesis and the decay of plant material during autumn and winter.

1.2 Photosynthesis

Plants use CO_2 together with H_2O for making sugars in the process of photosynthesis. The plant uses the energy of the sun for using the carbon atom and releasing O_2 trough the stomata in the atmosphere. With higher CO_2 levels the photosynthesis is boosted, which stimulates the growth of the plants (Deryng, et al., 2016) (Prior, et al., 2011).

Current rising atmospheric CO_2 levels are the cause of the greening of the earth as measured in leaf area index (LAI). CO_2 fertilization currently explains approximately 70% of this greening on 52% of the vegetated lands (Zhu, et al., 2016). Which will store atmospheric carbon by facilitate more plant growth, especially in regions with colder climates. Most models even underestimate photosynthetic carbon fixation by plants, which could have important implications on the carbon cycle and the world's climatic changes (Winkler, et al., 2019).

The current rising atmospheric CO_2 concentrations are also expected to enhance the future global photosynthesis and reduce crop water use (Kimbal, 2011). With enriched CO_2 concentrations, water-use efficiency in agriculture can be increased (Prior, et al., 2011) and with that the water use can be reduced 4 to 17 percent (Deryng, et al., 2016), biomass can increase with 23% and yield production can go up 10-27 percent (Vanuytrecht, Raes, & Willems, 2012).

Biome	GPP (Pg C year^{-1})	GPP = 2 × NPP* (Pg C year^{-1})
Tropical forests	40.8	43.8
Temperate forests	9.9	16.2
Boreal forests	8.3	5.2
Tropical savannahs and grasslands	31.3	29.8
Temperate grasslands and shrublands	8.5	14
Deserts	6.4	7
Tundra	1.6	1
Croplands	14.8	8.2
Total	121.7	125.2

*Based on integrated numbers for biomes (*6, 7*)

a

Figure 2: Global carbon uptake by biome (Beer et al., 2010)

The global CO_2 uptake is mostly by tropical forests, tropical savanna's and grasslands (Beer, et al., 2010). They account for 72.1 Pg C out the total 121.7 Pg C of yearly global CO_2 uptake (figure 2). In tropical forests this is balancing net deforestation. It is feasible that this rising CO_2 effect acts as a negative feedback in the worldwide carbon cycle, capturing up to 30% of anthropogenic CO_2 emissions (Schimel, Stephens, & Fisher, 2015).

1.3 C3, C4 and CAM-plants

In plants, there are two main types of photosynthesis: the C3- and C4-plants. C3 plants assimilate CO_2 with intermediates that have three carbon atoms, and C4 plants use four carbon atoms in the process before it is in both processes ultimately converted in glucose (Talapatra, 2015). In C4-plants CO_2 is concentrated by the metabolism in the bundle sheat (BS) tissue making it more efficient than the C3-metabolism, this process evolved independently at least 66 times in different plants (Sage, Christin, & Edwards, 2011) (Sage, Sage, & Kocacinar, 2012).

Another later evolved form of photosynthesis is Crassulacean Acid Metabolism (CAM), where CO_2 fixation is separated in time. During the day the stomata are closed, and at night CAM-plants take up CO_2 in the form of malate or isocitrate which are processed again during the day when stomata are closed (Ting, 1985).

In current low atmospheric CO_2 levels C3-plants have more photorespiration then under historical high CO_2 concentrations as shown in figure 1, where photorespiration probably was limited (Noctor & Mhamdi, 2017). Photorespiration is an ancient pathway present in every oxygen producing organism, it evolved to thrive in an oxygen-rich environment (Bauwe, Hagemann, & Fernie, 2010). CAM plants concentrate the CO_2 in the proximity of the *RuBisCO* enzyme, so the photorespiration is limited (Peterhansel, et al., 2008).

Photorespiration happens more often in C3 and C4 plants, this happens when an O_2 molecule is used instead of an CO_2 molecule which creates an harmful by-product *2-phosphoglycolate* in the Calvin Cycle, which has to be recycled and causes a loss of photosynthetic output, thus lower carbon fixation (Hagemann & Bauwe, 2017). There is even research done on synthetic glycolate metabolism pathways in Tobacco (*Nicotiana tabacum*), a C3 plant, to bypass this photorespiration and increase the photosynthetic yield by 20% (South, et al., 2019).

In literature it is known that an enriched CO_2 concentrations in the open fields is increasing photosynthesis (Caporn S. J., 1988) and affecting the yield of C3 plants like lettuce (*Lactuca sativa*) (Mckeehen, et al., 1996), where yields are going up 30% with an increase to 1000 ppm CO_2 (Caporn, et al., 1993) (Prior, et al., 2011). In C4 plants this can be 10-15% (Prior et al., 2003) (Prior, et al., 2011).

Also in greenhouses it is known that the increase of CO_2 to a recommended 1.000 ppm will increase the yield from some plants up to 50% over atmospheric CO_2 levels (Blom, et al., 2016). The reason for this increase is because there is a lower chance of an O_2 molecule used instead of an CO_2 molecule by the *RuBisCO* enzyme, so in this way the plant is more efficient and this creates a gain in average yield in C3 plants (Vanuytrecht, Raes, & Willems, 2012) (Kozai & Niu, 2016)

Most C3 plants in general like potatoes (*Solanum tuberosum*) respond well at levels of 1000 ppm CO_2 in the field (Wheeler R. M., 2006) and super-elevated concentrations of 10.000 ppm will reduce the growth of C3 plants like Radish (*Raphanus sativus*) and Lettuce (*L. sativa*). It is also known that sensitivities to CO_2 enrichment can differ among varieties (Wheeler, et al., 2000).

1.4 Limitations in crop growth

All plant growth on earth is dependent on the energy of the sun. Photosynthesis supports the global crop production. One limiting factor can limit the growth of an organism, as for example lowering the growth rate of a plant. This limiting factor can be water or CO_2 if sunlight and plant nutrients like Potassium or Phosphate are abundant. In worldwide ecosystems this limiting factor can also be Nitrogen. This is for example found in forest canopy's after a few years of CO_2 fertilization (Hiemann & Reichstein, 2008).

Most primary production of more than the half of the global ecosystems are limited by the availability of water. It is expected that in a warming world the evaporation of plants is expected to increase, but a rising concentration of CO_2 in the atmosphere will tend to mitigate this effect by increasing the water-use efficiency (Hiemann & Reichstein, 2008).

Another limiting factor is the decreasing agricultural land, despite the deforestation for new agricultural land. And with 10bn people to feed in 2050, which is an increase of 60% (Terazono, 2018), future severe extreme weather can be a problem from stable rising crop production (Romeo, et al, 2018). Photorespiration is seen as an prime target for crop improvement (Rashad, et al., 2007) .

Hydroponic crop production can be part of the solution for these limitations. Hydroponic crop production uses only 5 to 25% of the land and up to 5% of the water that conventional agriculture

(Kozai & Niu, 2016) and next to that the food production line can be shortened which will reduce the carbon emissions (Romeo, et al., 2018).

1.5 Urban farming: Hydroponics

In urban farming systems, plants are grown under controlled environments. And in hydroponics, a form of urban farming, crops are grown on water with necessary plant nutrients under light emitting diodes (LED) as seen in figure 3. The crops are grown in an controlled environment and with increased levels of CO_2 you can reduce photorespiration, and stimulate photosynthesis and crop growth (Kozai & Niu, 2016), and increase dry matter compared to conventional agriculture (Fuentes & King, 1989). And with hydroponic farming the industry can reduce the runoff and water use and with that improve cost-effectiveness compared to conventional agriculture (Viviano, 2017) (Romeo, et al., 2018).

Figure 3: Vertical hydroponic farming of Infarm in Berlin (Richard Steenvoorden, 2018).

In a country for example like Japan, urban farming is booming right now. In Japan there are already 180 plant factories that do vertical farming, this is mainly because of the lack of space and young farmers (Krajenbrink, 2018). Globally there was invested 146 million dollars in urban farming in the year 2018 alone, of which 90% was invested in the United States. And these numbers are rising (Kukotai, Fung, & Place, 2018).

There are not yet that many urban/vertical farming companies in the Netherlands. A few known companies and testing stations are: Philips GrowWise, Certhon, Staay Food Group, Proeftuin Zwaagdijk, Plantlab, GrowX, Hortilux, Priva Horticulture and Own Greens (Brakeboer, 2016). Most urban farming start-ups go bankrupt within a few years because of a missing good business plan, no efficiency and expensive technologies (De Leeuw & Boere, 2016) (Sijmonsma, 2018).

1.6 Knowledge gap

Because there are only a few companies in the Netherlands that specialize in hydroponics and maybe none or only very few are testing with the combination of enriched CO_2 concentrations, most of the testing goes to light or watering systems, and because of that there is in general not much known how a variety of crops respond and/or what their sensitivity is on these enriched concentrations of CO_2 in hydroponic controlled environments.

CO_2 enrichment is not tested on most grown modern varieties and their difference in sensitivity in modern hydroponic LED-based systems. What is missing is an optimal CO_2 concentration (between 1000 and 10.000 ppm) per variety and effect per CO_2 concentration on the development, and compactness of hydroponically grown varieties.

From this lack of knowledge of the effect on different varieties of Lettuce, an optimum CO_2 concentration is selected for an efficient as possible hydroponic vertical farming system. With these results the main- and sub questions will be answered.

Main question:

- *What is the effect of enriched CO_2 concentrations on the production, taste and water-use efficiency Lettuce (Lactuca sativa) varieties?*

Sub-questions:

- *What is the effect of 600, 1000, and 1500 ppm CO_2 on the fresh weight yield in gram of the chosen lettuce varieties: Cristabel, Red Span, Tough Red, and Ilema?*
- *What is the effect of 600, 1000, and 1500 ppm CO_2 on the height of a variety of the chosen lettuce varieties: Cristabel, Red Span, Tough Red, and Ilema?*
- *What is the effect of 600, 1000, ands 1500 ppm CO_2 on the stem length of the chosen lettuce varieties: Cristabel Red Span, Tough Red, and Ilema?*
- *What is the effect of 600, 1000, and 1500 ppm CO_2 on the water use efficiency of the chosen lettuce varieties: Cristabel, Red Span, Tough Red, and Ilema?*
- *What is the effect of 600, 1000, and 1500 ppm CO_2 on the taste of the chosen lettuce varieties: Cristabel, Red Span, Tough Red and Ilema?*

In this research there will be looked at what the beneficial effects of enriched CO_2 concentrations on the way it is cultivated at Own Greens in Burgh-Haamstede. The first three sub-questions will be used to answer the 'production' part of the main question.

Hypothesis and goal

Literature shows that plants in general benefit from enriched CO_2 concentrations when there is abundance of nutrients and light, and a right temperature and RV. Expected is that under enriched CO_2 concentrations fresh weight will increase by less photorespiration, water efficiency will increase, plants will be more compact by less height and stem length will be reduced by lengthening the vegetative growth and postponing the generative growth and with that the taste of the leaves is expected to be sweeter.

The goal is to gain more knowledge about the effects of enriched CO_2 concentrations in practice on lettuce varieties grown under hydroponic well-balanced indoor conditions and thereby improving and optimizing the growth of crops in hydroponic systems and reducing unwanted effects that are seen under 600 ppm CO_2 like wobbling and stretching of plants. And with the outcome of that, give an advice on the use of CO_2 per variety of crop.

2. Material and methods

Two experiments will be conducted for this research. These experiments will run in total, from sowing to harvesting, for five weeks:

- The first test will run for five weeks from the 21st of February till March 28th under 1500 ppm CO_2 and a control in office CO_2 concentrations (600 ppm);
- The second test will run for five weeks from the 19th of April till the 24th of May under 1000 ppm CO_2 and control also with office CO_2 concentrations (600 ppm).

After harvesting and gathering the data of these two experiments the sub-questions can be answered and with that ultimately the main question.

2.1 Two experiments

The goal of the first experiment is to find out the difference between 600 ppm and 1500 ppm CO_2 on three lettuce varieties. The goal of the second experiment is to find out the difference between 600 and 1000 ppm and to compare the data with the first experiment. The reason for choosing 1500 ppm was because of advice from Kaneya ltd. in Japan that had positive results in their facilities and 1000 ppm because of general positive results in literature. Another goal of the enrichment in general was to find out how the wobbling and stretching of plants, that was seen at lower levels of CO_2 in earlier research at the Kaneya ltd. and Own Greens companies, could be reduced under higher CO_2 levels.

In total there were in both experiments 72 lettuce plants from three varieties divided over two 3-layered home sets with LED from Own Greens. The reason for choosing the 3-layered home-set was that these can fit in the V-cube (figure 5). Each layer could fit twelve plants from the start (figure 6). Six plants were used for the end results at week 5 (figure 8), the other six were used to create a realistic as possible set-up until week 4 (figure 7), because in future cultivation the crops at Own Greens will be grown like that too.

2.2 Method

To start the experiments seeds were first sown. The seeds for the experiments are sown on two 140-plugs trays and after one week they will transplanted in the two home sets in room CO_2 levels and in a V-cube on 1500 ppm and in the second experiment on 1000 ppm CO_2 during light hours. The lettuce has grown for at least 5 weeks; this is the standard protocol for lettuce grown at Own Greens. The chosen lettuce seeds came from two different companies. The Black Rose, Red Span and Tough Red were coming from Japan, the Kaneya ltd. company. The Cook, Cristabel and Ilema varieties came from Bejo Zaden in the Netherlands.

The reason for choosing these varieties was because of earlier small-scale experiments in Japan at the Kaneya company produced the biggest visible difference in morphology and fresh weight. In the second experiment the results were compared to the first experiment.

2.2.1 Sowing

The company's protocol for sowing in a 140 holed tray in the laboratory was used. The medium used to soak the rockwool plugs was 1750 ml filtered water with one Calcinit and one Scarlet tablet (appendix I). The chosen tested seed varieties for the two experiments are described in table 1. In table 2 the sowing order for each experiment is written down. During the second experiment the Ilema variety was replaced by Though Red. Results from Tough Red on 1500 ppm and 'Ilema' on 1000 ppm CO_2 will be missing because of this. Next to the chosen lettuce varieties (table 1) some extra seeds from the Cook and Amica variety were sown on the outside of the tray to reduce a possible effect from the side shadow of the trays on the chosen varieties (table 2).

Table 1: lettuce varieties that are tested in the two experiments.

Tested lettuce varieties in experiment 1 on 600 and 1500 ppm CO_2	Tested lettuce varieties in experiment 2 on 600 and 1000 ppm CO_2
• Red Span • Cristabel • Ilema	• Red Span • Cristabel • Though Red

Table 2: order of sowing seeds in the two experiments in the 140 holed trays

First experiment	Second experiment
• 30 seeds Amica (three rows) • 20 seeds Ilema (two rows) • 20 seeds Red Span (two rows) • 20 seeds Cristabel (two rows) • 50 seeds Cook (two rows)	• 30 seeds Amica (three rows) • 20 seeds Though Red (two rows) • 20 seeds Red Span (two rows) • 20 seeds Cristabel (two rows) • 50 seeds Cook (two rows)

Figure 4: Seed germination for the first experiment after one week on the 28th of February. On the left for the V-cube and on the right the control (photo: Richard Steenvoorden, 2019)

After sowing was finished the two trays were marked with necessary information like: the date, the lettuce varieties, and the medium used and were then placed in the corresponding places: the V-Cube and in the office. Settings as described in table 3 were used. Moisture, light and temperature levels were kept approximately the same in both LED home-sets from Own Greens (table 3).

Table 3: V-Cube and Own Greens lab room atmospheric conditions

V-Cube settings	**Own Greens lab room**
RV on 50%. 20h light (5:00-01:00) and 4h dark (01:00-05:00). LED: ≈107mmol/s Experiment 1: 1500 ppm CO_2 during light, Experiment 2: 1000 ppm CO_2 during light, and both experiments 500 ppm CO_2 during dark hours. Temperature 21 degrees	RV measured in room between 35 and 55.0% 20h light (5:00-01:00) and 4h dark (01:00-05:00) LED: ≈107mmol/s Atmospheric room CO_2 levels (Average indoor 600 ppm CO_2) Temperature 21 degrees.
 *Figure 5: V-Cube with the Own Greens home-set and planted lettuce (Photo: Richard Steenvoorden, 2019).*	 *Figure 6: Lab room with the Own Greens Home-set and planted lettuce (Photo: Richard Steenvoorden, 2019).*

2.2.2 Planting seedlings

After growing for one week in the tray, the seedlings were transplanted to Kaneya containers. For the out planting, gloves were put on to try to work as sterile as possible to prevent possible contaminations. Kaneya containers were filled with one calcinate and one scarlet tablet which contained all of the necessary plant nutrients (appendix I) and last 700 ml of filtered water was added, the tablets were dissolved in the water after 30 minutes.

This was planned the day before because it took time to seal, cut and write down the corresponding codes on the Kaneya containers (table 4 and 5). For each experiment 72 Kaneya containers had to be sealed with white foil. The next step was that they got one hole in the foil for the plant plug with help of a soldering iron. The last step was a post-it with all the information that was placed on the white trays as extra information for other employees.

Table 4: experiment 1 set-up after planting out

# containers	Code	Variety	CO_2 ppm
12	0-(1 to 12)	Ilema	≈600
12	1-(1 to 12)	Red span	≈600
12	2-(1 to 12)	Cristabel	≈600
12	3-(1 to 12)	Ilema	1500
12	4-(1 to 12)	Red span	1500
12	5-(1 to 12)	Cristabel	1500

Table 5: experiment 2 set-up after planting out

# containers	Code	Variety	CO_2 ppm
12	0-(1 to 12)	Though Red	≈600
12	1-(1 to 12)	Red span	≈600
12	2-(1 to 12)	Cristabel	≈600
12	3-(1 to 12)	Though Red	1000
12	4-(1 to 12)	Red span	1000
12	5-(1 to 12)	Cristabel	1000

Seedlings of lettuce plants of approximately the same size were chosen for a minimum in growth variety. And the plugs were added directly in the holes, as far as the plugs touch the water. The containers were then placed according to table 4 and 5 in their LED home-sets from Own Greens. In the lab room there was minimal extra light from other sources in the room, this level was so low that it would not have had significant effect on the growth of the plants.

2.2.3 Harvesting

During the experiments, after four weeks, 36 plants (3 layers 600 ppm and 3 layers 1000/1500 ppm CO_2) were scored on fresh weight (table 4 and 5) and water use and were then discarded to make space for the remaining 36 plants that were used for final data collection that was used for answering the research question (table 6).

Table 6: measurements on different characteristics in week 4 and week 6

Measurements	**Week 4**	**Week 5**
Fresh weight in gr	6 of code #1, #2, #3, #4, #5, and #6	Remaining 6 plants per layer
Height in cm	Not scored	Remaining 6 plants per layer
Stem length in cm	Not scored	Remaining 6 plants per layer
Taste (1-5)	Not scored	Remaining 6 plants per layer
Water refill in ml	6 of code #1, #2, #3, #4, #5, and #6	Not scored
Water use in ml	6 of code #1, #2, #3, #4, #5, and #6	Remaining 6 plants per layer
Total # observations	2 measurements x 6 plants x 6 layers. = 72 data points.	5 measurements x 6 plants x 6 layers. = 360 data points.

Fresh weight and that was left was measured with a scale, just like the remaining water which was then deducted from the start 700 ml to get the total amount of water use in the first four weeks.

After the measurements and noting of the amount of refill with filtered water in the containers of the 36 remaining lettuce plants, they were placed more evenly distributed under the light (6 plants per layer) and left alone for another week. In week 5 they were again scored on the same characteristics (table 6, week 5) plus the height and stem length of the plants, which were measured with a ruler. The next measurement was the taste, which was measured with a score from bitter (1) to sweet (5) by two people per plant, which gave an average score. As last the water use in total was noted, by measuring the remaining ml of water that has been deducted from the starting 700 ml plus the refill that was given in week 4.

After these measurements of the lettuce plants that have grown in 600, 1000 and 1500 ppm CO_2, the data was put in Excel for creating average numbers per variety and CO_2 concentration. This data was then put in graphs to give a proper overview. Data was later also processed in SPSS with a paired T-test to find a possible significant difference between the applied CO_2 concentrations on these different lettuce varieties. With these results the sub-questions and main question are answered.

In total four tested lettuce varieties were scored: Ilema, Red Span, Cristabel, and Tough Red. Under room atmospheric level CO_2 600 ppm and enriched levels of 1000 and 1500 ppm CO_2. The variety Ilema was only tested in experiment 1 and Tough Red, as the replacement of Ilema, only in experiment 2 (table 7). From sowing to harvesting half of the plants per tray grew for four weeks (figure 7). The other half was spaced and grew for another week (figure 8).

Table 7: Test design of lettuce varieties and corresponding CO_2-levels with their number of replications.

CO_2 LEVEL	LETTUCE VARIETIES			
	Ilema	**Tough Red**	**Red Span**	**Cristabel**
600 ppm	1x	1x	2x	2x
1000 ppm		1x	1x	1x
1500 ppm	1x		1x	1x

Figure 7: Harvesting 6/12 of Cristabel lettuce in week 4 of experiment 2.

Figure 8: Spaced Cristabel plants in week 4 of experiment 2.

3. Results

In this chapter the results of this study will be presented and described. Data from experiment one can be found in Appendix III, data from experiment two can be found in Appendix IV, and statistics on combined data in SPSS can be found in Appendix V. All results noted are rounded off to two decimal places.

This chapter is divided into five paragraphs corresponding to the sub-questions:

- § 3.1 Yield in gram per variety under different CO_2 concentrations.
- § 3.2 Height in centimetre per variety under different CO_2 concentrations.
- § 3.3 Stem length in centimetre per variety under different CO_2 concentrations.
- § 3.4 Water-use efficiency in millilitre per gram measured per variety under different CO_2 concentrations.
- § 3.5 Taste from bitter to sweet (1-5) per variety under different CO_2 concentrations.

3.1 Yield

Lettuce plants of both experiments were harvested five weeks after sowing. There is a difference between average fresh yield of the varieties but also between CO_2 concentrations (figure 9). Ilema had less yield under 1500 ppm (95.67 vs. 81.5 gr). Tough Red had 2.9 gram less yield under 1000 ppm (47.5 vs. 44.6 gr.). Red Span had the highest yield under 600 ppm in control 1 (83.73 gr.), the lowest under 600 ppm in control 2 (54.67 gr.). Cristabel had the highest yield under 1500 ppm (104.33 gr.). Cristabel is the only variety that exceeded above an average 100 gram after five weeks under 1500 ppm. Tough Red is the slowest growing variety, only managing to reach the 47.5 gram after five weeks. Most difference between plant fresh weight yield in one treatment was found in Christabel at 1500 ppm CO_2 (St Dev = 12.99), and the least at Red Span under 600 ppm CO_2 (St Dev = 2.21).

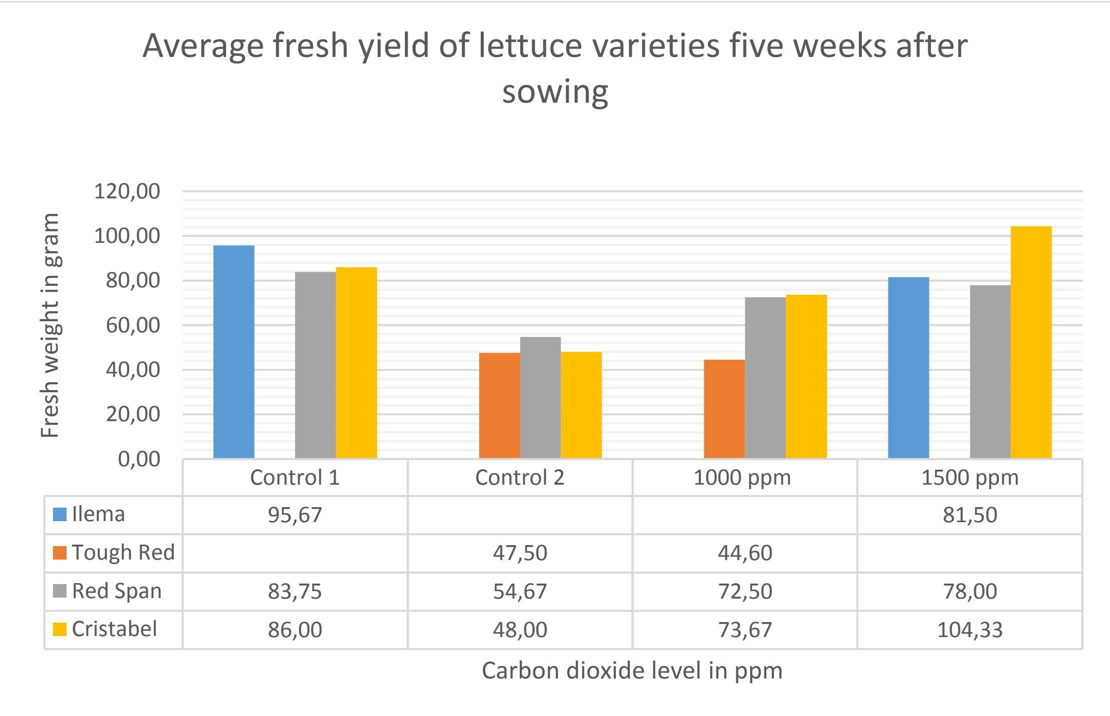


Figure 9: Average fresh yield of all lettuce varieties under 600, 1000 and 1500 ppm CO_2 five weeks after sowing

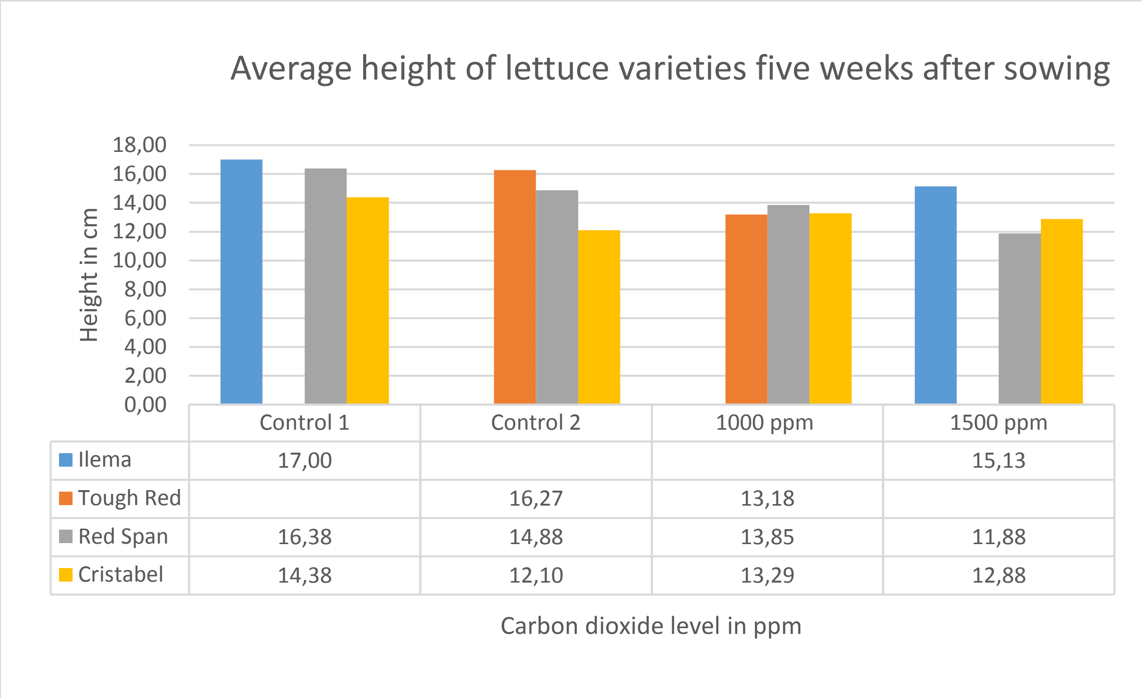
For statistical analysis (table 8) is chosen for a paired T-test between control (both 600 ppm) and the CO_2-enrichment (1000 or 1500 ppm). In pink the 0-hypothesis is not rejected, so there is no difference in effect of CO_2. In green the 0-hypothesis is rejected, so there is a significant difference of CO_2 on fresh weight yield of the lettuce variety. For Red Span (600 vs 1000 ppm), Cristabel (600 vs 1000 and 1500 ppm) the 0-hypothesis is rejected, so there is a difference in yield between the two CO_2 concentrations.

Table 8: Statistical analysis of fresh weight yield between control and CO_2 enrichment (Appendix V, figure II).

Null hypothesis	CO_2 level control	CO_2 level Vi-Cube	Variety	Statistic test	St Dev	Significance
There is no significant difference in effect on CO_2 levels on fresh weight yield in gram.	600	1500	Ilema	Paired T-test	7.23	P=0.08
	600	1000	Tough Red	Paired T-test	8.09	P=0.61
	600	1000	Red Span	Paired T-test	6.04	P=0.00
	600	1500	Red Span	Paired T-test	10.53	P=0.36
	600	1000	Cristabel	Paired T-test	11.00	P=0.03
	600	1500	Cristabel	Paired T-test	6.98	P=0.00

3.2 Height

On average Tough Red, Red Span and Cristabel were significant reduced in height with increasing levels of CO_2 (table 9). Ilema was also reduced in height, but not significant enough. Just like Cristabel on 1000 ppm compared to 600 ppm CO_2 (figure 10). In general there was a steady decline with every increasing amount of CO_2. Red Span is the variety that has the highest effect of CO_2 on height (figure 11). Most difference between plant height in one treatment was found in Christabel at 1500 ppm CO_2 (St Dev = 1.65), and the least at Ilema under 600 ppm CO_2 (St Dev = 0.48).



	Control 1	Control 2	1000 ppm	1500 ppm
Ilema	17,00			15,13
Tough Red		16,27	13,18	
Red Span	16,38	14,88	13,85	11,88
Cristabel	14,38	12,10	13,29	12,88

Figure 10: Average height in cm of lettuce varieties under 600, 1000 and 1500 ppm CO_2 five weeks after sowing.

Figure 11: Difference in height of Red Span control (600 ppm) and in the Vi-Cube (1500 ppm) after five weeks.

For statistical analysis (table 9) is chosen for a paired T-test between both control (both 600 ppm) and the CO_2-enrichment (1000 or 1500 ppm). In pink the 0-hypothesis is not rejected, so there is no difference in effect of CO_2 -level. In green the 0-hypothesis is rejected, so there is a significant difference of CO_2 on height of the lettuce variety. For Tough Red (600 vs 1000 ppm), Red Span (600 vs 1000 and 1500 ppm), Cristabel (600 vs 1500 ppm) the 0-hypothesis is rejected, so there is a difference in height between the two CO_2 concentrations.

Table 9: Statistical analysis of height in cm between control and CO_2 enrichment (Appendix V, figure III).

Null hypothesis	**CO_2 level control**	**CO_2 level Vi-Cube**	**Variety**	**Statistic test**	**St. Dev.**	**Significance**
There is no significant difference in effect on CO_2 levels on height in cm.	600	1500	Ilema	Paired T-test	1.80	P=0.13
	600	1000	Tough Red	Paired T-test	1.36	P=0.03
	600	1000	Red Span	Paired T-test	0.91	P=0.02
	600	1500	Red Span	Paired T-test	0.96	P=0.048
	600	1000	Cristabel	Paired T-test	0.58	P=0.19
	600	1500	Cristabel	Paired T-test	1.50	P=0.01

3.3 Stem length

With 600 ppm of CO_2 compared to 1000 or 1500 ppm CO_2, stem length of all varieties decreased (figure 12). Only the stem length of Cristabel and Red Span under 600 ppm (control) in the second experiment were shorter than under 1000 ppm CO_2. Ilema was in week 5 already in a later phase of generative growth under 600 ppm CO_2, this was greatly reduced under 1500 ppm CO_2. Most difference between plant stem length in one treatment was found in Ilema at 600 ppm CO_2 (St Dev = 0.26), and the least at Cristabel under 600 ppm CO_2 (St Dev = 0.08).

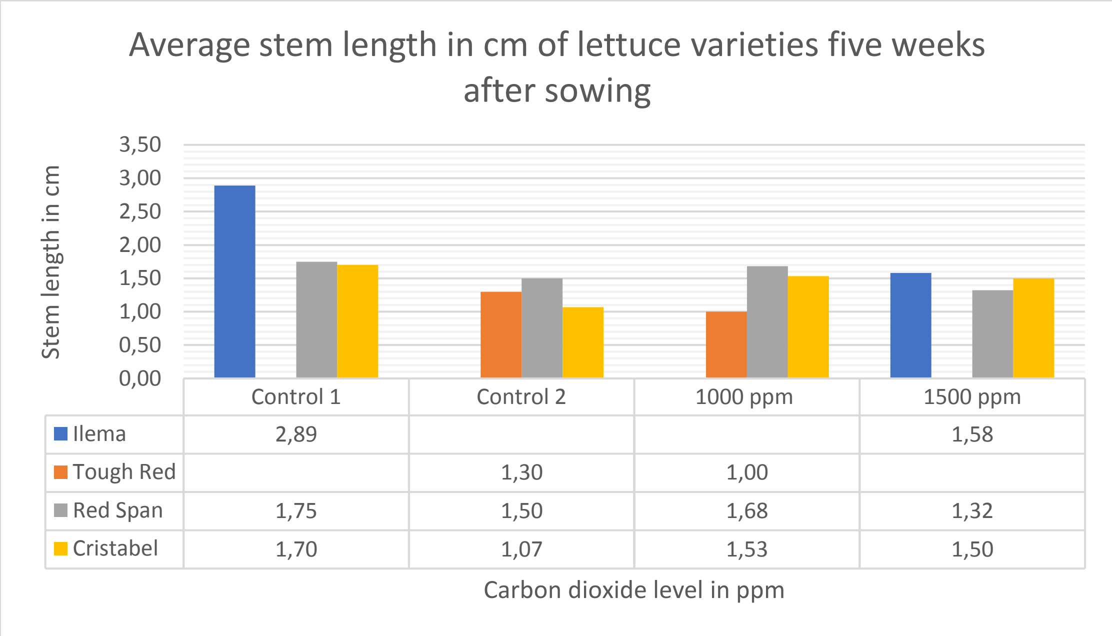


Figure 12: Average stem length in cm of lettuce varieties under 600, 1000 and 1500 ppm CO_2 five weeks after sowing.

For statistical analysis (table 10) is chosen for a paired T-test between both control (both 600 ppm) and the CO_2-enrichment (1000 or 1500 ppm). In pink the 0-hypothesis is not rejected, so there is no difference in effect of CO_2-level. In green the 0-hypothesis is rejected, so there is a significant difference of CO_2 on the stem length of the lettuce variety. For Ilema (600 vs 1500 ppm), Tough Red (600 vs 1000 ppm), Red Span (600 vs 1500 ppm), Cristabel (600 vs 1000 and 1500 ppm) the 0-hypothesis is rejected, so there is a difference in stem length between the two CO_2 concentrations.

Table 10:Statistical analysis of stem length cm between control and CO_2 enrichment (Appendix V, figure IV).

Null hypothesis	**CO_2 level control**	**CO_2 level Vi-Cube**	**Variety**	**Statistic test**	**St. Dev.**	**Significance**
There is no significant difference in effect on CO_2 levels on stem length in cm.	600	1500	Ilema	Paired T-test	0.22	P=0.01
	600	1000	Tough Red	Paired T-test	0.31	P=0.12
	600	1000	Red Span	Paired T-test	0.28	P=0.17
	600	1500	Red Span	Paired T-test	0.21	P=0.03
	600	1000	Cristabel	Paired T-test	0.15	P=0.04
	600	1500	Cristabel	Paired T-test	0.10	P=0.01

3.4 Water-use efficiency

There is a difference in water-use efficiency between varieties (figure 13). But only Ilema has a significant response on enriched CO_2 levels in a negative way, the variety used more water (+3.25 ml/g) at 1500 ppm (table 11). Tough Red has an decreased water-use efficiency under enriched levels (+1.9 ml/gr), while not significant (p=0.07). Cristabel and Red Span have an increased water-use efficiency with increasing levels of CO_2, but also not significant enough. Tough Red is the variety that uses the most water per gram fresh yield, while Cristabel is the variety that uses the least water overall per gram fresh yield. Most difference between plant water-use in one treatment was found in Cristabel at 600 ppm CO_2 (St Dev = 1.25), and the least at Cristabel under 1500 ppm CO_2 (St Dev = 0.28).

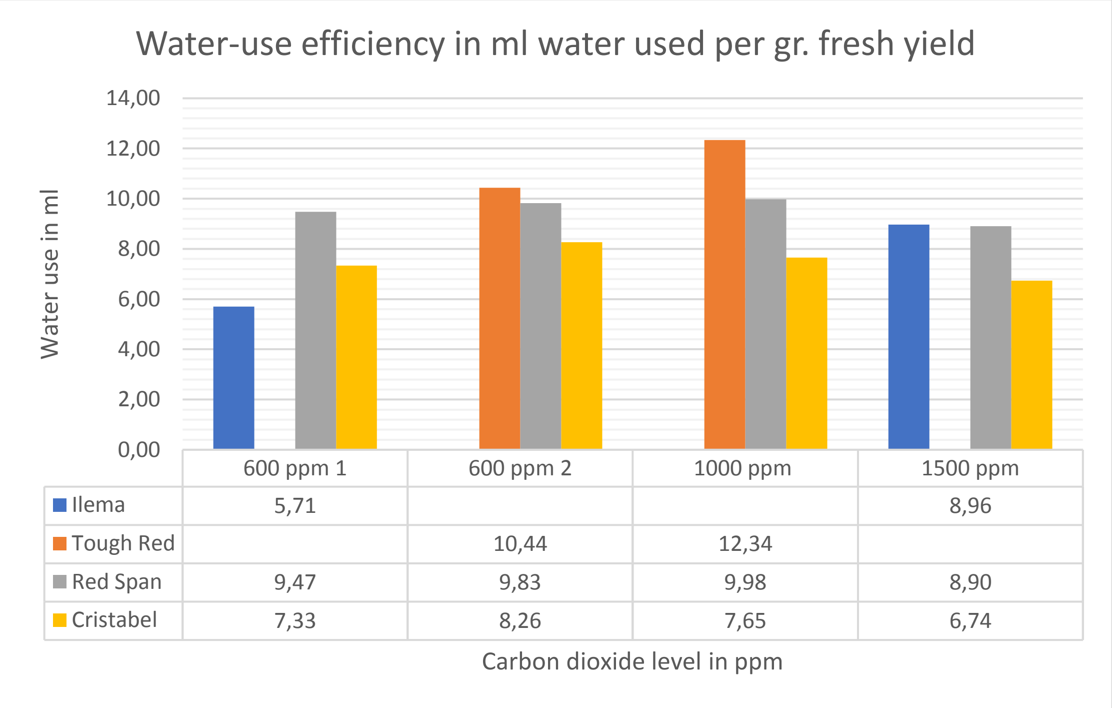


Figure 13: Average water-use efficiency in ml water used per gr. fresh yield of lettuce varieties under 600, 1000 and 1500 ppm CO_2 five weeks after sowing.

For statistical analysis (table 11) is chosen for a paired T-test between both control (both 600 ppm) and the CO_2-enrichment (1000 or 1500 ppm). In pink the 0-hypothesis is not rejected, so there is no difference in effect of CO_2 -level. In green the 0-hypothesis is rejected, so there is a significant difference of CO_2 on water-use efficiency of the lettuce variety. For Ilema (600 vs 1500 ppm) the 0-hypothesis is rejected, so there is a difference in water-use efficiency between the two CO_2 concentrations.

Table 11: Statistical analysis of water-use efficiency between control and CO_2 enrichment (Appendix V, figure V).

Null hypothesis	CO_2 level control	CO_2 level Vi-Cube	Variety	Statistic test	St. Dev.	Significance
There is no significant difference in effect on CO_2 levels on water use efficiency in ml per gram fresh yield.	600	1500	Ilema	Paired T-test	0.66	P=0.03
	600	1000	Tough Red	Paired T-test	1.17	P=0.07
	600	1000	Red Span	Paired T-test	0.48	P=0.17
	600	1500	Red Span	Paired T-test	0.59	P=0.52
	600	1000	Cristabel	Paired T-test	1.42	P=0.07
	600	1500	Cristabel	Paired T-test	0.43	P=0.25

3.5 Taste

The effect on the taste per variety and under different CO_2 concentrations is scored from bitter (1) to sweet (5) by two persons (figure 14). Ilema (+1.25), Tough Red (+0.18) and Cristabel 1000 ppm (+0.79) and 1500 ppm (+0.17) were all sweeter in score with increased levels of CO_2 compared to 600 ppm, but only Ilema (p=0.03) and Cristabel (600 vs. 1000 ppm, p=0.04) were significant enough (table 12). Red Span was the sweetest variety in score overall (4.8) and CO_2 didn't have significant effect on the taste (p=1 and p=0.7) (table 12). Most difference between plant taste in one treatment was found in Ilema at 600 ppm CO_2 (St Dev = 0.6), and the least at Cristabel under 600 ppm CO_2 (St Dev = 0.00).

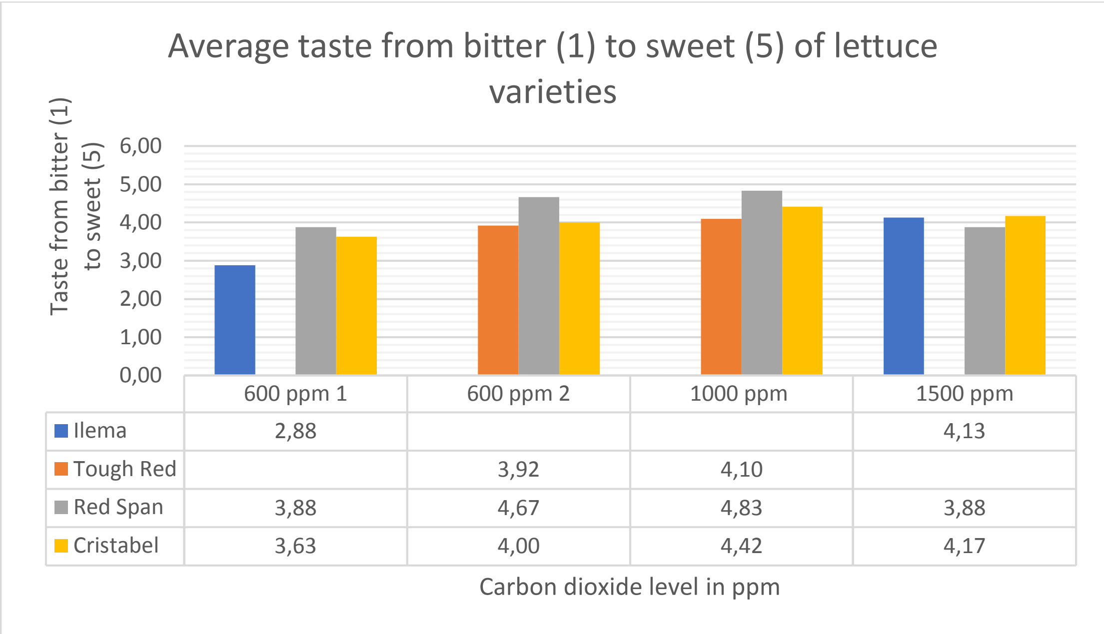


Figure 14: Average taste from bitter to sweet (1-5) of lettuce varieties under 600, 1000 and 1500 ppm CO_2 five weeks after sowing.

For statistical analysis (table 12) is chosen for a paired T-test between control (both 600 ppm) and the CO_2-enrichment (1000 or 1500 ppm). In pink the 0-hypothesis is not rejected, so there is no difference in effect of CO_2 -level. In green the 0-hypothesis is rejected, so there is a significant difference of CO_2 on the taste of the lettuce variety. For Ilema (600 vs 1500 ppm), and Cristabel (600 vs 1000) the 0-hypothesis is rejected, so there is a difference in taste between the two CO_2 concentrations.

Table 12: Statistical analysis of the taste from bitter to sweet between control and CO_2 enrichment (Appendix V, figure VI).

Null hypothesis	CO_2 level control	CO_2 level Vi-Cube	Variety	Statistic test	St. Dev.	Significance
There is no significant difference in effect on CO_2 levels on the taste of the lettuce variety	600	1500	Ilema	Paired T-test	0.65	P=0.03
	600	1000	Tough Red	Paired T-test	0.42	P=0.10
	600	1000	Red Span	Paired T-test	0.49	P=1
	600	1500	Red Span	Paired T-test	0.25	P=0.7
	600	1000	Cristabel	Paired T-test	0.38	P=0.04
	600	1500	Cristabel	Paired T-test	0.87	P=0.42

4. Discussion

The discussion is divided in three paragraphs, first paragraph (§ 4.1) has discussion about the yield, the second paragraph (§4.2) about height, the third paragraph (§4.3) about stem length, the fourth paragraph (§4.4) about water-use efficiency, the fifth paragraph (§4.5) about the taste and the sixth (§4.6) will give an reflection about the research.

The main goal of this research was to see what kind of effect enriched CO_2 levels has on the growth of production, water-use efficiency and taste of different lettuce varieties. CO_2-enrichment clearly has an effect on plant growth but noted that there was in general a lot of variety per characteristic and per lettuce variety.

4.1 Yield

As in seen in figure 7 and table 8 is that only Red Span (600 vs 1000 ppm, p= 0.00) and Cristabel (600 vs 1000 ppm, p=0.03 and 600 vs 1500 ppm, p=0.00) had a significant effect of CO_2 enrichment on the fresh weight yield. While for Ilema there was a reasonable difference of 14.7 gr., it was not significant enough (p=0.07). Also noticed was that the Ilema plants grown at 600 ppm CO_2 were very wet, so excess water was removed before harvesting. Tough Red had no significant effect in growth (p=0.71), and compared to the other varieties it is a slow growing race, only reaching the 47.5 gram after five weeks. So for trustworthy results the variety should grow for at least one more week to reach a comparable fresh yield weight with the other varieties.

In general it was expected that the CO_2 enrichment would decrease photorespiration, which is a prime target for crop improvement (Rashad, et al., 2007), with which it is possible to improve the yield of crops. In greenhouses and open fields the yield can be 30 to 50% improved. (Blom, et al., 2016) (Mckeehen, et al., 1996) (Vanuytrecht, Raes, & Willems, 2012) (Kozai & Niu, 2016). This because higher CO_2 levels will lead to higher carbon uptake by stimulating photosynthesis and inhibiting photorespiration (Prior, et al., 2011).

This hypothesis has been proven true for the varieties Red Span and Cristabel. Red Span under 1000 ppm CO_2 saw an increase of 32.61%, Cristabel under 1000 ppm CO_2 53.48% and Cristabel under 1500 ppm CO_2 saw an increase of 21,34% fresh weight yield compared to 600 ppm CO_2.

4.2 Height

In total four out of six CO_2 enriched treatments from the two experiments were significant smaller. These were Tough Red (1000 ppm CO_2, P=0.03), Red Span (1000 ppm CO_2, P=0.02, and 1500 ppm CO_2, P=0.048) and Cristabel (1500 ppm CO_2, P=0.01). Ilema had a reduction in growth under 1500 ppm CO_2, from 17.3 cm to 15.13 cm, but this was not significant. For Cristabel the height under 1000 ppm CO_2 was 13.29 cm compared to 12 cm at 600 ppm CO_2, but noted that there was a huge difference in fresh weight yield of 25 gram more under CO_2 enrichment (figure 9). Ilema, Tough Red and Red Span are in general comparable in size, while Cristabel is a more compact variety in general because of the thicker leaves.

In advance it was not really clear what the effect of CO_2 enrichment would be on the compactness of the different Lettuce varieties. There were some earlier experiences at the Own Greens company with lettuce grown under higher CO_2 concentrations, which pointed to some more compact plants, but this was never tested in an extensive study. Under 600 ppm CO_2 there was a common problem with wobbling plants, it was hoped that an enriched concentration of 1000 or 1500 ppm would reduce that problem. This research shows that if there is no big difference in fresh weight yield as seen at Cristabel, all the varieties will have a reduced height with higher concentrations of CO_2, which also reduced the wobbling of the crop on the container.

4.3 Stem length

The variety Ilema (p=0.01) was the variety with the highest significant reduction of stem length (table 10), 2.83 cm for 600 ppm CO_2 versus 1.58 for under 1500 ppm CO_2, which is a reduction of 1.31 cm (figure 12). Also stem length of Red Span (600 vs 1500 ppm CO_2, p=0.03) and Cristabel (600 vs 1000 CO_2, p=0.04 and 600 vs 1500 ppm CO_2, p=0.01) were significant shorter, the only thing that was noticed that under 1000 ppm CO_2 average Cristabel stem length was longer than at 600 ppm, the opposite of under 1500 ppm CO_2. Both Tough Red (600 vs 1000 ppm, p=0.12) and Red Span (600 vs 1000 ppm, p=0.17) stem length were not significantly reduced.

There were some signals that stem length would be shortened under CO_2 enrichment, but during the preliminary research yet no literature was found about it yet. During the research an article was found about delaying generative growth by improving the growth conditions (Park, et al., 2012). Which means that there should be an optimum in CO_2 concentration for reaching an optimum in environmental condition. In that way the vegetative growth can be elongated while the generative growth is delayed. The hypothesis for lettuce stem length would be then that lower CO_2 concentrations have a shorter vegetative growth thus making the stem length shorter compared to 1000 or 1500 ppm CO_2. The hypothesis was true for three out of six treatments, namely Red Span, Cristabel and Ilema at 1500 ppm CO_2.

4.4 Water-use efficiency

The only significant difference in water use-efficiency was seen at Ilema (p=0.03), in a negative way. It used more water under 1500 ppm compared to the 600 ppm CO_2, this was the same for Tough Red, while not significant (600 vs 1000 ppm, p=0.07). In average numbers Red Span (p=0.17 and p=0.52) and Cristabel (p=0.07 and p=0.25) were more efficient with water use compared to 600 ppm, but not significant enough. These results could be different if the plants would have grown for 6 weeks, reaching the 100 grams of fresh yield.

The hypothesis was here that water use efficiency would increase by a more enhanced photosynthesis (Prior, et al., 2011), which would reduce crop water use (Kimbal, 2011) (Hiemann & Reichstein, 2008). Which is also seen in conventional agriculture, where the water use was reduced 4 to 17 percent (Deryng, et al., 2016). This hypothesis was not proven for hydroponic farming, it doesn't improve the water-use efficiency on such a small scale. This could be explained by that the hydroponic system already is very efficient and that the amount of water used in the Kaneya containers (700 ml) is too little to see any significant difference after five weeks of crop growth. If the lettuce is grown for six weeks this might change because lettuce uses the most water in the final week.

4.5 Taste

CO_2 enrichment had effect on the taste of Ilema (600 vs 1500 ppm, p=0.03) and Cristabel (600 vs. 1000 ppm, p=0.04). Cristabel saw the average taste going up from 2.88 to 4.13, this is comparable with the reduction in stem length. The CO_2 enrichment had absolutely no effect on the taste of Red Span (p=1 and p=0.7), which also is the sweetest variety overall. Cristabel under 1500 ppm had, in contrast to the treatment of 1000 ppm, also no significant effect on the taste (p=0.42). The taste of Tough Red had also no significant influence under 600 vs 1000 ppm (p=0.1).

The hypothesis was that with delaying the generative growth, the taste of the leaves could be sweeter because during generative growth the sugars are concentrated to the elongating stem (Park, et al., 2012). This is because sugars help in the transition from the vegetative to the generative phase in plants (Rolland, Baena-Gonzalez, & Sheen, 2006). This hypothesis was also proven significant for

Ilema at 1500 ppm and Cristabel at 1000 ppm. So the effects of CO_2 on taste also depends on the variety, the green leaf lettuce varieties had that effect, the two red leaf varieties didn't.

It was also noticed that some of the results are comparable with the reduction in stem length. This was the case for Ilema at 1500 ppm, Tough Red at 1000 ppm, Red Span at 1000 ppm and Cristabel at 1000 ppm. This is because leaf sweetness is preserved by elongating vegetative growth.

For a greater difference in results, the plants should be harvested at least a week later, when the plants enter the generative growth phase when they are putting more energy in stem elongation. Harvesting five weeks after sowing is too short for most varieties. Only Ilema had entered generative growth under 600 ppm.

4.6 Reflection

The research was divided into two experiments. Where the first experiment was to find out the difference between 600 ppm and 1500 ppm CO_2 on three lettuce varieties, the reason for choosing 1500 ppm was from positive results in the Kaneya ltd. company in Japan where it had positive results in their facility and they recommended Own Greens in the Netherlands to test it on their Lettuce varieties. The second experiment was to find out de difference between 600 ppm and 1000 ppm and compare this with the first experiment. The first reason that 1000 ppm CO_2 was chosen is because of the damage that was seen on the new leaves in the Cristabel variety under 1500 ppm CO_2, to check whether the CO_2 enrichment was the cause for this. The second reason was because 1000 ppm had in general positive results in literature. In general multiple levels of CO_2 concentrations were chosen to find out if there is also difference in CO_2-sensitivity per variety, which in literature was earlier also proven different (Wheeler, et al., 2000).

The reason for choosing the number of 72 lettuce plants per experiment was because of the number of plants that could fit in the LED home-set of the Own Greens company. Only one set with a total of 36 plants could fit in the Vi-Cube, the machine where CO_2 levels could be controlled (figure 5).

The data from the 36 plants grown for 5 weeks was used for answering the sub- and main questions. The argument for choosing this duration of the experiment is to check whether the plants could reach a recommended 100 grams of fresh weight under CO_2 enrichment, with a fresh weight that is comparable with conventional grown lettuce when it is sold to costumers. After four weeks of growth the plants were spaced for more room, because when fully grown only six plants per total could fit on one layer in the LED home-set.

The reason for choosing the tested lettuce varieties was because of earlier small-scale experiments in Japan at the Keneya company where Red Span and Tough Red had a good visible difference in morphology and fresh weight, expected was that there should be an interesting difference under CO_2 enrichment. The Cristabel and Ilema variety were tested because there was not much known about the growth in hydroponic conditions of these varieties.

Ilema was a variety that didn't respond well on both 600 and 1500 ppm CO_2. The crop was too heavy for the stem which is why the crops fell over, the variety was too wet at harvest time under 600 ppm CO_2, some of the plants had tip burns on the new leaves at 1500 ppm CO_2, and it had also a bad marketable appearance in general. This happened only with this variety. Concluded was that Ilema doesn't grow well on hydroponic cultures, as it was bred for the open field. That is also the cause for replacing this variety with Tough Red in the second experiment.

In general the most significant results were observed at 1000 ppm CO_2, which is comparable with the research of Wheeler on hydroponic cultivated potatoes, where the maximum rates were observed at

1000 ppm (Wheeler R. M., 2006) and with other research on lettuce plants where at 1000 ppm the best results were observed (Caporn, et al., 1993) (Prior, et al., 2011). Choosing 1000 ppm CO_2 should be a starting point for cultivating lettuce for companies that do hydroponic farming, but noted that for every variety the optimum concentration of CO_2 should also first be examined if the facilities are available this because CO_2-sensitivity can differ per variety, which is also observed in the research of Wheeler (2000).

Some minor issues have occurred during the two experiments. Plants coded #5-7 (experiment 1, week 5) and #3-12 (experiment 2, week 5) have not been used for data analysing. #5-7, a Cristabel plant under 1500 ppm CO_2 enrichment in the first experiment ,was a plant that was lacking growth and tasted very bitter. #3-12, a Tough Red plant under 1000 ppm CO_2 enrichment in the second experiment was far behind in growth and had a severe fungal infection. This is 3.78% of the total plants measured in week 5.

In the first test in the Vi-cube, which was then set on 1500 ppm CO_2, the fungal infection rate was high, this due problems with the machine moist suction at the rear of the machine what appeared at the end of the first experiment. This might have had an effect on the average outcome of the varieties tested. As is seen in the difference in yield in gram between 1000 and 1500 ppm CO_2, but this could also have been oversensitivity of some varieties to an more enriched concentration of CO_2.

Extra noticed was that at enriched CO_2 concentrations more side- and air roots forming was visible. This was also noticed in the first week of the first experiment during seed sprouting under 1500 ppm CO_2 (appendix II). It was also visible that there was a difference in leaf surface, first cotyledons developed more early under CO_2 enrichment. There were also less ‘crawlers’, as it is called by employees at Own Greens when the roots have problems finding the way downwards through the plug. Finally, extra noted, it was clearly visible that the stems were thicker and shorter compared to the plants grown under 600 ppm. This was not measured as it was not part of the research, but this could be interesting for future research.

5. Conclusion

The conclusion is divided in three paragraphs, first paragraph (§ 5.1) will give conclusions on the sub questions, the second paragraph (§5.2) will give an conclusion on the main question, and the third paragraph (§5.3) will give an recommendation.

The goal of this research was to gain more knowledge about the effects of enriched CO_2 concentrations on the production, water-use efficiency and taste of lettuce varieties in practice, cultivated under hydroponic well-balanced indoor conditions and thereby improving and optimizing the growth of crops in hydroponic systems, and give an advice on the concentration of CO_2 per variety of crop and what the consequences are on the yield, height, stem length, water-use efficiency and taste of those varieties.

5.1 Sub questions

- *What is the effect of 600, 1000, and 1500 ppm CO_2 on the fresh weight yield in gram of the chosen lettuce varieties: Cristabel, Red Span, Tough Red, and Ilema?*

Fresh yield weight is different per variety and CO_2 concentration, Cristabel and Red Span have an significant effect in yield under more enriched levels of CO_2 compared to 600 ppm. While Ilema and Tough Red don't have an significant effect on enriched levels of CO_2.

- *What is the effect of 600, 1000, and 1500 ppm CO_2 on the height of a variety of the chosen lettuce varieties: Cristabel, Red Span, Tough Red, and Ilema?*

Compactness increased with higher concentrations of CO_2. Height is under all varieties less under enriched CO_2 levels. But for Ilema at 1500 ppm and Cristabel under 1000 ppm it was not significant enough, but there was a significant difference for Cristabel under 1500 ppm. The biggest difference in cm can be seen in Red Span.

- *What is the effect of 600, 1000, ands 1500 ppm CO_2 on the stem length of the chosen lettuce varieties: Cristabel Red Span, Tough Red, and Ilema?*

Stem length under enriched CO_2 was significant less with Ilema, Red Span and Cristabel, especially under 1500 ppm CO_2. But with Red Span and Cristabel there was a difference between the treatments in the two experiments. Under 1000 ppm the stem length was longer under enrichment compared to 600 ppm, while it was reduced at 1500 ppm compared to 600 ppm CO_2. There was no significant effect seen at the Tough Red variety. The biggest difference overall was seen with Ilema, were the generative growth was greatly reduced under CO_2-enrichment.

- *What is the effect of 600, 1000, and 1500 ppm CO_2 on the water use efficiency of the chosen lettuce varieties: Cristabel, Red Span, Tough Red, and Ilema?*

Water-use efficiency under enriched CO_2 levels is very different per variety but there was no positive significant effect seen in the treatments. Only Ilema under 1500 ppm was significant, in a negative way, where it used more water under enriched levels of CO_2.

- *What is the effect of 600, 1000, and 1500 ppm CO_2 on the taste of the chosen lettuce varieties: Cristabel, Red Span, Tough Red and Ilema?*

The variety Ilema was significant more sweeter at an enriched CO_2 level of 1500 ppm CO_2, Cristabel at 1000 ppm CO_2. Tough Red and Red Span are not significantly effected in their taste after five weeks of growth. The CO_2 sensitivity on taste is very dependent on the chosen variety. Here in this research the CO_2 enrichment had no significant effect on the taste red leafed lettuce varieties.

5.2 Main question

With answering these sub-questions the main question can now be answered:

- *What is the effect of enriched CO_2 concentrations on the production, taste and water-use efficiency Lettuce (Lactuca sativa) varieties?*

The outcome of the research is that extra CO_2 had an significant effect on the production characteristics of all varieties, but the effect differs per variety. Two out of four varieties had significant increased fresh weight on more CO_2. Height was significantly reduced at three out of four varieties. Stem length was significantly reduced at three out of four varieties. Extra CO_2 had no significant positive effect on water-use efficiency, only one negative significant effect. Extra CO_2 had on two out of four varieties effect on the taste, where the taste was sweeter.

5.3 Recommendation

With current results it is recommended to grow the tested lettuce varieties Red Span and Cristabel under enriched CO_2 levels, these were the varieties with most significant differences, so these are the varieties recommended to start cultivating under CO_2 enrichment if vertical Farming companies have the availability of a controlled environment where CO_2 levels can be set. Further testing on Tough Red is necessary to give trustworthy conclusions. Ilema is not recommended for cultivation in hydroponic environments.

1000 ppm CO_2 gives the best average results on measured plant characteristics. Especially the variety Red Span, that has a high plant height under normal atmospheric levels, which is reduced under enriched CO_2 levels. This variety is more compact while increasing the yield, this will work great in hydroponic cultures for mass production where the yield can rise per m^2. This improvement in efficiency be a small part of the solution for the decreasing agricultural land and the increasing worldwide population which will mostly in the future live in the bigger cities (Terazono, 2018).

For every different or new variety it is important to test which CO_2 concentration give the most optimal condition. It is also recommended to let all the lettuce varieties grow for at least six weeks, to reach an average crop weight of 100 gram, which is closer to the average weight of conventional cultivated lettuce.

For a next research it is recommended to retry the 1500 ppm CO_2 test on Cristabel, Red Span, and including the Tough Red variety to exclude that the decline in yield could come from the first fungal infections and to also have results and a conclusion on 1500 ppm CO_2 for Tough Red. During follow-up experiments even higher CO_2 enrichment above the 1500 ppm CO_2 could be tested to see where the decline or rise in production, water-use efficiency, and taste per variety starts or ends. The most decline is seen in literature at lettuce at super-elevated levels of 10.000 ppm CO_2 (Wheeler, et al., 2000), so that will be the recommended limit of CO_2 enrichment.

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Appendix I – Plant nutrients

YARA KRISTALON SCARLET NUTRIENT COMPOSITION

Nutrient	Weight percentage
N-Total	7.5%
NO_3-N	7.5%
P_2O_5	12%
K_2O	36%
MgO	1%
S	4%
B	0.027%
Cu-EDTA	0.004%
Fe-DTPA	0.075%
Fe-EDTA	0.075%
Mn-EDTA	0.06%
Mo	0.004%
Zn-EDTA	0.027%
Chlorine free	

YARALIVA CALCINIT NUTRIENT COMPOSITION

Nutrient	Weight percentage
N-Total	15.5%
NO_3-N	14.4%
NH_4-N	1.1%
CaO	26.5%

Appendix II – Seed sprouting

Seed sprouting in experiment 1 after day 2, 5 and 8. Noted in number of seeds sprouting against the total number of present seeds of the variety in the tray.

Table I: number of seeds sprouting of total seeds in experiment 1 on days 2, 5 and 8.

Race	Day 2 control	Day 2 CO_2	Day 5 control	Day 5 CO_2	Day 8 control	Day 8 CO_2
Amica	29/29	23/29	29/29	29/29	29/29	29/29
Red Span	16/19	19/20	17/19	19/20	19/19	20/20
Ilema	20/20	20/20	20/20	20/20	20/20	20/20
Cristabel	19/20	20/20	19/20	20/20	19/19	20/20
Cook	41/44	41/44	41/44	44/44	42/44	44/44

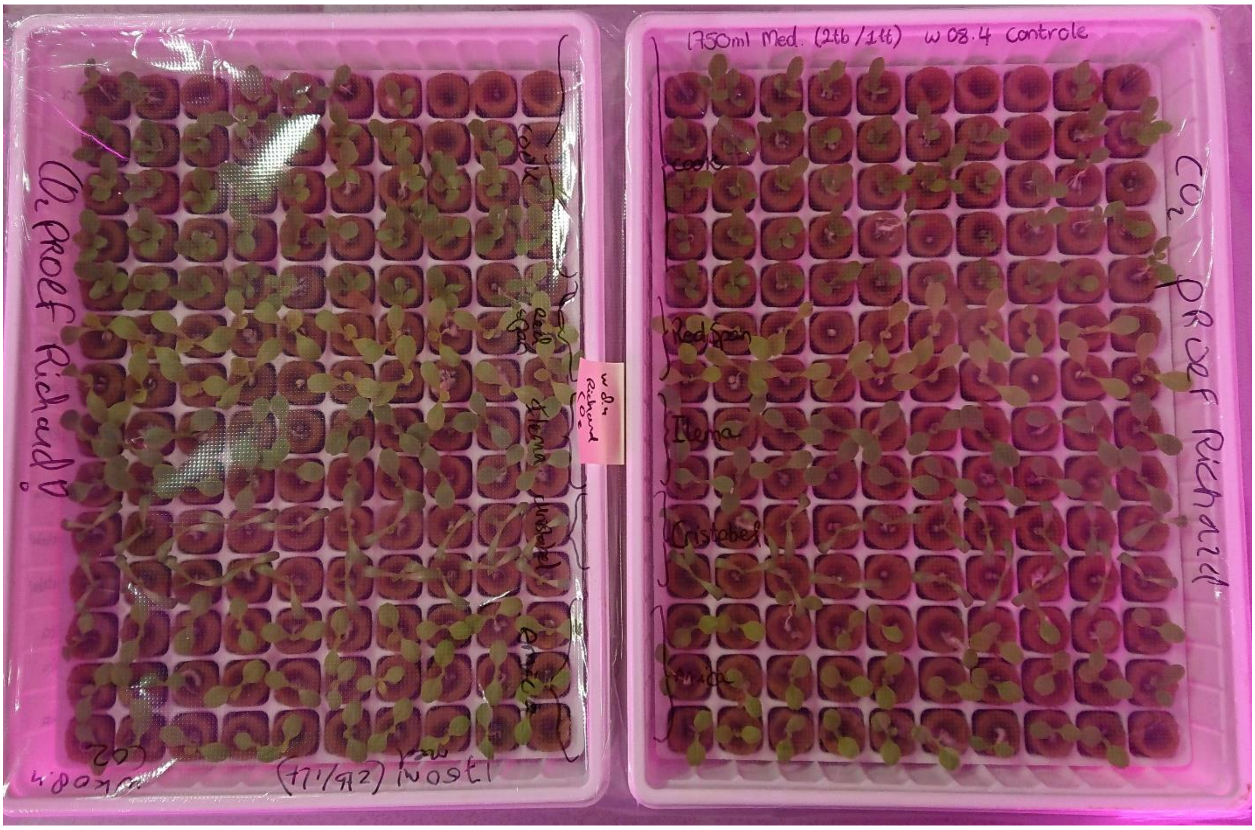
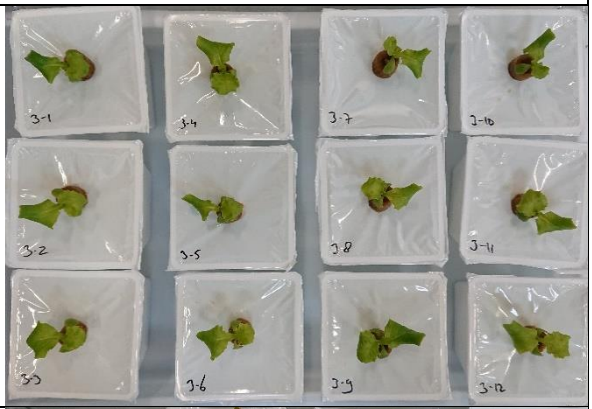
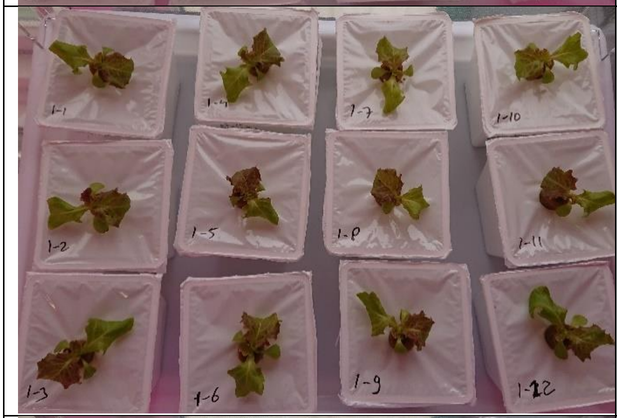
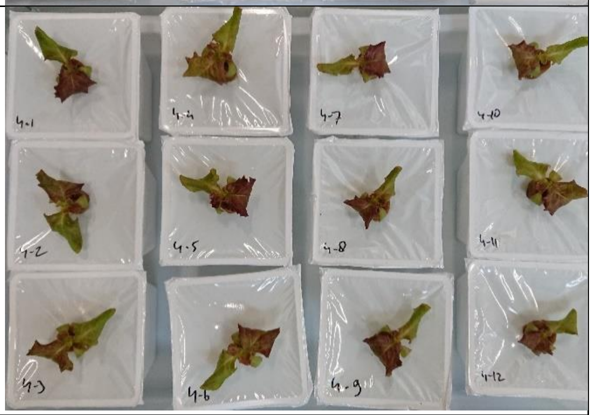
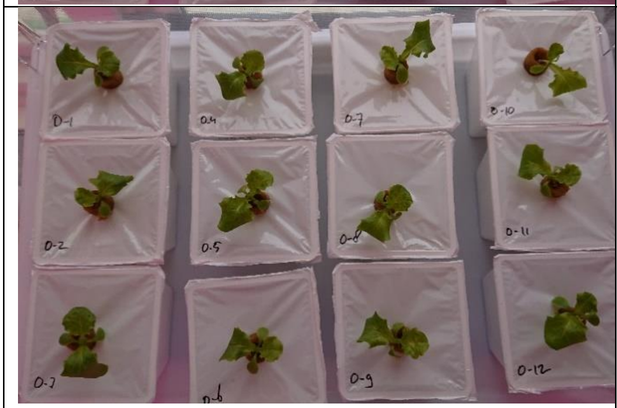
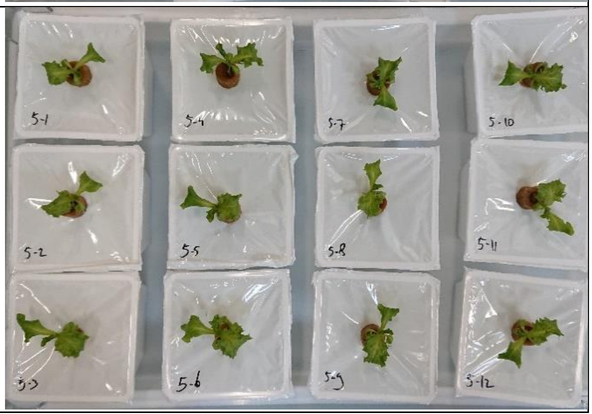


Figure I: Lettuce varieties seed sprouting during experiment one in tray 140 after one week.

Appendix III – Experiment 1

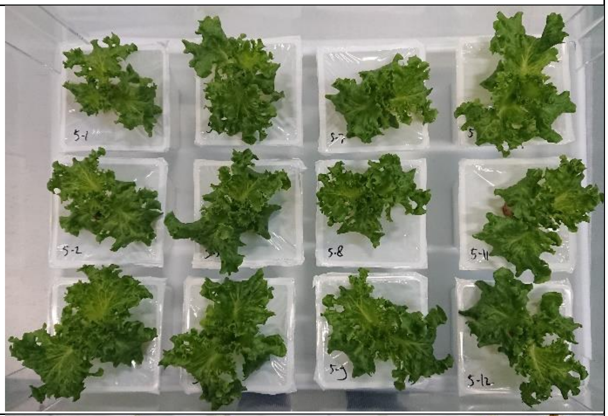
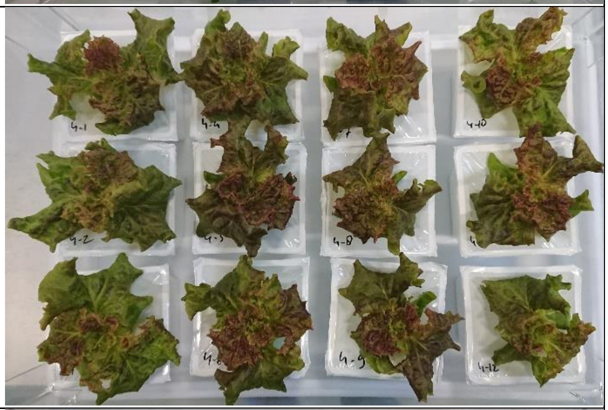
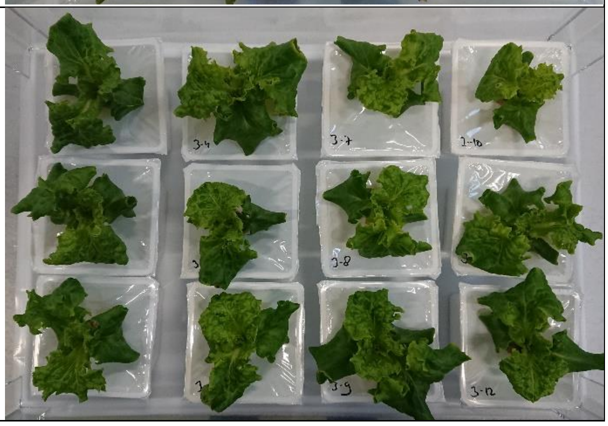
2 weeks after sowing, 1 week after planting (7th of march)

Table II: Lettuce plants development in experiment 1 two weeks after sowing.

Normal atmospheric levels 2. Cristabel 1. Red Span 0. Ilema	**1500 ppm CO_2 in Vi-Cube** 5. Cristabel 4. Red Span 3. Ilema
	
	
	

Three weeks after sowing (14^{th} of march)

Table 13: Lettuce plants development during experiment 1 three weeks after sowing

Normal atmospheric levels 2. Cristabel 1. Red Span 0. Ilema	**1500 ppm CO_2 in Vi-Cube** 5. Cristabel 4. Red Span 3. Ilema
	
	
	

4 weeks after sowing

Table IV: combined data of experiment 1, four weeks after sowing

Plant	Fresh weight in gr	Average per treatment in gr	Water left in ml	Total use water in ml	ml use per gr	Extra
0-1	24	25 (20,6)	499	201	177/25 = 7,08 (8,37)	
0-2	19		531	169		
0-3	20		539	161		
0-4	23		503	197		
0-5	28		501	199		
0-6	17		565	135		
1-1	19	20,8	475	225	243,75 / 20,8 = 11,72	
1-2	21		465	235		
1-3	19					
1-4	22		439	261		
1-5	21					
1-6	23		446	254		
2-1	19	22,3	508	192	211,75 / 22,3 = 9,49	
2-11	27		467	233		
2-3	20		491	209		
2-4	22		494	206		
2-5	26		477	223		
2-6	20		493	207		
3-3	20	20,7	462	238	249,8 / 20,7 = 12,06 (238,25 / 19,4 = 12,28)	Fungi
3-4	25		443	257		Lots of air roots
3-6	17		464	236		Fungi
3-8	23		478	222		Fungi
3-9	27		404	296		Fungi
3-10	12					Very small
4-1	21	20,7	480	220	221 / 20,7 = 10,67	
4-2	22		464	236		
4-3	18					Fungi
4-4	21		500	200		Fungi
4-5	21		458	242		Fungi
4-6	21		493	207		
5-1	17	25,8	524	176	222,2 / 25,8 = 8,61	Fungi
5-2	20		490	210		Fungi
5-3	33		462	238		Fungi
5-4	28		463	237		Fungi
5-5	26		463	237		Fungi
5-6	31		465	235		Fungi

5 weeks after sowing (28th of march)

Table V: Lettuce plants development of experiment 1 five weeks after sowing.

Normal atmospheric levels 2. Cristabel 1. Red Span 0. Ilema	**1500 ppm CO_2 in Vi-Cube** 5. Cristabel 4. Red Span 3. Ilema
	
	
	

5 weeks after sowing (28th of march)

Table VI: combined data of experiment 1, five weeks after sowing.

Plant	Fresh weight	Average	ml water left	Stem Length	SL/ Aver	Taste	Taste Aver	Height	Height Aver	Extra
0-7	99		273	3	2,89	3	2.88	19	17	Wet and unstable
0-8	95	96 (corrected)	345	2.9		3		16		Wet and unstable
0-9	93		352	2.5		3.5		16		Wet and unstable
0-10	72		407	3.1		2		17		Wet and unstable
1-9	83	83.75	208	1.9	1.75	4	3.88	15.5	16.38	
1-10	85		242	1.5		3.5		16		
1-11	86		230	1.8		4		17		
1-12	81		202	1.8		4		17		
2-2	78	86	313	1.4	1.7	3.5	3.63	13.5	14.38	Small stem
2-7	78		321	1.9		3.5		15		Very crispy
2-8	98		274	1.7		4		14		
2-9	90		274	1.8		3.5		14		Unstable
3-1	77	81.5	278	1.7	1.58	4	4.13	14.5	15.13	'Soft' sweet
3-7	85		201	1.8		4.5		15.5		
3-11	84		214	1.3		4		15		
3-12	80		196	1.5		4		15.5		Thick stem
4-7	93	78	188	1.3	1.32	4	3.88	12	11.88	
4-8	73		290	1.3		3.5		10.5		
4-10	75		277	1.5		4		13		
4-12	71		266	1.2		4		12		Different sweet
5-7	83	104.3	333	1.3	1.5	1	4.17	10.5	12.88	Bitter and small
5-8	94		303	1.7		4.5		13		Damaged heart
5-9	108		249	1.4		4.5		14		
5-10	111		265	1.6		3.5		14		

Table VII: Water use of the lettuce varieties 5 weeks after sowing.

Plant	Start	Weight wk4	New-Old weight	End water	Water used
0-7	700	483	217	273	644
0-8	700	536	164	345	519
0-9	700	567	133	352	481
0-10	700	549	158	407	451
0-11	700	555	145	NA	NA
0-12	700	604	107	NA	NA
1-7	700	417	289	NA	NA
1-8	700	413	298	NA	NA
1-9	700	382	323	208	815
1-10	700	394	312	242	770
1-11	700	401	305	230	775
1-12	700	389	316	202	814
2-2	700	502	201	313	588
2-7	700	496	206	321	585
2-8	700	447	253	274	679

2-9	700	463	245	274	671
2-10	700	526	174	NA	NA
2-12	700	505	195	NA	NA
3-1	700	454	251	278	673
3-2	700	398	298	NA	NA
3-5	700	450	250	NA	NA
3-7	700	477	226	201	725
3-11	700	436	268	214	754
3-12	700	436	266	196	770
4-7	700	429	271	188	783
4-8	700	484	220	290	630
4-9	700	430	270	NA	NA
4-10	700	452	250	277	673
4-11	700	441	259	NA	NA
4-12	700	444	258	266	692
5-7	700	507	189	333	556
5-8	700	441	266	303	663
5-9	700	412	287	249	738
5-10	700	433	273	265	708
5-11	700	452	246	NA	NA
5-12	700	443	257	NA	NA

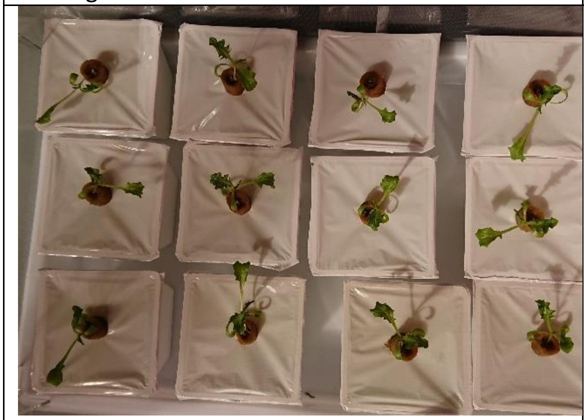
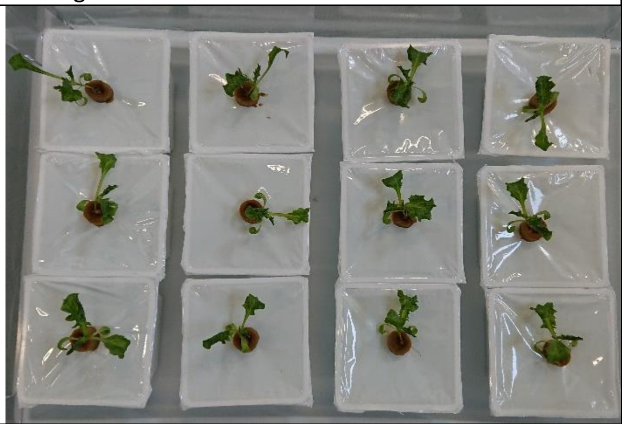
Table VIII: Water use efficiency (water use divided by yield) per variety of lettuce in experiment 1 after five weeks.

Lettuce variety	**Av. Water use wk 5**	**Av. Yield**	**ml / g**
0 Ilema	548	96	5.71
1 Red Span	793.5	83.75	9.47
2 Cristabel	630.75	86	7.33
3 Ilema CO_2	730.5	81.5	8.96
4 Red Span CO_2	694.5	78	8.90
5 Cristabel CO_2	703	104.3	6.74

Appendix IV – Experiment 2

Experiment 2: 2 weeks after sowing, one week after planting (3th of may)

Table IX: lettuce plants development during experiment 2 two weeks after sowing.

Normal atmospheric levels 2. Cristabel 1. Red Span 0. Tough Red	**1000 ppm CO_2 in Vi-Cube** 5. Cristabel 4. Red Span 3. Tough Red
	
	
	

Experiment 2: 4 weeks after sowing (17th of May)

Table X: Picture of lettuce varieties 4 weeks after sowing, just before the first harvest, in experiment 2.

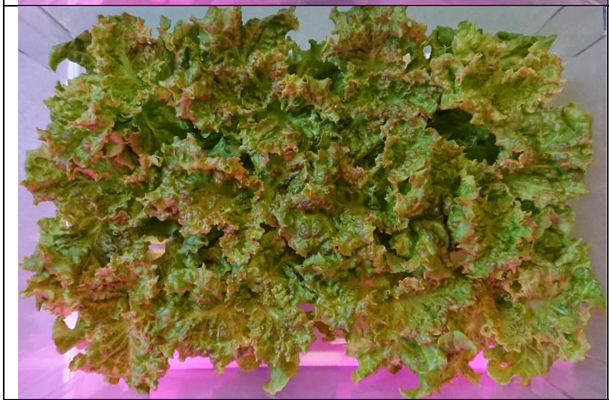
Normal atmospheric levels 2. Cristabel 1. Red Span 0. Tough Red	**1000 ppm CO_2 in Vi-Cube** 5. Cristabel 4. Red Span 3. Tough Red
	
	
	

Table XI: combined data of experiment 2, four weeks after sowing.

Plant	Fresh weight in gr	Average per treatment in gr	Water left in ml	Total use water in ml	Average water use	ml use per gr	Extra
0-1	16	17.5	507	193	211	12.06	
0-2	19		489	211			
0-3	17		496	204			
0-4	18		457	243			
0-5	20		490	210			
0-6	15		495	205			
1-1	24	27.5	412	288	263.5	9.58	
1-2	28		460	240			
1-3	26		438	262			
1-4	30		429	271			
1-5	34		449	251			
1-6	23		431	269			
2-1	13	19.5	548	152	185.67	9.52	
2-2	19		533	167			
2-3	18		524	176			
2-4	20		492	208			
2-5	20		526	174			
2-6	27		463	237			
3-1	30	22.33	338	362	288.83	12.93	Fungi
3-2	30		362	338			
3-3	12		465	235			Fungi
3-4	29		384	316			Fungi
3-5	21		428	272			
3-6	12		490	210			
4-1	33	30.83	370	330	305.83	9.92	
4-2	24		458	242			
4-3	33		392	308			
4-4	32		375	325			
4-5	32		398	302			
4-6	31		372	328			
5-1	32	37.33	469	231	276.5	7.41	
5-2	35		431	269			
5-3	34		432	268			
5-4	40		428	272			
5-5	48		353	347			
5-6	35		428	272			

Experiment 2: 5 weeks after sowing (24th of May)

Table XII: Tough Red, Red Span and Cristabel varieties five weeks after sowing, during harvest.

#0 (left)and #3 (right) Tough Red	
#1 (left)and #4 (right) Red Span	
#2 (left) and #5 (right) Cristabel	

Table XIII: combined data of experiment 1, five weeks after sowing.

Plant	Fresh weight	Average	ml water left	Stem Length	SL/ Aver	Taste	Taste Aver	Height	Height Aver	Extra
0-7	47	47.5	374	1.2	1.3	3.5	3.9	17	16.27	
0-8	45		386	1.1		4		15.5		
0-9	44		366	1.6		3.5		15		Slight bitter
0-10	45		416	1.2		3.5		17.6		
0-11	52		395	1.3		4		16.5		
0-12	52		364	1.4		4.5		16		
1-7	51	54.67	331	1.5	1.5	5	4.67	14	14.88	
1-8	51		350	1.5		5		15.5		
1-9	58		357	1.6		4.5		13.9		
1-10	53		356	1.1		4.5		15.5		
1-11	64		335	1.7		4.5		15.7		
1-12	51		354	1.6		5		14.7		
2-7	38	48	486	1.1	1.07	4	4	12	12.1	
2-8	57		397	1.1		4		12		
2-9	53		393	1.1		4		12.4		
2-10	54		402	1.1		4		13.2		
2-11	52		406	1.1		4		12		
2-12	34		459	0.9		4		11		
3-7	46	44.6	281	1.1	1	4	4.1	13.6	13.18	Algae
3-8	45		286	0.9		4.5		13.8		All light fungi
3-9	35		359	0.8		4		13.5		Algae
3-10	55		264	1.2		4		14.8		Algae
3-11	42		356	1.0		4		12.4		
3-12	22		456	0.6		1		11		Extrem funghi - exit
4-7	66	72.5	231	1.4	1.68	5	4.83	14.5	13.85	All light fungi
4-8	77		220	1.7		4.5		14.7		
4-9	67		244	1.6		5		12.8		Algae
4-10	73		220	1.8		5		14.8		Algae
4-11	79		217	1.8		5		13.4		
4-12	73		204	1.8		4.5		12.9		
5-7	73	73.67	304	1.5	1.53	4	4.42	13.2	13.29	No damage
5-8	81		253	1.6		4		13.3		No damage
5-9	79		290	1.6		4.5		14		No damage
5-10	80		294	1.7		4.5		13.8		Algae
5-11	58		385	1.3		4.5		12.3		Taai
5-12	71		351	1.5		5		13.5		Light funghi

Table 14: water use per lettuce variety of experiment 2 after five weeks.

#	Start	Weight wk4	new weight - old	End water	Water used
0-7	700	561	128	374	454
0-8	700	538	155	386	469
0-9	700	570	118	366	452
0-10	700	517	166	416	450

0-11	700	554	138	395	443
0-12	700	525	163	364	499
1-7	700	520	161	331	530
1-8	700	539	149	350	499
1-9	700	473	212	357	555
1-10	700	499	191	356	535
1-11	700	464	218	335	583
1-12	700	512	176	354	522
27	700	600	103	486	317
28	700	592	105	397	408
29	700	567	118	393	425
210	700	532	162	402	460
211	700	586	109	406	403
212	700	569	126	459	367
37	700	543	147	281	566
38	700	508	183	286	597
39	700	541	144	359	485
310	700	528	156	264	592
311	700	535	167	356	511
312	700	567	129	456	373
4-7	700	468	224	231	693
4-8	700	482	213	220	693
4-9	700	495	200	244	656
4-10	700	417	277	220	757
4-11	700	419	285	217	768
4-12	700	418	279	204	775
5-7	700	544	157	304	553
5-8	700	510	176	253	623

5-9	700	500	195	290	605
5-10	700	505	186	294	592
5-11	700	527	164	385	479
5-12	700	507	180	351	529

Table XIV: Water use efficiency (water use divided by yield) of the lettuce varieties from experiment 2 after five weeks

Lettuce variety	**Water use wk 4**	**Water use wk 5**	**Av. Yield**	**ml / g wk 5**
0 Tough Red	144.67	461.17	44.17	10.44
1 Red Span	184.5	537.33	54.67	9.83
2 Cristabel	120.5	396.67	48	8.26
3 Tough Red CO_2	154.33	550.2	44.6	12.34
4 Red Span CO_2	246.33	723.67	72.5	9.98
5 Cristabel CO_2	176.33	563.5	73.67	7.65

Appendix V – SPSS statistics

Yield

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	llema_control_1 - llema_1500ppm	13,66667	7,23418	4,17665	-4,30403	31,63736	3,272	2	,082
Pair 2	Tough_Red_control_2 - Tough_Red_1000ppm	2,00000	8,09321	3,61939	-8,04904	12,04904	,553	4	,610
Pair 3	Red_Span_control_1 - Red_span_1500ppm	5,75000	10,53170	5,26585	-11,00828	22,50828	1,092	3	,355
Pair 4	Red_Span_control_2 - Red_span_1000ppm	-17,83333	6,04704	2,46869	-24,17931	-11,48736	-7,224	5	,001
Pair 5	Cristabel_control_1 - Cristabel_1500ppm	-13,00000	6,97615	3,48807	-24,10061	-1,89939	-3,727	3	,034
Pair 6	Cristabel_control_2 - Cristabel_1000ppm	-25,66667	11,00303	4,49197	-37,21364	-14,11969	-5,714	5	,002

Figure II: paired samples test on combined data from two experiments on fresh yield..

Height

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	llema_control_1 - llema_1500ppm	1,87500	1,79699	,89849	-,98441	4,73441	2,087	3	,128
Pair 2	Tough_Red_control_2 - Tough_Red_1000ppm	3,08333	1,36443	,55703	1,65145	4,51521	5,535	5	,003
Pair 3	Red_Span_control_1 - Red_span_1500ppm	4,50000	,91287	,45644	3,04742	5,95258	9,859	3	,002
Pair 4	Red_Span_control_2 - Red_span_1000ppm	1,03333	,97091	,39637	,01443	2,05224	2,607	5	,048
Pair 5	Cristabel_control_1 - Cristabel_1500ppm	1,25000	1,50000	,75000	-1,13683	3,63683	1,667	3	,194
Pair 6	Cristabel_control_2 - Cristabel_1000ppm	-1,25000	,77653	,31702	-2,06492	-,43508	-3,943	5	,011

Figure III: paired samples test on combined data from two experiments on plant height.

Stem Length

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	llema_control_1 - llema_1500ppm	1,30000	,21602	,10801	,95626	1,64374	12,036	3	,001
Pair 2	Tough_Red_control_2 - Tough_Red_1000ppm	,28000	,31145	,13928	-,10671	,66671	2,010	4	,115
Pair 3	Red_Span_control_1 - Red_span_1500ppm	,42500	,20616	,10308	,09696	,75304	4,123	3	,026
Pair 4	Red_Span_control_2 - Red_span_1000ppm	-,18333	,27869	,11377	-,47580	,10913	-1,611	5	,168
Pair 5	Cristabel_control_1 - Cristabel_1500ppm	,17500	,09574	,04787	,02265	,32735	3,656	3	,035
Pair 6	Cristabel_control_2 - Cristabel_1000ppm	-,46667	,15055	,06146	-,62466	-,30867	-7,593	5	,001

Figure IV: paired samples test on combined data from two experiments on stem length.

Water-use efficiency

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ilema_control_1 - Ilema_1500ppm	-3,12000	,66663	,33332	-4,18076	-2,05924	-9,360	3	,003
Pair 2	Tough_Red_control_2 - Tough_Red_1000ppm	-2,69800	1,17093	,52365	-4,15190	-1,24410	-5,152	4	,007
Pair 3	Red_Span_control_1 - Red_span_1500ppm	,54250	,59422	,29711	-,40303	1,48803	1,826	3	,165
Pair 4	Red_Span_control_2 - Red_span_1000ppm	-,13667	,47994	,19594	-,64034	,36700	-,698	5	,517
Pair 5	Cristabel_control_1 - Cristabel_1500ppm	,61750	,43177	,21588	-,06954	1,30454	2,860	3	,065
Pair 6	Cristabel_control_2 - Cristabel_1000ppm	,75667	1,42989	,58375	-,74391	2,25724	1,296	5	,252

Figure V: paired samples test on combined data from two experiments on water-use efficiency.

Taste

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ilema_control_1 - Ilema_1500ppm	-1,25000	,64550	,32275	-2,27713	-,22287	-3,873	3	,030
Pair 2	Tough_Red_control_2 - Tough_Red_1000ppm	-,40000	,41833	,18708	-,91943	,11943	-2,138	4	,099
Pair 4	Red_Span_control_2 - Red_span_1000ppm	-,08333	,49160	,20069	-,59923	,43257	-,415	5	,695
Pair 5	Cristabel_control_1 - Cristabel_1500ppm	-,50000	,86603	,50000	-2,65133	1,65133	-1,000	2	,423
Pair 6	Cristabel_control_2 - Cristabel_1000ppm	-,41667	,37639	,15366	-,81166	-,02167	-2,712	5	,042

Figure VI: paired samples test on combined data from two experiments on taste.

Appendix VI – Checklist schriftelijk rapporteren

Checklist Schriftelijk Rapporteren

Naam: Richard Steenvoorden Klas: 4TBb Datum: 10 juni 2019

Titel verslag/rapport: The effect of enriched CO2 concentrations on hydroponically grown Lettuce (Lactuca sativa)

Nadat **jij** je verslag/rapport hebt gecontroleerd met behulp van deze checklist, voeg je deze toe als bijlage. Zonder de ingevulde checklist vindt er geen beoordeling plaats. De assessor controleert met deze checklist je rapport/verslag. De beoordelingscriteria die met een * zijn aangegeven, zijn de zogenaamde *'killing points'*. Indien de assessor meer dan vijf *'killing points'* heeft aangekruist, dien je het rapport/verslag op alle onvoldoende onderdelen te verbeteren. Voor de herbeoordeling moet je ook de oude versie inleveren. In het afstudeerwerkstuk zijn geen *'killing points'* toegestaan! AANVINKEN WAT NIET IN ORDE IS!

1. Het taalgebruik:
- ☐ Bevat niet meer dan drie grammaticale, spel- en typefouten per duizend woorden*
  Bij meer dan drie fouten per duizend woorden is het rapport/verslag afgekeurd!
- ☐ Heeft een adequate interpunctie*
- ☐ Is afgestemd op de gekozen doelgroep (juiste stijl)*
- ☐ Laat een zakelijke en actieve schrijfstijl zien*
- ☐ Bevat geen persoonlijke voornaamwoorden*

2. Het rapport/verslag:
- ☐ Is ingebonden (hard copy)*
- ☐ Is vrij van plagiaat* (zie onderwijsexamenregeling)

3. De omslag:
- ☐ Bevat de titel
- ☐ Vermeldt de auteur(s)

4. De titelpagina/het titelblad:
- ☐ Heeft een specifieke titel*
- ☐ Vermeldt de auteur(s)*
- ☐ Vermeldt de plaats en de datum*
- ☐ Vermeldt de opdrachtgever(s)*

5. Het voorwoord:
- ☐ Bevat de persoonlijke aanleiding tot het schrijven van het rapport/verslag
- ☐ Bevat persoonlijke bedankjes (persoonlijke voornaamwoorden toegestaan)

6. De inhoudsopgave:
- ☐ Vermeldt alle genummerde onderdelen van het rapport/verslag*
- ☐ Vermeldt de samenvatting en de bijlage(n)
- ☐ Is overzichtelijk
- ☐ Heeft een correcte paginaverwijzing

7. De samenvatting:
- ☐ Is een verkorte versie van het gehele rapport/verslag
- ☐ Bevat conclusies
- ☐ Bevat geen persoonlijke mening
- ☐ Is gestructureerd
- ☐ Is zakelijk geschreven
- ☐ Staat direct na de inhoudsopgave

8. De inleiding (toelichting op intranet):
- ☐ Is hoofdstuk 1*
- ☐ Beschrijft het grotere kader en aanleiding
- ☐ Beschrijft inhoudelijke achtergrondinformatie*
- ☐ Formuleert het probleem/de onderzoeksvraag*
- ☐ Vermeldt het doel*
- ☐ Bevat een leeswijzer voor het rapport/verslag*

9. Materiaal en methode:
- ☐ Beschrijft de gevolgde onderzoeksmethode
- ☐ Past bij de onderzoeksvraag/vragen*
- ☐ Beschrijft de variabelen/eenheden
- ☐ Beschrijft de methode van data-analyse

10. De (opmaak van de) kern:
- ☐ Bestaat uit genummerde hoofdstukken en (sub)paragrafen (maximaal drie niveaus)*
- ☐ Deze zijn verschillend in opmaak*
- ☐ De hoofdstukken en (sub)paragrafen hebben een passende titel
- ☐ Een hoofdstuk beslaat ten minste één pagina
- ☐ Een nieuw hoofdstuk begint op een nieuwe pagina
- ☐ De zinnen lopen door (geen 'enter' binnen een alinea gebruiken)
- ☐ De figuren zijn (door)genummerd en hebben een passende titel (onder de figuur)*
- ☐ De tabellen zijn (door) genummerd en hebben een passende titel (boven de tabel)*
- ☐ Tabellen en figuren zijn zelfstandig te begrijpen
- ☐ In de tekst zijn er verwijzingen naar figuren en/of tabellen*
- ☐ De tekst bevat verwijzing naar de desbetreffende bijlage(n)
- ☐ De tekst is ook zonder verwijzingen te begrijpen
- ☐ De pagina's zijn genummerd*

11. De discussie:
- ☐ Bevat een vergelijking met relevante literatuur
- ☐ Geeft de valide argumentatie weer
- ☐ Evalueert de gebruikte onderzoeksmethode
- ☐ Bevat een kritische reflectie op de eigen bevindingen (zie toelichting op intranet)

12. De conclusies en aanbevelingen:
- ☐ De conclusies zijn gebaseerd op relevante feiten
- ☐ De aanbevelingen zijn gebaseerd op relevante feiten
- ☐ Bevatten geen nieuwe informatie*

13. De bronvermelding:
- ☐ In de tekst is conform de geldende APA-normen* (zie toelichting op intranet)

14. De literatuurlijst:
- ☐ Is opgesteld conform de geldende APA-normen* (zie toelichting op intranet)

15. De bijlagen:
- ☐ Zijn genummerd
- ☐ Zijn voorzien van een passende titel
- ☐ Bevatten geen eigen analyse