Norwegian University
of Life Sciences

Master's Thesis 2024 60 ECTS
Faculty of Biosciences

The Effect of Red/Blue Light and CO_2 Enrichment on Development, Photosynthesis, Yield and Quality of *Vicia faba* Grown in Controlled Environment

Effekten av rødt/blått lys og CO_2 økning på utvikling, fotosyntese, avling og kvalitet av *Vicia faba* dyrket i kontrollert klima

Karoline Vestland
MSc Biology – Plant Biology

Karoline Vestland

The Effect of Red/Blue Light and CO_2 Enrichment on Development, Photosynthesis, Yield and Quality of Vicia faba Grown in Controlled Environment

Master´s thesis, Biology

Norwegian University of Life Sciences (NMBU)

Spring 2024

Supervised by Prof. Sissel Torre

Norwegian University of Life Sciences (NMBU)

Faculty of Biosciences

Department of Plant Sciences

P.O. Box 5003

1432, Ås,

Norway

Preface and Acknowledgments

This master thesis in biology was part of, and funded by, the project "Virkemidler for Omstilling av matsystemet (VOM)", led by Cicero Centre for Climate Research, Oslo, Norway.

First, I would like to thank my supervisor Prof. Sissel Torre for the opportunity to be a part of the VOM-project. Thank you for all help and guidance throughout the experiment and writing, and for all the interesting discussions the last year and a half. A big thank you to Ida Kristin Hagen for all help with the practical experiment, from sowing and watering, to light adjustments and many hours of harvest and recordings. Without you there wouldn't have been any faba beans. Thank you to Anders Bakke for being so excited about faba beans and helping me getting started with the subject, and many valuable suggestions during the experiment.

Thank you to Silje Evensdatter Roksti and Gry Skjeseth for watering and helping with the harvest. I would like to extend my gratitude to Svein Andrè Kolltveit for helping me with light recordings, and Dr. Sheona Noemi Innes for guiding me through the gas exchange measurements. Thank you to Lene Cecilie Hermansen at the Imaging Center for help and guidance with microscopy. I would also like to thank Eirik Høyheim from Skrivesenteret with statistical help and for giving me some confidence in programming. Thank you to the technical staff at SKP for setting up the growth chambers and answering a lot of my emails.

Thank you to Marte, for loving biology and plants as much as me, and for help and corrections in the writing process.

Last but not least, I would like to thank my friends and family, who in spite of me only talking about faba beans for the last year have continued to invite me for dinners and walks. You helped me take breaks, and lifted my spirits when I needed it.

I am so grateful for you all!

Karoline Vestland
Ås, Mars 2024

Sammendrag

Det er behov for å undersøke nye metoder innen matproduksjon ettersom klimaendringer påvirker avling og kvalitet av planter dyrket i åker. Økende frekvens av ekstremvær, nye sykdommer og skadegjørende insekter, i tillegg til økte produksjonskostnader er argumenter for å utforske produksjon i kontrollert klima. Ved å effektivisere fotosyntese ved CO_2 økning, LED-belysning og løsninger for å kontrollere klima er det muligheter for å oppnå økte og mer forutsigbare avlinger, samtidig som matkvaliteten ivaretas. Det er også økende interesse for å forbedre nasjonal produksjon av protein til menneskekonsum, for å begrense avhengigheten av import. Derfor ble to sorter (´Ratio´ og ´Witkiem major´) av fababønner (*Vicia faba*) dyrket i 3-liters potter i vekstkammer gitt 16 timer lysperiode med 22°C og 8 timer mørkeperiode med 16°C, med en lysintensitet på mellom 2-300 μmol m^{-2} s^{-1} under enten høy rød (R)/blå lys (BL) eller lav R/BL, og med enten a[CO_2](ambient nivå, ca. 415 ppm) eller e[CO_2](økt nivå, ca. 1000 ppm).

Resultatene viser potensial for å dyrke fababønner som grønnsak i kontrollert klima, ettersom nedgangen i sykdom vil være en fordel, spesielt med tanke på sjokoladeflekk (*Botrytis fabae*), som er en ødeleggende soppsykdom i åkerproduksjon. Til tross for dette, bladrandskade ble observert, og mer forskning på skaden er nødvendig. ´Ratio´ utviklet seg raskest dyrket ved høy R/BL med e[CO_2]. ´Witkiem major´ viste samme utvikling for høy R/BL med e[CO_2] og lav R/BL med a[CO_2]. Høy R/BL ga generelt økt avling og større tørrvekt sammenlignet med lav R/BL, og det samme mønsteret kan sees for e[CO_2], som økte biomassen og total friskvekt av bønner sammenlignet med a[CO_2]. Uansett, for ´Witkiem major´ var proteininnholdet i bønner signifikant lavere i høy R/BL (26%) sammenlignet md lav R/BL (30%) uavhengig av CO_2 konsentrasjonen. Resultatene viser at til tross for potensialet som grønnsak i kontrollert klima, så blir utvikling av nye sorter mer tilpasset kontrollert klima viktig, ettersom den genetiske variansen og forskjellen mellom sorter er stor.

Abstract

There is a need to explore new methods of food production as effects of climate change influence yield and quality of crops in field production. Higher frequency of extreme weather, new diseases and insect pests, in addition to increased expenses of production are all arguments for examining production in controlled environment. Efficient photosynthesis by CO_2 enrichment, LED-lighting and solutions for climate control give the potential to attain more predictable and higher yields, while securing quality of produce. There is also an increased interest in improving national protein production, to limit the dependability on import. With that in mind, two cultivars (´Ratio´ and ´Witkiem major´) of faba beans (*Vicia faba*) were grown in 3-liter pots in growth chambers given 16 hour photoperiod with 22°C, and 8 hour dark period with 16°C, with between 200-300 μmol/m^2/s under high red (R)/blue light (BL) (4:1) or low R/BL (1:1) and with a[CO_2](ambient level, approx. 415ppm) or e[CO_2](elevated level, approx. 1000ppm).

The results show potential for production of faba beans as a vegetable in controlled environment, as it will benefit from reduction in fungal diseases, especially chocolate spot (*Botrytis fabae*), which is a detrimental infection in field production. However, the appearance of the injury leaf edge/tip burn is present, which needs to be further studied. For ´Ratio´ high R/BL with e[CO_2] developed the fastest, while ´Witkiem major´ showed the same development for high R/BL with e[CO_2] and low R/BL with a[CO_2]. High R/BL gave generally higher yield and larger DW compared to low R/BL, the same pattern was seen for e[CO_2], which increased biomass and total seed FW compared to a[CO_2]. However, for ´Witkiem major´ the protein content of the seeds was significantly lower in the high R/BL (26%) compared to the low R/BL (30%), independent of the CO_2 concentration. This study showed the potential for controlled environment production of faba beans. However, it highlights the importance of further breeding and selection of cultivars more suitable for controlled environment, as the genetic variance and the differences between cultivars is substantial.

Abbreviations

RH	Relative air humidity
PAR	Photosynthetic active radiation
PPFD	Photosynthetic photon flux density
PFD	Photon flux density
FR	Far-red
BL	Blue light
R	Red light
SEM	Scanning electron microscope
DW	Dry weight
FW	Fresh weight
EC	Electrical conductivity
HPS	High pressure sodium
LED	Light emitting diode
NR	Nitrate reductase
N	Nitrogen
Mg	Magnesium
Ca	Calcium
Cl	Chlorine
K	Potassium
P	Phosphorous
RuBisCO	Ribulose 1,5-Bisphosphate Carboxylase Oxygenase
GHG	Greenhouse gas
PFAL	Plant factory with artificial lighting
IRGA	Infra-red gas analyzer
NADH	Nicotinamide adenine dinucleotide + hydrogen
a[CO_2]	Ambient concentration of CO_2
e[CO_2]	Elevated concentration of CO_2

List of Figures

List of Tables

Table of Contents

1. Introduction

As a result of climate change and loss of nature, food production and food systems need to be revised in order to maintain food security (Landbruksdirektoratet, 2022; Nemali, 2022). Already many areas around the world experience more frequent weather extremes, such as drought and flooding, which negatively affects field production (Gomez-Zavaglia et al., 2020). This food insecurity is expected to increase further as the global mean temperatures rise (Intergovernmental Panel on Climate Change, 2023). There is an increased frequency of unfavorable field conditions in addition to an expected rise in pests and diseases in a warmer climate. Because of this new production methods might need to be considered. Recent events such as a global pandemic and wars have shown the dependability on import and export of food, as well as energy production (Landbruksdirektoratet, 2023). With increased prices on electricity, fertilizers, building materials and imported food, there is a growing need to explore national solutions to make the food system more predictable for both producer and consumer.

In addition to these physical challenges there is the ethical aspect of trying to change food production in a more sustainable direction, although this could be a costly investment for many producers. Animal husbandry is known to have higher greenhouse gas (GHG) emissions compared to production of plant protein. This is mainly because of water usage (Farchi et al., 2017), transport, and the relative energy inefficient aspect of calories fed to livestock compared to how much becomes food suitable for human consumption (González et al., 2020). Multiple reports have emphasized the possibility for changing protein production from livestock to plant protein to lower the carbon footprint for production of human food (Abrahamsen et al., 2019; Foyer et al., 2016; González et al., 2020). There is also an increased interest in improving national protein production, to limit the dependability on import (Bakken & Mittenzwei, 2023; Grieu et al., 2021).

This master thesis is part of the project “Incentives for Measures for Food system Transition” (VOM), which is a collaborative research project owned by CICERO (Centre for climate research, Oslo, Norway). The main objective of the project is to investigate possibilities for sustainable low-emission food production in Norway, including what challenges and incentives that could be important for both producers and consumers in the pursuit of this (Cicero, 2021)

The faba bean (*Vicia faba*) has been an important crop all around the globe, and can be utilized in many different ways. The young seeds can be boiled and eaten as a vegetable, while mature seeds can be used as feed for livestock (Yitayih & Azmeraw, 2018). Many cultivars exist, and there are new varieties being developed based on nutritional value, size and taste of the beans, disease resistance, as well as flood and drought adaptability to name a few. Faba beans are susceptible to weather conditions, such as drought and high temperatures (Karkanis et al., 2018), and it may be vulnerable in the face of increasingly extreme weather conditions in field production.

Therefore it could be valuable to examine the possibility of production in controlled environment, such as walk-in tunnel production or greenhouse. Especially prolonging the growth season with more optimal temperatures and lighting would be favorable to the crop grown in Norway. One of the greatest advantages of greenhouse production is the ability to improve conditions to optimize photosynthesis and plant growth. The greenhouse technology is developing more energy efficient equipment. Among them, Light emitting diodes (LED) has become increasingly popular, and many greenhouse growers have invested in new lighting (Dutta Gupta & Agarwal, 2017). LEDs have given the opportunity to design the specific light spectrum suitable for their different production. This has also increased energy efficiency in plant factories with artificial lighting (PFALs), which are completely closed high-tech horticultural systems, where all environmental conditions can be adjusted, often in a vertical farming set-up (Stanghellini et al., 2019).

All plants have specific optimums in which they thrive and it is possible to change their environment to what is most suitable. This will depend on what is the wanted outcome; shorter growth season, higher yield, bigger leaves, sweeter fruit etc. Changing one of the conditions surrounding the plants are nevertheless not as straight forward as it first seems. Plants have a highly fine-tuned physiology and morphology to their environment, as well as many acclimation responses if the conditions change (Mittler, 2006). In effect, changing one condition might also affect the plants´ response to other conditions. Therefore exploring the effect of the interactions of changing environmental conditions is important when finding the optimal growth program for different plant species.

Light is one of the most important factors regarding plant growth and development, and the plants have responses to both intensity and quality, as well as direction and duration. These responses are important to the plants´ survival as they are sessile, and therefore dependent on being able to adapt to the changing environment, such as light conditions. During the day solar radiation will change in intensity, numbers of hours with light, angle and quality, and there are also great geographical differences which will affect the actual light conditions the plants experience, as well as competition for light which can happen in denser canopies. Because the light conditions will often vary the plants have evolved several different photoreceptors, sensitive to different wavelengths. When different light hits these receptors it activates reaction pathways leading to morphological and/ or physiological changes in the plant. In order to optimize light conditions in plant production it is necessary to examine these responses and how it affects the final sellable product.

Another factor important to plant growth is the concentration of carbon dioxide (CO_2). Elevating CO_2 concentration in greenhouse production can be beneficial as it is often the limiting factor in photosynthesis in nature (Ainsworth & Rogers, 2007). In other words, increasing the levels could lead to an increased photosynthetic rate (Zheng et al., 2020), which could again lead to increased biomass production (Ainsworth & Long, 2021). However, there are concerns that increased levels of CO_2 could be connected to a decrease in nitrogen (N) uptake and protein content in seeds (Cotrufo et al., 1998; Parvin et al., 2019). As the plant production research and industry is always looking for methods to lower energy consumption, while keeping or increasing yield as well as the nutritional value of the produce this is an important aspect to explore.

Additionally, another aspect to consider is the excess CO_2 gas being released into the atmosphere because of fossil fuel burning in other industries. Increasing levels of CO_2 gas is a problem both at the atmospheric level as well as an air pollutant in the air we breathe (Willey, 2018). Therefore, to be able to decrease the amount being released, and instead use it in the production of food could be greatly beneficial for both industries. The technology already exists, as it is being used in the project “Den Magiske Fabrikken” (The magical factory), where a greenhouse uses green CO_2 from a biogas facility, as a step towards a more circular economy (Den Magiske Fabrikken). These types of initiatives are crucial on the path to a more sustainable food production system and demonstrates the potential of a greener industrial sector.

1.1 Aim of the Study

Based on the above, there is a need to explore new methods for food production with regards to food security and unstable environmental conditions, in order to increase predictability for both producers and consumers. Also, to try to get closer to energy efficient methods of greenhouse production to limit the reliability on import. Identifying possible challenges that may arise with this type of plant production will be important. To examine this, faba beans will be grown in growth chambers with controlled environment, two different red (R)/blue light (BL) treatments with LED lighting, and with ambient (a[CO_2]) and enriched (e[CO_2]) CO_2 levels. Three research questions have been developed for the experiment:

1. What are the possibilities and challenges regarding growing *V. faba* in controlled environment?
2. How will two morphologically different cultivars respond to variation of R/BL light and CO_2 enrichment with regards to development, photosynthesis and growth?
3. How will the yield and quality of beans of *V. faba* be affected by R/BL light and CO_2 enrichment?

2. Background

2.1 Faba Beans (*Vicia faba* L.)

2.1.1 History and use

Faba bean is an annual legume field crop. It is nutritious and protein rich and has been widely used both as animal feed (Staniak et al., 2014) and human food (Crépon et al., 2010). It is an old crop that has been used for a long time, which have resulted in many different names; tick beans, horse beans, field beans, broad beans etc. (Cubero & Nadal, 2005). It has been widely used in the Mediterranean area and has been popular as food because of its nutritional value. In addition to high protein content (approximately 30% of dry matter, depending on cultivar), it is also rich in fiber and some vitamins and minerals (Crépon et al., 2010; Cubero & Nadal, 2005).

Contrary to this, there is also a challenge regarding the nutritional value in faba beans; the content of tannins, and vicine and convicine. Tannins can combine with the protein in the beans and limit the correct processing in the intestines. As this reduces the biological value of the faba bean protein, cultivars with low tannin content have been selected in some breeding. These cultivars are morphologically recognized by white flowers (Øverland & Viken, 2020), and these are the types most common in European production (Cubero & Nadal, 2005). The faba bean varieties are divided into three botanical types; *major*, *equina* and *minor*. Where *major* have the largest seeds (up to 2 g/seed), *equina* middle sized (between 0.6 and 1.1 g/seed), and *minor* have the smallest seeds (up to 0.6 g/seed) (Cubero & Nadal, 2005). There is big differences in quality, seed and pod shape and size, determinant and indeterminant growth, and typical uses, but the horticultural cultivars are of the *major* type.

Its increasing popularity in field production today is because of its ability to form symbiotic relations with some nitrogen fixating rhizobium bacteria, increasing nitrogen availability for the sequential crop (Khazaei et al., 2021; Staniak et al., 2014; Zong et al., 2019). Thus limiting the need for extra nitrogen fertilizer. This decrease in fertilizer usage is both economically positive for the farmer and more sustainable as it limits pollution in nearby areas, previously affected by nitrate leaching (Confalone et al., 2010). In addition, the increased popularity of plant protein in Europe has led to an increased use of beans and peas in new products. However, in Norway most of these products are imported, or are based on imported crops (Abrahamsen et al., 2019).

2.1.2 Challenges and Potential in Controlled Environment

One of the challenges discovered with legume production in Europe is that legume crop yields vary more than grain production (Alharbi & Adhikari, 2020; Cernay et al., 2015). This is largely due to environmental conditions, such as drought. Recent studies showed that faba beans are not likely to benefit from increased atmospheric CO_2 concentration if they are exposed to drought conditions (Alza et al., 2024; Parvin et al., 2019). There has also been reports of negative effects on seed yield in response to drought in Norwegian fields (Grieu et al., 2021). New genetic lines of legumes more tolerable to variable environmental conditions is being explored, but similarly, production in controlled environment should be considered as it also offers a solution to the uncertainty. A meta study done by López-Bellido et al. (2005) found that different developmental stages and also the faba bean yield was affected by environmental factors such as temperature and water availability (López-Bellido et al., 2005). It also showed great differences because of planting densities. The big variations found for the environmental conditions affecting the growth and development shows that there is great potential for finding the optimal conditions of each stage and utilizing them in a controlled growth program in order to optimize yield.

Another reason farmers are not choosing faba beans is because they are prone to many pests and fungal diseases (Jensen et al., 2010; Stoddard et al., 2010), which can decrease the yield. Chocolate spot (caused by *Botrytis fabae*) is one of the most destructive fungal diseases affecting faba bean production. In fact, *B. fabae* is present in almost all cultivation areas of faba beans (Cubero & Nadal, 2005; Stoddard et al., 2010), and there have been substantial losses of yield in fields in Norway due to chocolate spot (Aamot et al., 2023). From a study conducted in northern Ethiopia in the 2004/2005 cropping season 261 fields of faba bean production was observed in 12 different districts, and chocolate spot was found in all fields (Sahile et al., 2008). The severity was dependent on the density of weed populations, where high density led to higher severity of chocolate spot infection. Generally, the presence of weeds will require more management and higher costs in the field compared to controlled environment.

Additionally, many insect pests are known for targeting legumes. In order to treat these forms of attack, pesticide treatment is often required, leading to increased expenses for the farmer, as well as exposure to potentially harmful chemicals (Stoddard et al., 2010). Pesticides is still playing a key role in field management of faba bean production. Although there have been developed genetic lines resistant to some of the most common diseases, few of the available cultivars are resistant to more than one (Stoddard et al., 2010; Yitayih & Azmeraw, 2018). Recently, *Botrytis* resistant to chemical fungal treatment have been discovered in faba beans (Aamot et al., 2023). The overall effect of these biological threats to faba bean field production is both expensive and time-consuming for the producer. This is one of the challenges that would greatly benefit from production in controlled environment, as the isolation in a closed system such as a greenhouse or a PFAL would drastically reduce the disease attacks caused by contaminated soil, insects, or nearby weeds. Although equipment and installations for more controlled environment would also be an expensive investment (Shamshiri et al., 2018), the need to control pests and fungal diseases could decrease, and therefore lower the production cost.

From previous studies and reports from greenhouse production there is a recurring challenge of leaf edge or tip burn. This has been a problem for many different species like cabbages (*Brassica oleracea*)(Kim et al., 2021), tomatoes (*Lycopersicon esculentum*)(Shamshiri et al., 2018), and lettuce (*Lactuca sativa*)(Bert & Honma, 1975). It can be difficult to assess the reason behind plant injuries, as it is difficult to determine what is an effect of the injury and what is the cause. However, numerous hypotheses have been tested regarding leaf edge or tip burn in different species. Many conclusions have been drawn on the effect of calcium (Ca) deficiency (Kim et al., 2021), humidity (Shamshiri et al., 2018), rhizosphere parameters, and nutrient accumulation or deficiency (Bert & Honma, 1975). At this point no clear cause have been identified, and it will therefore be a point of interest in studies regarding greenhouse production as it can lower the plant quality. Hence, pinpointing the source of stress on the crop in question will be important in the pursuit to diminish the injury.

The main advantage of greenhouse or PFAL production in the northern regions is the opportunity to extend the growth season. The growth season in the north is short, and faba beans require approximately four months from seeding to harvest for the earliest cultivars, but later cultivars in seasons with below optimal temperature can take as much as 4,5 months in the fields (Abrahamsen et al., 2019). It is a cool-season crop and can withstand frost in the seedling stage (Murphy-Bokern et al., 2017), but will nevertheless thrive in warmer climates. Optimizing temperature could prove favorable to maximize quality and yield as it is an important regulator for many developmental stages in plant growth (Hatfield & Prueger, 2015). As temperatures in the north some summers can be lower than optimal, it could be possible to explore the use of walk-in tunnel production, as it will to some extent allow for a higher degree of temperature control than free-air. However, the means of harvest will probably also have to be altered, which could be a challenge. If the harvest methods have to be changed it could be even more favorable to use greenhouse or PFAL production, as it would open for more opportunities to optimize the environmental parameters, and therefore increase yield to a point where the extra time spent on harvest could be worth it.

In conclusion, to increase national production there is a need to find a solution where the faba bean production is more reliable from year to year. the most beneficial aspects of growing faba beans in a controlled environment could be to prolong the growth season and limit the variance in yield and quality based on water availability as the species is susceptible to negative effects of drought conditions in the fields. Furthermore, it would be easier to isolate the crop, limiting infection of fungal diseases and pests, especially with regards to chocolate spot. In addition, it would also be possible to try to find the most optimal growth program for faba beans, and in effect increase yield or quality by adjusting for example light quality and intensity, temperature, nutrients and available CO_2.

2.2 Light and Plant Responses

2.2.1 Nature of Light

Light can be described as both a particle and a wave. This dual nature is important for the effect light has on its surroundings. The light particle is called a photon, and the number of photons decide the energy of the incoming light on a surface. The number of photons is also how we can quantify the amount of light reaching a canopy. In photobiology an important parameter to examine or control when researching plants is the irradiance, which is described as number of photons or amount of energy per unit area per unit of time (μmol m^{-2} s^{-1}). In natural sun light the irradiance on a day with full sun is about 2000 μmol m^{-2} s^{-1} (Taiz et al., 2015).

In addition, light act as waves. In photobiology this is described as light quality, and its characteristics is decided by the wavelength given in nanometers, where longer wavelengths have less energy than shorter wavelengths. In the spectrum of visible light (approx. 400-700 nm) this also decides the color of light; blue light have shorter wavelengths (approximately 400-500 nm) and higher energy than red light (approx. 600-700 nm). This region of the electromagnetic spectrum is also recognized as photosynthetically active radiation (PAR) (Taiz et al., 2015).

2.2.2 Photosynthesis

Light is an important factor regarding plant growth, as it functions both as an energy source and a signal for regulation. The process of converting light energy to chemical energy is photosynthesis and occurs in chloroplasts in leaves or other plant structures. In higher plants photosynthesis is mainly driven by light absorption in chlorophyll *a* and *b* (Fig. 1), but carotenoids are also important in photochemistry (Taiz et al., 2015). Previous findings show that red light is the most photosynthetically efficient light quality (Sun et al., 1998), this is because the antenna pigment of the light harvesting complex has wavelength optimums of 680 nm and 700 nm. In other words, these are the wavelengths that most easily will react and lead to photosynthesis. Blue light is used by chlorophyll for photosynthesis, but the incident blue light is also absorbed by auxiliary photoreceptors, called carotenoids (Dutta Gupta & Agarwal, 2017). The chemical energy then has to be transferred to the chlorophylls. In this process energy is lost, and the blue light is therefore somewhat less energy efficient compared to red light.

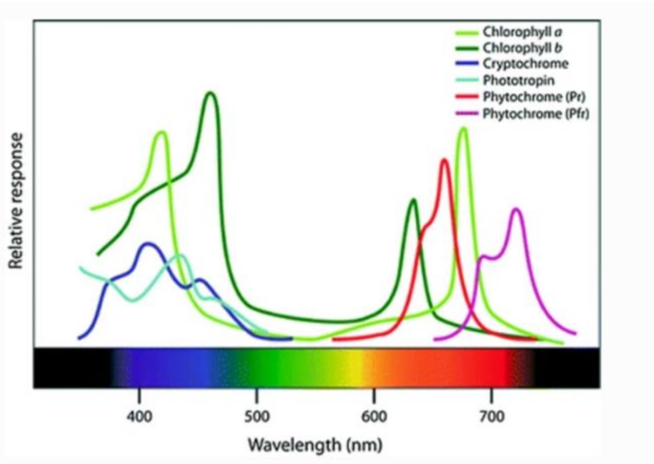


Figure 1: Absorption spectra from photoreceptors; chlorophyll a/b, cryptochrome, phototropin and phytochrome P_r/P_{fr}. (Dutta Gupta & Agarwal, 2017)

The green light wavelength spectrum has been debated. It has been considered less efficient in plant photochemistry as it is not as easily absorbed as red and blue light (Sun et al., 1998). Red and blue wavelengths are often absorbed in the upper parts of the leaf, and in the upper parts of the canopy. As it is so easily absorbed, the leaves will absorb more than the photosystems are able to use for photochemistry. This can lead to photodamage if the plant is not able to dispatch the energy in safe processes. In contrast, more of the green light is transmitted and reflected, which means that it can reach other parts of the leaf or the canopy, and therefore be absorbed by parts of the canopy or leaf that is not as readily available for the more efficient wavelengths. When more of the leaf tissue and leaves in less advantageous parts of the canopy to some extent is able to perform photosynthesis, this could lead to an overall increase in photosynthetic rate (Sun et al., 1998; Terashima et al., 2009). Photosynthesis is recognized as one of the key determinants in genetic development, when considering the potential yield and the plants´ response to environmental stress (Mathan et al., 2016). Monitoring photosynthesis and its related traits are therefore important in breeding programs when aiming for improved crop production or yield increase (Khazaei et al., 2019).

2.2.3 Photomorphogenesis

As previously mentioned, sunlight is comprised of different wavelengths. And the different light qualities will affect the receiving plant according to the wavelength, and how the plant is able to absorb it. When light interacts with plant photoreceptors and induce a morphological change in structure or form it is called photomorphogenesis. In plants there are many light absorbing photoreceptors, such as cryptochromes, phytochromes or phototropins (Taiz et al., 2015). These interactions drive light induced responses in the plant, and there are found many effects of different spectra interacting with a wide range of horticultural crops with regards to seed yield and protein content (Kasperbauer & Loughrin, 2004), nutritional value (Alrajhi et al., 2023; Naznin et al., 2019), and flowering (Nanya et al., 2012) to name a few.

BL wavelengths are mostly absorbed by cryptochromes and phototropins, which are groups of flavoprotein photoreceptors (Möglich et al., 2010). Some of the well-known responses include phototropism, movement of chloroplast and stomatal opening (Matthews et al., 2020). The effect of BL is mostly responses increasing capacity for photosynthesis. For the effect on the stomata the BL induces a phototropin mediated opening. In nature when the sun is low in the sky, the distribution of different wavelengths will vary throughout the day as the light have a bigger proportion of the atmosphere to travel through. BL photoreceptors are assumed to be involved in the detection of high and low fluence rates, and therefore the interaction between light quality and quantity (Anderson et al., 1995). However, the BL effect on stomata has previously been found to decrease in growth chamber conditions (Frechilla et al., 2004).

R and FR wavelengths are mostly absorbed by phytochromes. Where the status of the pigment is altering between active (P_{fr}) and passive (P_r) with absorption maxima at 660 nm and 730 nm respectively (Wang & Deng, 2004). The alteration occurs based on the last light given and is reversible. This interaction affects many events of photomorphogenesis such as, seedling emergence (Chen & Chory, 2011), and flowering and shade avoidance response (Wang & Deng, 2004).

Not only will the plants show different response to R and BL, but also to different combinations of the two. High R/BL has been found to increase biomass production in lettuce (Okamoto et al., 1996). Low R/BL has previously lead to a decrease in dry matter in kale (*Brassica oleracea*) (Zhang et al., 2020), but with an increased accumulation of soluble protein. When regarding the aspect of protein production, increasing the accumulation of soluble proteins would be of great interest, as it would increase the amount of protein produced per unit area. In other studies, BL has been found to increase photosynthetic capacity (Wang et al., 2015), while R led to larger increase in nutrient accumulation (Miao et al., 2019). Because plants show so many different responses to light quality, affected by other abiotic conditions and light intensity, there is a need to further examine the combined effects of different light quality ratios to find the optimum.

These different responses to light quality and quantity can be viewed as stress responses. Because plants are sessile organisms they are specialized to the conditions the species or population normally grow in. What this entails is generational adaptation for traits favorable in their normal environment, while being able to acclimatize to changes in the environmental conditions. This acclimation process can be viewed as a stress response, where physiological or morphological responses of an organism change dependent on the surrounding conditions. Because plants show such a strong impact by light conditions it is possible to manipulate the crops largely by creating appropriate conditions with artificial lighting.

2.2.4 Artificial Lighting

In plant production artificial lighting have been important for many years (Stanghellini et al., 2019). Especially in high latitude locations where the natural light conditions vary greatly from season to season artificial lighting allows for longer growth season and potential year-round production (Moe et al., 2005; Stanghellini et al., 2019). As both light intensity, quality and duration affects biomass, yield and the plant quality it is a vital aspect to control in order to optimize production. Many electrical light sources have been in use over the years, including high-pressure sodium lamps (HPSLs), high-pressure mercury lamps (HPMLs), metal-halide lamps (MHLs), fluorescent lamps (FLs) and incandescent lamps (ILs) (Dutta Gupta & Agarwal, 2017).

In the last years the use of LEDs have become more common. They have lower energy consumption, and long life expectancy (Dutta Gupta & Agarwal, 2017), compared to many of the other types of electrical lighting. They do not produce heat, which makes it possible to conduct crop interlighting, leading to higher productivity in the lower leaves of the canopy (Stanghellini et al., 2019). The use of LEDs has also opened for the possibility of designing the exact emission spectra of incident light, making it possible to manipulate the light conditions for each specific crop, and therefore improve production (Demotes-Mainard et al., 2016; Mitchell et al., 2015). Many studies have shown the benefits of finding the optimal R/BL ratio on yield and quality of different crops (Naznin et al., 2019; Piovene et al., 2015; Samuolienė et al., 2010; Zha & Liu, 2018).

2.3 CO_2 and Plant Responses

2.3.1 Background and Biochemistry

CO_2 is an important molecule for life on earth. It is essential in photosynthesis, hence fundamental for the oxygen-rich atmosphere. CO_2 is the carbon-based molecule that enables carbohydrate production and chemical energy to the plant, and its presence in the plant environment is vital. However, since the post-industrial revolution human activities have led to a rapid increase in atmospheric CO_2 concentration, especially with regards to the burning of fossil fuels (Willey, 2018). This have given a negative impact on the environment with the greenhouse effect and global warming. As the amount of CO_2 is expected to increase further, an important question is how plants will respond to the increase, and how it will affect both wild and cultivated plants. As the human existence in many areas relies on plant production, it will be important to try to anticipate the effect an increase in atmospheric CO_2 will have, especially on crop plants used in food or feed production.

CO_2 enters the plant leaves through the stomatal opening. The stomata are responsible for both CO_2 assimilation and water vaporization, and its regulation is important for photosynthesis. The carbon fixation occurs when CO_2 reacts with RuBisCO in the Calvin-Benson cycle. The interaction between the stoma aperture and plant metabolism is divided into three metabolism types; C3, C4 and CAM, where C3 and C4 are the two relevant for horticultural science in Norway. C3 is the most common metabolism type and includes the faba beans. In C3 the CO_2 is fixated by the enzyme RuBisCO. As RuBisCO can act as both a carboxylase and an oxygenase this method is not very efficient. In other words, the efficiency of the photosynthesis is dependent on the CO_2:O_2 ratio in the intracellular space. Hence, increasing available carbon could have a positive effect, as long as other growth factors are not limiting (Kant et al., 2012).

The C4 metabolism is to a larger degree adapted to low CO_2 concentrations, as it separates the carbon fixation and photosynthetic activity in different parts of the leaf. CO_2 is fixated by the enzyme PEP carboxylase, which is not affected by the CO_2:O_2 ratio. The CO_2 is transformed to malate, which is then translocated to a neighbor cell where photosynthesis occurs. This means C4 metabolism is more efficient than C3. However, by manipulating the environmental conditions surrounding the C3 crops, it is possible to maximize the efficiency of these crops as well. This point has been further emphasized as it has become clear that RuBisCO activity is strongly negatively affected by changing abiotic factors caused by climate change (Galmés et al., 2013). This suggests that more research is needed in order to maintain a sufficient level of photosynthesis, in the face of changing environmental conditions.

2.3.2 Effect on Plants

The stomata are pores on one or two leaf surfaces (abaxial/adaxial), each surrounded by two guard cells. The control of stomata regulate the water status of the plant and the intracellular CO_2 concentration in response to the environmental conditions. This control is important in C3 plants as it will affect the CO_2:O_2 status in the intracellular space of the leaves and therefore affect the carboxylase/oxygenase activity of RuBisCO. One of the important aspects regarding the stomatal opening is the balance in allowing enough CO_2 to enter the leaf, while not losing too much water at the same time. Especially in C3 plants this will be important to keep an efficient photosynthesis. Therefore, to increase the CO_2 concentration outside the leaves allows the stomata to operate at smaller apertures, and therefore limiting water loss while at the same time providing sufficient amounts of CO_2 to photosynthesis. Because of this elevated levels of CO_2 have often led to an increased photosynthetic rate, while at the same time a decrease in stomatal conductance (Taub, 2010). Although the photosynthetic rate is expected to increase with elevated CO_2, there are big differences between species, where trees have shown a large response, while legumes showed the smallest (Ainsworth & Rogers, 2007).

From previously conducted FACE-experiments (Free Air CO_2 Enrichment) where CO_2 concentration was elevated with 200 ppm from ambient level, an average of 18% increase in yield was found under non-stress conditions, and legumes showed an even greater increase (Ainsworth & Long, 2021). However, this increase was diminished under nitrogen deficiency or under wet conditions. As greater variation in seasons and more extreme weather is expected to be more common, the weather in the future could mitigate the benefits from an increase in CO_2. There is also found evidence that elevated CO_2 led to inhibition in assimilation of nitrate in wheat and *Arabidopsis* (Bloom et al., 2010). Both are examples showing the advantage of production in controlled environment, as increased CO_2 could lead to increased yield, as long as other environmental factors are kept stable. Moreover, elevated CO_2 have previously led to a decrease in leaf nitrogen concentration which is closely linked to plant protein content. This decrease will be crucial to plant production as a fall in food quality is important to avoid. One hypothesis is that the reduction in stomatal opening and conductance limits the nutrient uptake, and therefore reduces the protein content of the plant tissue (Taub, 2010). This will mostly be an issue in fields and in wild plants, as it could be possible to solve this issue with increased nutrient concentrations in the irrigation system in a greenhouse or plant factory.

2.3.3 Potential for Circular Economy

An interesting aspect of CO_2 is that it is usually viewed as one of the problematic GHGs. More so because it is one of the pollutants associated with industry production and fossil energy sources. Therefore, to decrease the amount of CO_2 from human activity that is released into the atmosphere is important to reduce human impact on global warming and climate change. One alternative to reducing CO_2 emissions as well as making greenhouse production more efficient could be to connect the two. An example of this is the previously mentioned "The Magical Factory" (Den Magiske Fabrikken). Another example is GreenCap Solutions AS, which is a system created to capture CO_2 from ambient air (GreenCap Solutions). This technology can be connected to greenhouses, circulating the greenhouse air, and both optimize the CO_2 concentration suitable for the crop in production in addition to dehumidify the air. The dehumidifying process produces excess heat that can be used in temperature control, and the excess water can then be re-used for other purposes.

This method of production shows great potential with regards to both better control of greenhouse production parameters, as well as reducing the amount of CO_2 in the surrounding air. The system was tested on tomato production in a greenhouse in Norway and the experiment concluded that the GreenCap Solution system worked as planned, with potential of maximum optimalization for photosynthesis, yield and quality (Verheul & Maessen, 2021). These examples of food production systems leaning towards circular economy could be a solution to many challenges, as the food production can be optimized utilizing excess CO_2, hopefully limiting GHG effects, in addition to reaching maximum production potential.

3. Materials and methods

3.1 Plant Material and Establishment

Seeds of faba bean (*Vicia faba* L.) were sown in 3-liter pots (1 seed per pot) filled with Sphagnum peat mixture medium (Gartnerjord, 86% Sphagnum peat, 10% sand, 4% granulated clay, Tjerbo Torvfabrikk, Rakkestad, Norway). The peat mixture contained the following fertilizer; 35mg/L P, 190 mg/L K, and 900 mg/L total-N, with pH 5,5-6,5 and electrical conductivity (EC) of 25 mS/m. The two varieties sown was ´Witkiem major´ (LOG AS), a well-established landrace known for having big seeds, and being robust with high yield, and ´Ratio´ (Solhatt Økologiske Frø, Norge), a newer variety expected to be smaller, and therefore possibly more suitable for greenhouse horticulture.

The pots were placed in a growth chamber with high pressure sodium lamps (HPS, LU400/XO/T/40, GE Lighting, General Electric Company Nela Park, Cleveland, USA) giving approximately 150 μmol m^{-2} s^{-1} for ten days. The pots were watered with tap water. The photoperiod was 16 hours, temperature kept constant at 20°C, and relative humidity (RH) of 60%. Temperature and RH were controlled by a PRIVA system (Priva, De Lier, The Netherlands). After the ten days the 20 plants at approximate the same leaf number were chosen of each cultivar and divided between two growth chambers (Fig. 2).

Figure 2: The plants at the beginning of treatment. From left: high R/BL with a[CO_2], low R/BL with a[CO_2], high R/BL with e[CO_2], low R/BL with e[CO_2].

3.2 Experimental Set-up and Controlled Conditions

After the treatment started the temperature was kept at 16°C during the dark period (8h) and 22°C during the photoperiod (16h)(Fig. 3), and the relative air humidity (RH) was aimed at 60% (Table 1). The plants were watered with fertilized water using an equal mixture of YaraTera™ Calcinit™ (15.5% N, 14.4% NO_3, 1.1% NH_4, and 19.0% Ca, Yara Norge AS, Oslo, Norway) and YaraTera™ Kristalon™ (9.0% N, 8.0% NO_3, 1% NH_4, 4.8% P, 24.9% K, 4.2%

Mg, 5.7% S, 0.027% B, 0.004% Cu, 0.20% Fe, 0.06% Mn, 0.004% Mo, 0.027% Zn, Yara Norge AS, Oslo, Norway). In the chambers with a[CO_2] the EC of the fertilizer water was 1.5 dSm^{-1}, while in the chambers with e[CO_2], as the transpiration was expected to decrease 30% (Bævre & Gislerod, 1999), the EC was increased to 2.0 dSm^{-1} in order to limit the risk of nutrient deficiency.

Table 1: Overview of the ideal and actual conditions for the chambers with different CO_2 concentration. SD calculated from recorded data every five minutes for the a[CO_2] and every 15 minutes for the e[CO_2], during one photoperiod or one dark period.

	Temp photoperiod	**Temp dark period**	**Relative humidity**	**CO_2 concentration in photoperiod**
Ideal conditions a[CO_2]	22°C	16°C	60%	415 ppm
Actual conditions a[CO_2]	22°C±0.3°C	16°C±0.1°C	60-95%	415 ppm
Ideal conditions e[CO_2]	22°C	16°C	60%	1000 ppm
Actual conditions e[CO_2]	22°C±0.5°C	16°C±0.2°C	60-95%	1000 ppm±45 ppm

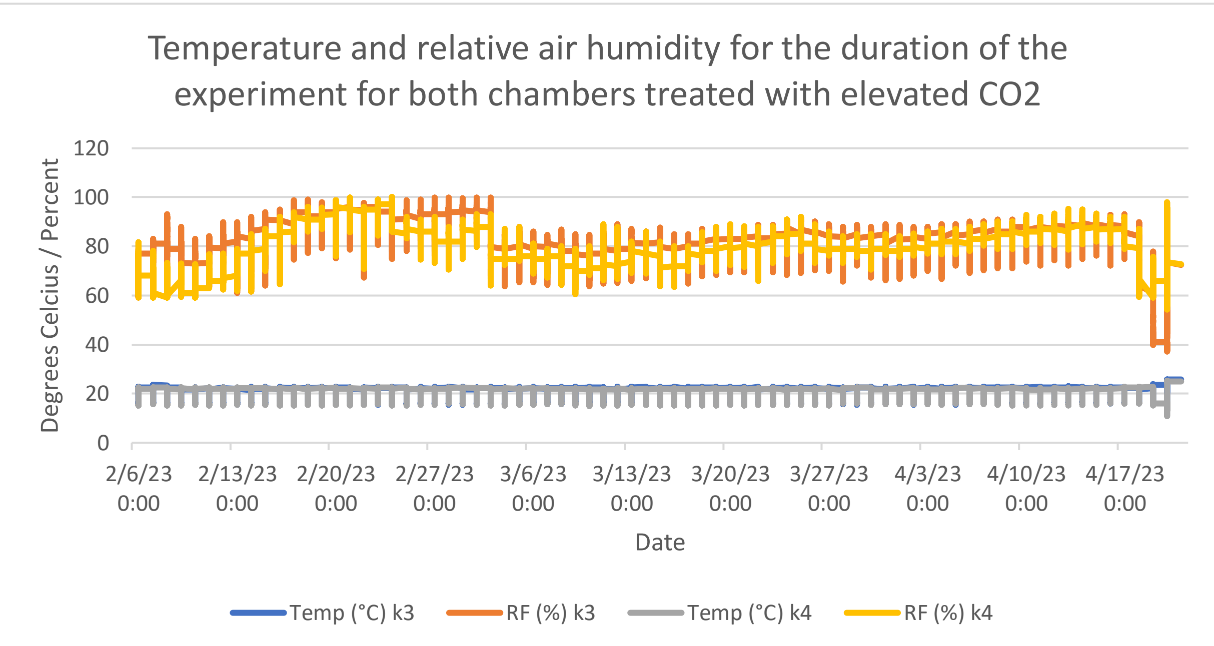


Figure 3: Logging of RH (RF) and temperature for the two chambers with e[CO_2] for the duration of the experiment, gathered from the PRIVA system. Where k3 represents the high R/BL treatment, and k4 represents the low R/BL treatment.

In order to test light with different R and BL ratios, two different light quality designs were used. Light emitting diodes (LED, Evolys, Norway) with additional far-red (700-800 nm) light emitting diodes (LED, Evolys, Norway), adjusted to give the same red/far-red (R/FR) in all chambers. In chambers 1 and 3 there was a peak in the R spectrum (R/BL approx. 4.1) of approximately 650 nm (Fig. 5, left), whereas in chambers 2 and 4 there was a peak in the BL spectrum (R/BL approx. 1.1) of approximately 450 nm (Fig. 5, right), and a bigger portion of green light compared to chambers 1 and 3. The light environment was measured at six points spread out in the chamber at the plant surface using a LI-COR spectrometer (LI-180 Spectrometer, LI-COR Biosciences, NE, USA) multiple times during the experiment to make sure the treatment remained constant and not exceeding 300 μmol m^{-2} s^{-1} at the top of the canopy. Some important light levels and ratios are gathered in Table 2. In addition, the chambers were enriched with two different CO_2 concentrations during the photoperiod; chambers 1 and 2 were treated with a[CO_2] of approximately 415 ppm, while chambers 3 and 4 were treated with e[CO_2] of approximately 1000 ppm (Fig. 4). The additional CO_2 was attained by connecting CO_2 storage tanks (Karbondioksid, AGA AS, Oslo, Norway) to the ventilation system circulating air through the chambers. This was monitored using PicoLog 6 data logging software (PicoLog 6, Pico Technology, England and Wales) on a Linux mini-computer.

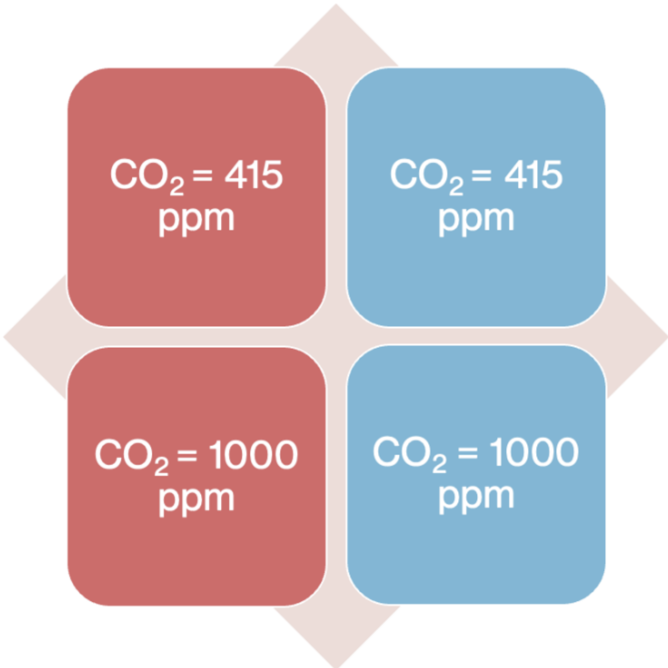


Figure 4 The experimental set-up of the four growth chambers, where two chambers were given a high R/BL of approximately 4.1 (red), and two were given a low R/BL of approximately 1.1 (blue). Across the light treatments, two chambers were given a[CO_2],, approximately 415 ppm, while the two others were given e[CO_2], approximately 1000 ppm.

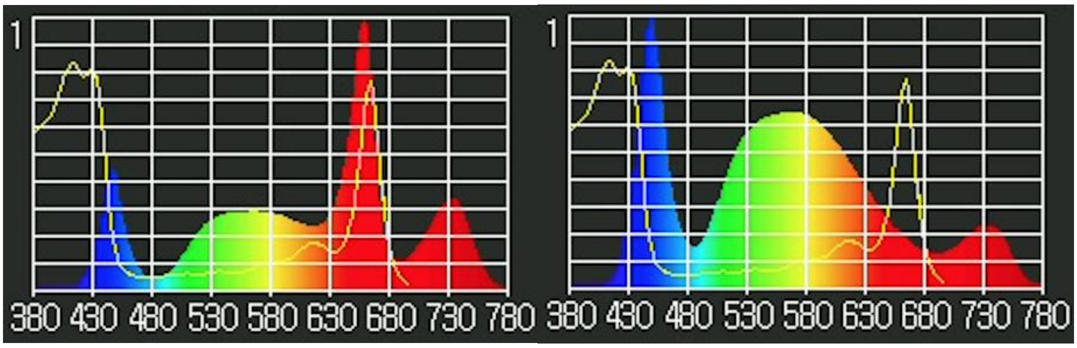


Figure 5: Light spectrum of the two LED treatments. Left: high R/BL treatment, right: low R/BL treatment. The absorption spectrum of chlorophyll A is added as a reference (yellow line).

Table 2: Overview of light treatments at the experiment start of the four chambers. Mean values calculated from six measurements at different spots in each chamber, and given in μmol m^{-2} s^{-1} for PPFD and PFD values. The three bottom rows gives the ratios of red and blue light (R:B), red and far-red light (R:FR) and blue and far-red light (B:FR).

	High R/BL + a[CO_2]	Low R/BL + a[CO_2]	High R/BL + e[CO_2]	Low R/BL + e[CO_2]
Total PPFD 400-700nm	209.51	212.37	211.13	211.57
Blue (PFD) 400-500nm	27.86	51.44	28.48	50.99
Red (PFD) 600-700nm	115.70	58.87	116.22	58.65
Green (PFD) 500-600nm	65.95	102.06	66.44	101.94
FR (PFD)700-780nm	51.77	25.98	52.31	27.82
R:B	4.15	1.14	4.08	1.15
R:FR	2.23	2.27	2.22	2.11
B:FR	0.54	1.98	0.54	1.83

To maintain an equal light intensity as much as possible the plants were cut to limit height differences. The main stem and four first side shoots were cut after they had developed three nodes with completely open flower buds. The shoots were cut one leaf above the third flower bud, the fourth bud was also removed from this leaf. When the side shoots were cut down the chlorophyll content was measured using a chlorophyll content meter (Model CL-01, Hansatech Instruments Ltd, England) on the fifth leaf on the main shoot, and on an equal leaf on the side shoots. Five repeats were measured on each leaf, and three randomly chosen repeats of the first side shoot values were selected for statistical analysis. For the side shoots growing on nodes higher up on the plant the tops were cut when reaching 75 cm from the top of the pot, as this kept the light intensity at a maximum of 300 μmol m^{-2} s^{-1}. The tops that were cut was dried in a drying cabinet at 56°C and added to the total DW of the plant at the end of the experiment.

3.4 Observations and Measurements

3.4.1 Observations of Plant Development

In the first weeks of the experiments some weekly observations were carried out to follow the development. Emergence of first flower bud was registered as days from treatment start of each plant (Fig. 6, left). Similarly, the first fully open flower (Fig. 6, middle) and the emergence of first visible pod (Fig. 6, right) was registered for each plant. Number of side shoots were counted, given that the shoot had a completely unfolded leaf pair, or developed buds. The number of side shoots were registered throughout the experiments, until the final harvest. As a rule, there were normally two main side shoots on every node, but the side shoots could also develop secondary side shoots. These were removed towards the end of the experiment approximately 50 days after treatment start, to limit shading and better the air circulation in the chamber. The secondary side shoots were then dried and added to the total DW of each plant.

Figure 6: Development stages recorded. Left: Emergence of first bud. Middle: Example of fully open flower. Right: Emergence of first visible pod.

3.4.2 Final harvest

The final harvest was conducted at approximately the same time for both the a[CO_2] and e[CO_2] chambers, around day 70 after treatment began. Chlorophyll measurements were taken of leaf number five or similar for the main stem and first side shoot. For each plant the total FW was measured and dried in a drying cabinet (56°C) for approximately three weeks, until completely dry, for total DW measurements. The main stem in addition to the first four side shoots to appear were separated. The number of unfolded leaves of each was counted, and leaf area measured using a leaf area meter (LI-3100 Area Meter, LI-COR Biosciences, Lincoln, NE, USA). The number of mature pods were counted, number of seeds registered, and both pods and seeds were weighed and dried in a drying cabinet for DW measurements. For each cultivar and treatment three tubes of three healthy and mature seeds from the same stem was frozen at -80°C for protein analysis. The seeds were freeze-dried in a freeze drier (LyoQuest -55 NO PLUS, Telstar LyoQuest Laboratory Freeze Drier, Spain) and sent to LabTek for protein analysis with the Dumas method for total nitrogen (N) (Jung et al., 2003). The results gathered from the analysis were multiplied with 6.25 as a conversion factor to attain total protein (Müller, 2017). In the same way, dried leaves from the third side shoot was gathered from each treatment and cultivar (*n*=3) and sent to LabTek for total nitrogen analysis. The thickness of each stem was measured at 20cm from the bottom of the stem, using a caliper.

3.4.3 Leaf Gas Exchange

The leaf gas exchange measurements were done once during the growth period, where light response curves with seven levels of irradiance were registered using a portable infrared gas analyzer (IRGA, LI-6400 XT Portable Photosynthesis System, LI-COR Biosciences, Lincoln, NE, USA), with a cuvette with an internal light source (LCF, LI-COR 6400-40, LO-COR Biosciences, NE, USA), giving light containing 90% R and 10% BL to the plants grown in high R/BL, while plants grown in low R/BL were given 80% R and 20% BL. From three randomly chosen plants, from both cultivars and all treatments, one leaf of approximately the same age at the top of the canopy, and therefore likely to be photosynthetically important to the plant, was selected for measurements. The measurements were carried out between 2-8 hours from the beginning of the photoperiod, and the gas analyzer was placed inside the growth chambers for the entirety of the recordings (Fig. 7).

Figure 7: The gas analyzer when measuring light response in the chamber with a low R/BL treatment and ambient CO_2.

The logging recorded photosynthetic rate (A) and stomatal conductance (g_s). For the measurements the auto-program LightCurve2 was used, giving irradiances of 1000, 600, 300, 150, 100, 50 and 0μmol m^{-2} s^{-1}, conducting a logging of each after reaching stability. The block temperature was set at 22°C, with flow rate 300 μmol s^{-1}. For the a[CO_2] chambers the reference CO_2 was set to 415 μmol mol^{-1}, and for the e[CO_2] chambers the reference CO_2 was set to 1000 μmol mol^{-1}. RH was set to match the chamber conditions and varied from 59-67% in the ambient chambers with an average of 65%, while varying from 69-78% in the enriched chambers, with an average of 74%.

3.4.4 Microscopy

3.4.4.1 Light microscopy of stomata density

Healthy leaves from approximately the fifth leaf pair height were sampled from both cultivars, and all treatments at the day of the final harvest. The leaves were slowly divided to pull off the epidermis layer from the abaxial side of the leaf, placed directly in 100% ethanol for fixation, and placed in 4°C for storage until further use. Three peels from each treatment were randomly selected, placed on a sample plate, and examined using 10x magnification in a light microscope (DM 5000B Automated Upright Microscope, Leica, Wetzlar, Germany). Three areas of 0.9 mm^2 in each peel were randomly chosen (Fig. 8), and stomata counted using the Multi-point Tool in ImageJ. When counting stomata the ones touching the edges were excluded, and only the ones entirely in the frame of view included.

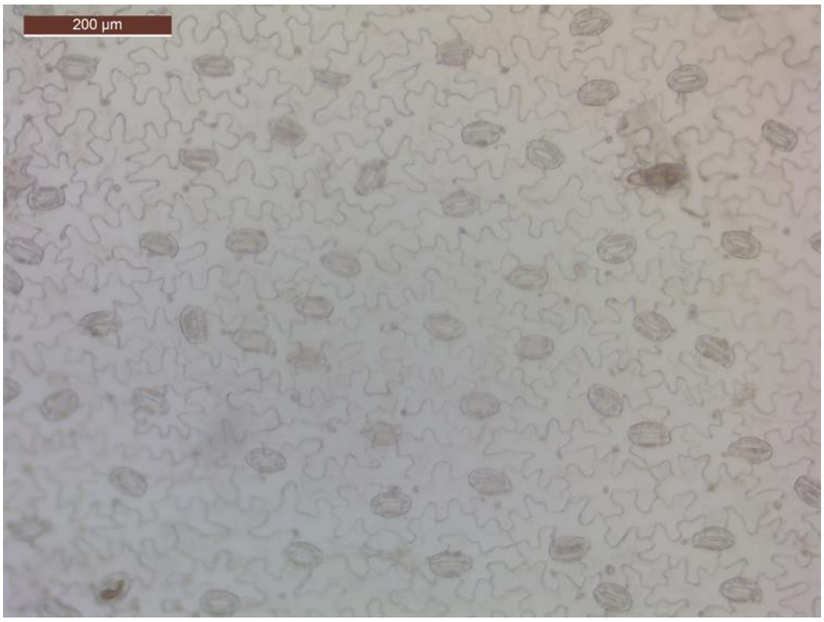


Figure 8: One of the counted areas of ´Ratio´ in the high R/BL treatment, with ambient CO_2.

3.4.4.2 SEM topographical of leaf edges

Edges of ten leaves towards the top of the plants were cut from ´Ratio´ in high R/BL and e[CO_2], five light green and five healthy, put in a fixative (paraformaldehyde solution) and stored in

4°C. After three weeks the samples were washed in PIPES buffer and dehydrated using an ethanol series according to Table 3. Three random samples from each, the healthy and the light green, were picked out for critical point drying (CPD 030, BAL-TEC AG, Pfäffikon Switzerland), and sputter coated (Sputter coater, EM ACE200, Leica, Wetzlar, Germany) with 20.2 nm platinum (Fig. 9), mounted on sample blocks and photographed in SEM (EVO50 EP Scanning electron microscope, ZEISS, Oberkochen, Germany) under high vacuum conditions at two different magnifications to examine stomatal location.

Table 3: Dehydration process to prepare for critical point drying for SEM.

Step	Solution	Duration
Fixation	2% PF + 1.25% GA in 0.05M PIPES buffer, pH 7	
Washing	0.1M PIPES buffer, pH 6.75	5 minutes x 3
Dehydration	30 % EtOH	10 minutes
	50 % EtOH	10 minutes
	70 % EtOH	15 minutes
	90 % EtOH	15 minutes
	96 % EtOH	15 minutes
	100% EtOH (Absolute alcohol)	15 minutes x 4

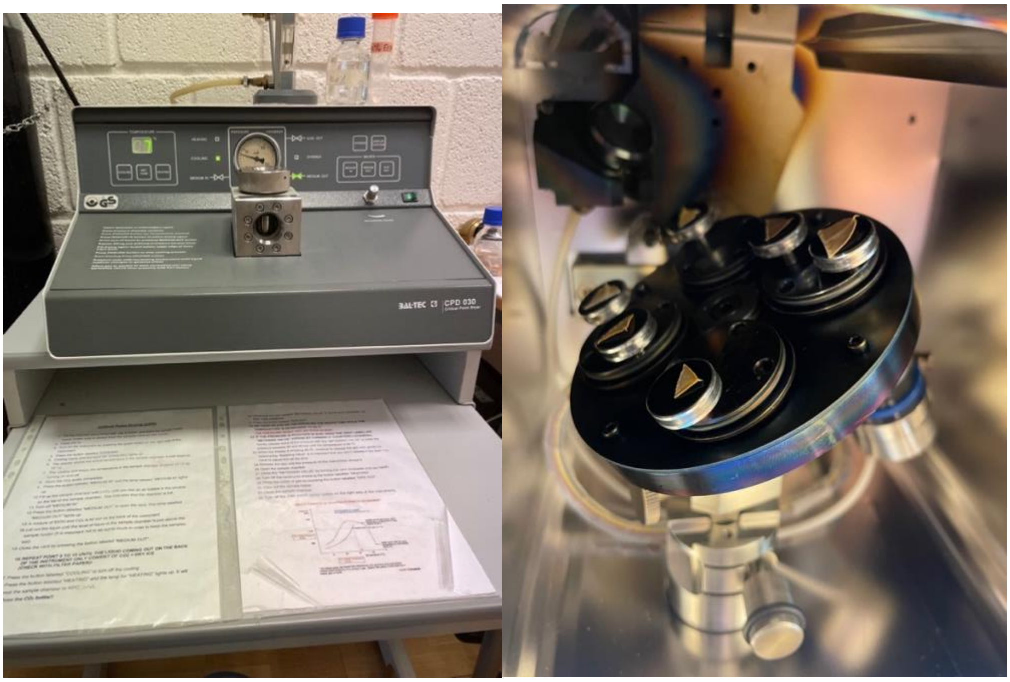


Figure 9: The preparation for SEM. The critical point drier (left), and leaf tips mounted on preparation blocks before being sputter coated (right).

3.4.4.3 SEM x-ray micro-analysis

One pair of healthy leaves (control) and one pair of damaged leaves were collected from ´Ratio´ grown in high R/BL. The leaves were placed in paper towels and pressed between tree blocks for a few weeks until completely dry. The dry leaves were mounted on a sample holder and analyzed using x-ray microanalysis (EDS Analysis system, Oxford Instruments NanoAnalysis, UK) under environmental pressure conditions in order to find the location and distribution of different elements. For each leaf three points were randomly selected at the middle of the leaf and at the front tip of the leaf (point of necrotic tissue on damaged leaves) and analyzed using INCA software. The middle of the leaf appeared to be healthy for both categories. Images of the areas where the random sampling occurred are presented in Figure 17. For the atomic percentage carbon, nitrogen, oxygen and hydrogen were excluded.

3.5 Statistics

All statistics were performed using RStudio for macOS (The R project for Statistical Computing and R-studio, R version 2023.6.1, Build 524, Posit Software, PBC, Boston, MA), except mean values and standard error of the development of first bud, first open flower and first pod, which were executed in Microsoft Excel for MacOS (Version 16.80, Microsoft Corporation). Only the difference between the two cultivars was tested using one-way ANOVA on the complete dataset. As there were expected to often be differences between the two varieties, the dataset was divided so that ´Ratio´ and ´Witkiem major´ were analyzed separately for other variables recorded. For statistical purposes the different treatments are separated into groups as shown in Table 4.

Table 4: Showing the group abbreviations used in some of the statistical presentations and analysis.

Variety	Light treatment	CO_2 concentration	Group abbreviation
Ratio	High R/BL	Ambient	RHA
Witkiem Major	High R/BL	Ambient	WHA
Ratio	Low R/BL	Ambient	RLA
Witkiem Major	Low R/BL	Ambient	WLA
Ratio	High R/BL	Enriched	RHE
Witkiem Major	High R/BL	Enriched	WHE
Ratio	Low R/BL	Enriched	RLE
Witkiem Major	Low R/BL	Enriched	WLE

First, the data was checked for homogeneity of variance using Levene´s test with a significance level of 5% (p<0.05). Secondly, the distribution was checked by visual inspection in the form of density plots and QQ-plot of normality, in addition to the Shapiro-Wilk´s test for a significance test of normality. In order to check for statistical differences of means, one-way and two-way ANOVA (where applicable) were performed, followed by post-hoc Tukey HSD if a significant difference was found. For the data that did not meet the assumption of normal distribution, Kruskal-Wallis test was performed, and pairwise Wilcox in order to compare treatments in cases with significant differences. Significance level was set to 5% ($p<0.05$) for all tests. The results that were not normally distributed and therefore tested with the Kruskal-Wallis significance test and pairwise Wilcox includes: side shoot production, number of pods per plant, number of seeds per plant, number of seeds per pod, average FW per seed, average leaf area, stomatal conductance, stomata density and atomic percentage of the microanalysis.

4. Results

4.1 Development and Growth

Two-way ANOVA was performed in RStudio, and no significant differences based on the light treatments of the appearance of first bud and first open flower was found in any of the cultivars. In addition, no interaction between R/BL ratio and CO_2 was found. However, e[CO_2] led to a significantly earlier appearance of first flower bud (Table 5) and first open flower. This was the same for both light treatments and varieties. ´Ratio´ developed slower than ´Witkiem Major´ for all treatments. Looking at the emergence of first pod the differences were greater. There was still a significant difference between the two cultivars ($p<0.05$), where the first pods of ´Witkiem major´ emerged earlier than those in ´Ratio´. For ´Ratio´ the high R/BL treatment led to significantly earlier emerged first pod ($p=0.012$), and the e[CO_2] was earlier than the a[CO_2] ($p=0.028$), however, no significant interaction of the two variables was found. For ´Witkiem major´ overall no significant difference was observed based on the treatments independently. However, there was a significant interaction between light conditions and CO_2 concentration ($p=0.013$). Where the high R/BL led to more days until pod emergence for the a[CO_2] treatment compared to e[CO_2], while the low R/BL led to the opposite.

Table 5: The effect of [CO_2] and R/BL treatment on development time. Mean values of counted days after beginning of treatment ± standard error (*n*=10), of the appearance of the first bud, first fully open flower and first visible pod in ´Ratio´ (Top panel) and ´Witkiem Major´ (Bottom panel) for both light treatments and CO_2 concentrations. *P*-values were found using two-way ANOVA. ´*´denotes that the mean values and statistical analysis were adjusted to *n*=9, the results were tested using two-way ANOVA.

´RATIO´	HIGH R/BL		P-VALUE	LOW R/BL		P-VALUE
	a[CO_2]	e[CO_2]		a[CO_2]	e[CO_2]	
FIRST BUD	14.1±0.53	12.0±0.42	0.006	15.2±0.97	12.5±0.40	0.015
FIRST OPEN FLOWER	22.0±0.15	19.6±0.40	<0.001	22.7±0.73	20.5±0.17	0.007
FIRST POD	38.8±3.40*	31.7±1.3*	0.099	43.4±2.16*	39.7±2.30*	0.355

´WITKIEM´	HIGH R/BL		P-VALUE	LOW R/BL		P-VALUE
	a[CO_2]	e[CO_2]		a[CO_2]	e[CO_2]	
FIRST BUD	13.1±0.47	11.2±0.49	0.009	13.3±0.42	11.6±0.50	0.018
FIRST OPEN FLOWER	20.5±0.37	18.3±0.37	0.001	20.6±0.31	18.8±0.33	0.001
FIRST POD	33.1±2.14*	28.9±0.39*	0.044	29.7±1.54*	36.2±2.90*	0.039

After approx. 50 days of treatment the number of side shoots was recorded. Mean values were calculated for each group (*n*=10), and significance was tested using Kruskal-Wallis test, with pairwise Wilcox as the values was not normally distributed. In addition, the values of total shoot production were summarized (Fig. 10). There was a large difference in the effect of CO_2 on side shoots between the two cultivars, where ´Ratio´ was greatly affected, while ´Witkiem Major´ was not. The side shoot production in ´Ratio´ was affected by CO_2 concentration (*p*=0,023), with a significantly higher production in the chamber treated with e[CO_2] (RLE and RHE) compared to the a[CO_2] (RLA and RHA), while no difference was detected for ´Witkiem Major´. No effect of the light quality treatment on side shoot production was found.

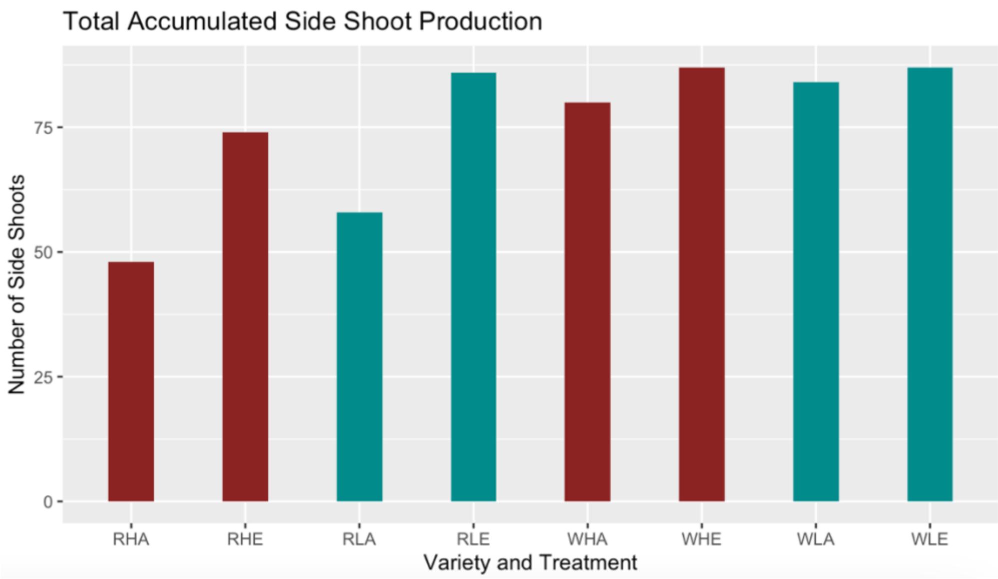


Figure 10: The effect of [CO_2] and R/BL on total number of accumulated side shoots produced by both varieties after 50 days of treatment. The blue bars showing the low R/BL treatment, and the red bars showing the high R/BL treatment. The groups are as follows, ´Ratio´ with high R/BL and a[CO_2] (RHA), ´Ratio´ with high R/BL and e[CO_2] (RHE), ´Ratio´ with low R/BL and a[CO_2] (RLA), ´Ratio´ with low R/BL and e[CO_2] (RLE), ´Witkiem major´ with high R/BL and a[CO_2] (WHA), ´Witkiem major´ with high R/BL and e[CO_2] (WHE), ´Witkiem major´ with low R/BL and a[CO_2] (WLA), and ´Witkiem major´ with low R/BL and e[CO_2] (WLE).

To further explore the morphological impact, mean leaf area for each treatment was calculated (*n*=10). The data was not normally distributed, so Kruskal-Wallis was used as analysis of variance, while pairwise Wilcox was used for significance testing between the groups. No difference was found between the two cultivars, or between the light treatments. However, the plants treated with e[CO_2] showed a trend of smaller leaf areas, although the only significant difference was in ´Ratio´ treated with low R/BL for the two CO_2 concentrations (*p*=0.005).
Average leaf size was calculated by dividing the total measured leaf area for each plant and divide it by number of leaves of the same plant. No significant differences were found for average leaf size (*n*=10) or the average thickness of the stem (*n*=10).

4.2 Plant Quality and Yield

´Witkiem Major´ produced significantly (p=0.005) higher total DW than ´Ratio´ (Fig. 11). After testing two-way ANOVA the plants exposed to e[CO_2] also showed a significantly larger DW compared to the plants cultivated in a[CO_2] concentration (p=0.033), but no significant difference between the light treatments was found (p>0.05). The interaction between light and CO_2 treatment was also tested, but no significant interaction was found. There seemed to be a weak trend of the high R/BL having an increased DW compared to the low R/BL treatment, although not significant. The lowest average of total DW is that of ´Ratio´ grown in low R/BL treatment and a[CO_2] which had a total DW of 42.34g, while the highest average of total DW was produced by ´Witkiem Major´ grown in the high R/BL treatment with the e[CO_2] concentration, with an average of 71.51g.

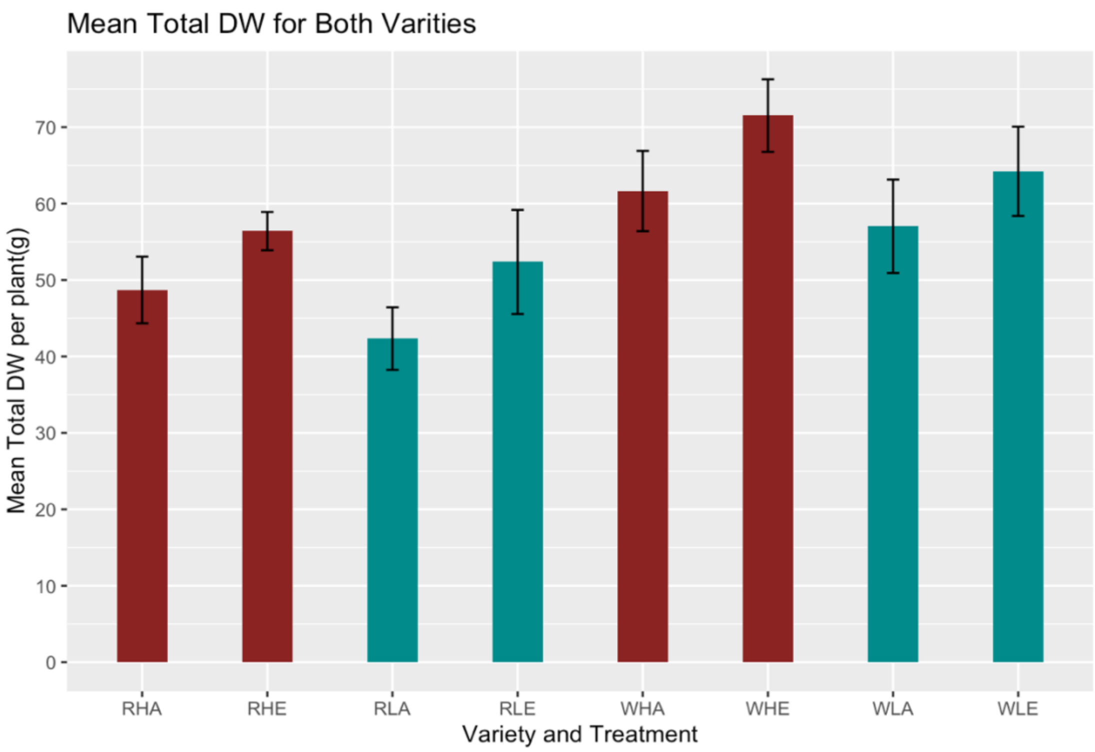


Figure 11: The effect of R/BL and [CO_2] on mean total DW per plant, shown as mean value ± SE (n=10). The low R/BL treatment shown as blue bars, and the high R/BL treatment shown as red bars. The groups are as follows, ´Ratio´ with high R/BL and a[CO_2] (RHA), ´Ratio´ with high R/BL and e[CO_2] (RHE), ´Ratio´ with low R/BL and a[CO_2] (RLA), ´Ratio´ with low R/BL and e[CO_2] (RLE), ´Witkiem major´ with high R/BL and a[CO_2] (WHA), ´Witkiem major´ with high R/BL and e[CO_2] (WHE), ´Witkiem major´ with low R/BL and a[CO_2] (WLA), and ´Witkiem major´ with low R/BL and e[CO_2] (WLE).

For the yield in pods and beans there were big differences between the two varieties dependent on the treatments. ´Ratio´ produced mature pods for the harvest of the a[CO_2] treatment in both R/BL treatments. But for the chambers treated with e[CO_2], although the plants had normal flowering compared to the other plants, only one plant had mature pods on the day of the final harvest in the low R/BL treatment. In effect, the results from ´Witkiem Major´ is the one that will be presented in this section (Fig. 13). In order to get a first impression of the actual yield produced, the total FW of the ´Witkiem Major´ beans was summarized (Fig. 12).

Figure 12: The effect of R/BL and e[CO_2] on summarized total FW of beans (without pods), for ´Witkiem Major´. The low R/BL treatment shown in blue color, and the high R/BL treatment shown in red color. The groups are as follows, ´Witkiem major´ with high R/BL and a[CO_2] (WHA), ´Witkiem major´ with high R/BL and e[CO_2] (WHE), ´Witkiem major´ with low R/BL and a[CO_2] (WLA), and ´Witkiem major´ with low R/BL and e[CO_2] (WLE).

From the total FW of the beans, plants treated with high R/BL had a bigger yield than the low R/BL treatment. In addition, the e[CO_2] induced a higher FW than the plants cultivated in a[CO_2]. Furthermore, the average weight per seed was calculated. There was a trend of the e[CO_2] treatment giving higher average FW per seed, although, after testing with Kruskal-Wallis (n=7) it was only found significant (p=0.025) in the low R/BL treatment.

Figure 13: Example of seeds harvested of ´Witkiem Major´ from a[CO_2] chamber (left) and from e[CO_2] chamber (right).

When looking at the mean number of seeds per pod there was a trend of the high R/BL treatment having a slightly higher number (2.5 per pod) compared to the low R/BL treatment (approx. 1.8

per pod). But the numbers varied greatly within the groups (*n*=7) and no significant difference was found. In addition, no significant difference was found with regards to average bean fresh weight per plant (*n*=10), or average number of pods per plant (*n*=10).

The next step was to look at the quality of the faba beans produced in controlled environment. As a measure of plant quality, the protein content was considered for the seeds and the total N for leaves (Fig. 14).

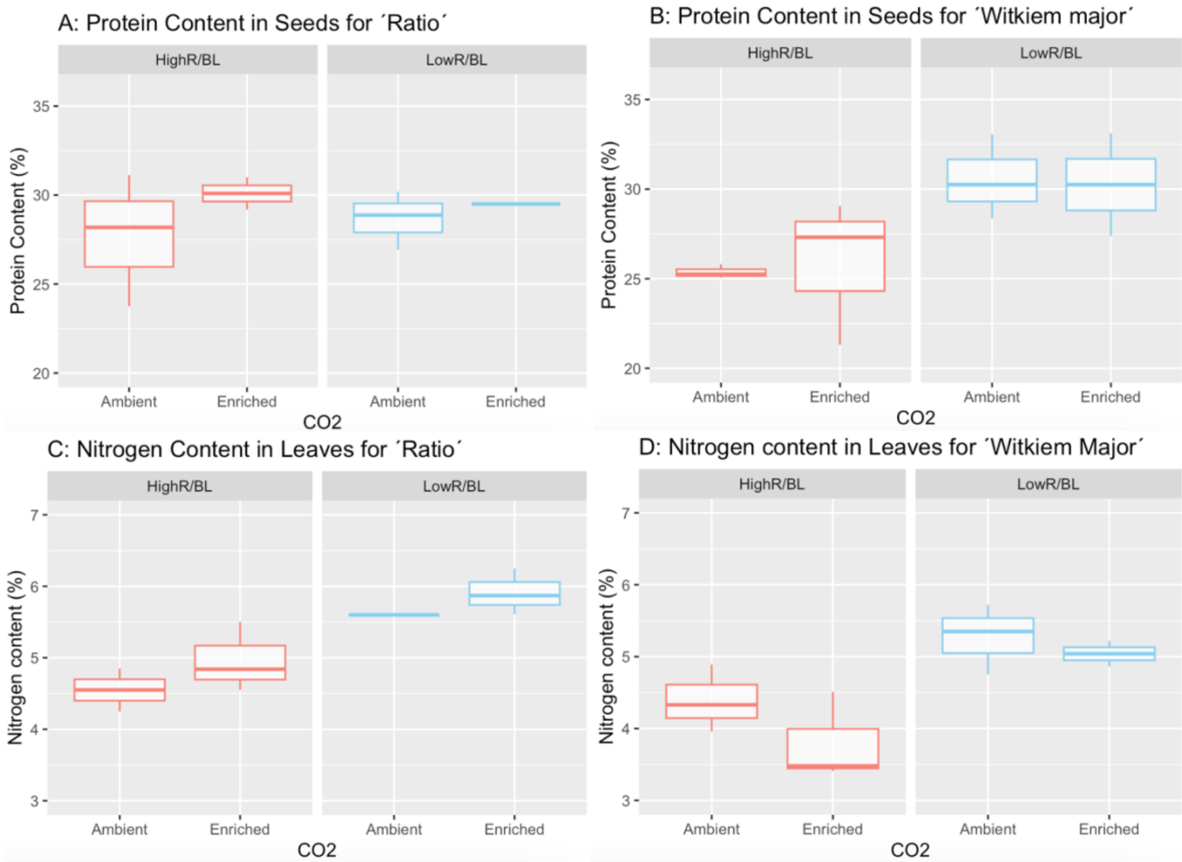


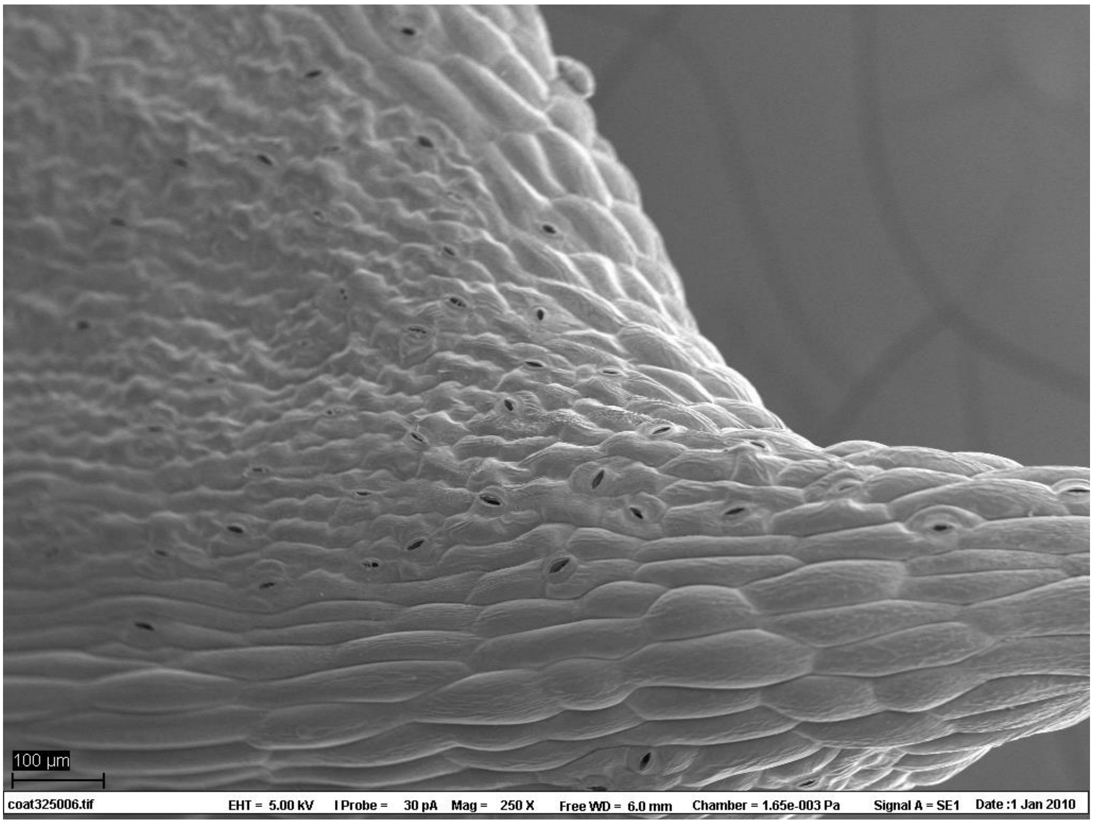
Figure 14: The effect of [CO_2] and R/BL on percentage of protein content in seeds and N content in leaves of both varieties gathered from the Dumas analysis for total N. A: Protein in seeds of Ratio. As only one plant of Ratio with low R/BL treatment and e[CO_2] had reached maturity for the final harvest this value is shown as a single horizontal line, but as there are no replicates no statistics was done for Ratio with this treatment. B: Protein in seeds of Witkiem Major (*n*=3). C: N content in leaves of Ratio (*n*=3). D: N content in leaves of Witkiem Major (*n*=3).

The percentage of protein content for the freeze-dried seeds was not affected by the CO_2 enrichment in the low R/BL treatment, but there was a trend of slightly higher protein content in the e[CO_2] chambers of the high R/BL treatments of both varieties, although not significant. However, for ´Witkiem Major´, there was a significant decrease in protein content in the high R/BL ratio treatment compared to the low R/BL (*p*=0.008)(Fig. 14 B), independent of the CO_2 treatment. For the N content in the leaves ´Ratio´ had a significantly higher N content (*p*=0.002) than ´Witkiem Major´ (Fig. 14 C, D), and the low R/BL treatment was also found to be significantly higher than the high R/BL treatment (*p*=0.020). No significant difference was found between the CO_2 treatments for either of the varieties. The main similarity between the protein content of seeds and the N content of the leaves was that there seems to be a higher percentage in the plants that was treated with the low R/BL.

Another aspect of plant quality is injuries and diseases. The fungal disease chocolate spot was not observed at all in any of the chambers. However, there was an injury on the tips of young leaves that was present in every chamber (Fig. 15, right). additionally, some of the pods were infected, most likely with the fungal disease Botrytis blight (*Botrytis cinerea*) (Fig. 15, left).

Figure 15: Two examples of the plant damage observed during the experiment. Left: A small pod with fungal disease. With parts of the withered flower attached. Right: Damaged/necrotic leaf tips.

Leaf edge burn is common in controlled environment, and it was hypothesized that the necrotic edges could be related to some sort of deficiency or accumulation of inorganic ions in the leaf tips. Leaf tips of ´Ratio´ were therefore topographically scanned in a SEM to find locations of stomata on the leaf tips. From the images (Fig. 16) the stomata appeared to be located towards the middle regions of the leaves, and not at the edges, nor the tip, although they are present in the region close to the tip. This seems to be the pattern for both the damaged and healthy leaves. In order to examine the reason for the withered leaf tips (Fig. 15, right), both leaves with necrotic tissue and healthy leaves were analyzed with x-ray to see if there were any differences in element composition (Table 6, Fig 17).



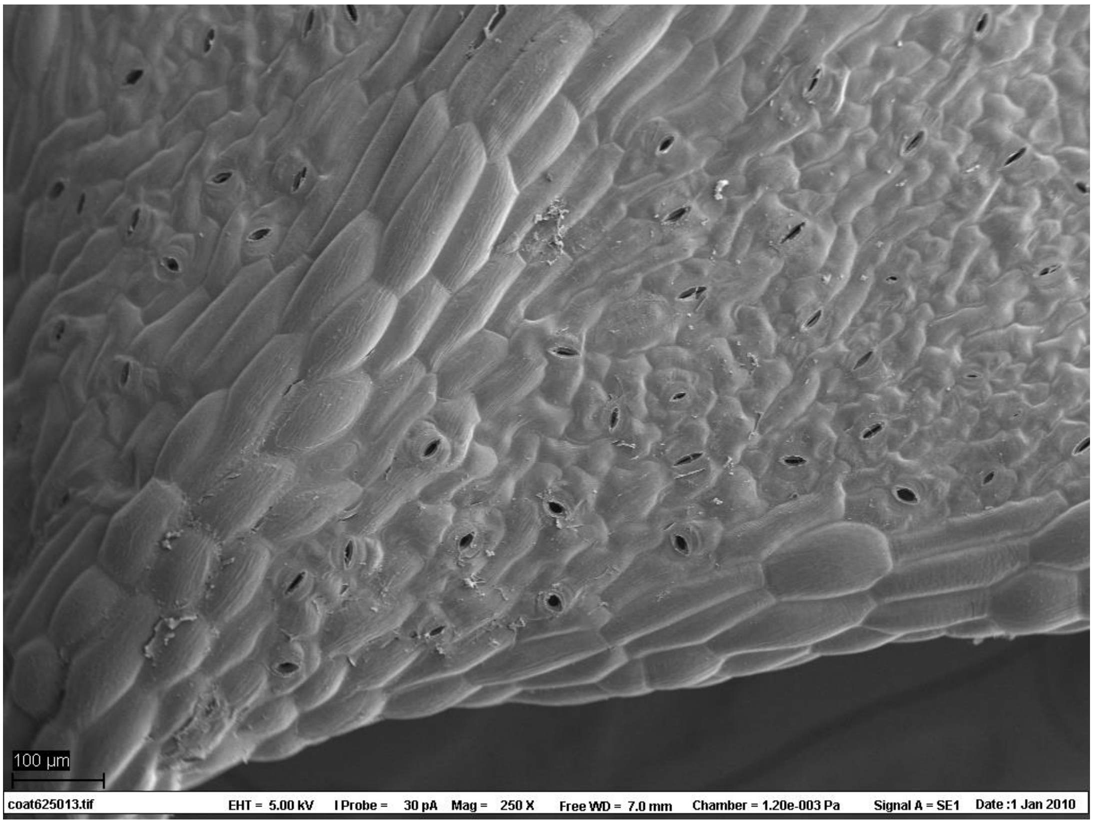


Figure 16: Images captured in the SEM at magnification of 250X of healthy (upper) and damaged (lower) leaf tips from ´Ratio´ treated with high R/BL and e[CO_2] concentration. See appendix for more images (Fig. 22, 23 and 24).

Table 6: Average accumulated elements registered using x-ray microanalysis from three randomly chosen areas in tip and middle leaf of four leaves of ´ Ratio´ (Fig. 17). Shown as average atomic percentage ± SE (*n*=3). The brown leaf tips (Fig. 15) were considered necrotic, while the green leaves and the middle leaf areas were considered healthy. The data was not normally distributed, and the letters are therefore showing significance after Kruskal-Wallis and pairwise Wilcox test, performed for each element in RStudio. Same letters indicate no significant difference.

CO_2	Color of leaf area	Area	Mg	Al	P	K	Cl	Ca
E	Brown	Tip	5.92±0.40ac	71.84±1.23a	14.73±0.67ab	2.72±0.72a	0.00a	0.00a
E		Mid	1.27±1.27a	0.00b	7.45±0.70b	68.46±3.77b	7.84±0.73b	9.18±1.93b
E	Green	Tip	8.41±1.25c	41.19±2.98c	6.22±0.63a	20.47±1.32c	4.73±0.56c	12.57±2.96b
E		Mid	15.86±1.30bc	0.00b	10.77±0.48bc	46.57±1.68d	7.65±0.12b	10.45±1.02b
A	Brown	Tip	4.97±3.11ac	80.15±7.68a	7.46±3.34ab	4.62±0.26a	0.00a	0.00a
A		Mid	3.27±0.35a	0.00b	11.45±0.60b	67.64±1.49b	8.27±0.59b	4.87±0.46b
A	Green	Tip	1.94±1.07c	56.29±9.73c	4.49±0.75a	27.93±6.97c	4.59±1.44c	2.41±0.51b
A		Mid	4.19±0.24bc	0.00b	16.72±0.50bc	59.49±2.01d	9.53±0.58b	1.51±1.51b

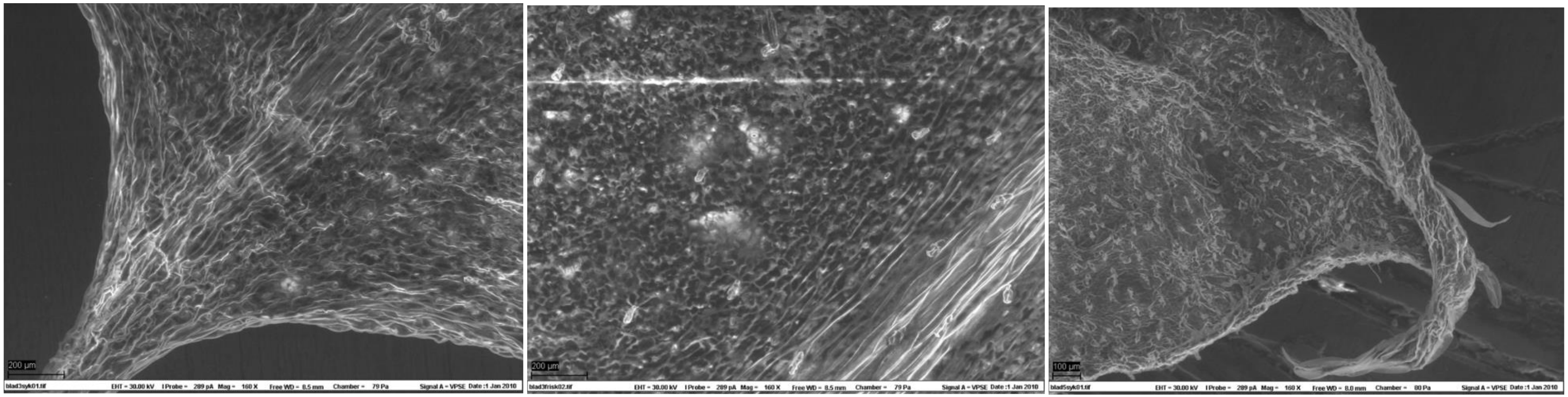


Figure 17: The leaf sections where three areas were randomly sampled for x-ray microanalysis from a green leaf tip (left), green middle area of leaf (middle) and brown leaf tip (right).

The clearest difference in element composition from this experiment was aluminum (Al) present in the leaf tips, while not registered at all in the middle of the leaves for neither the healthy leaves nor the leaves with necrotic brown tips. There were also registered significantly higher values of Al in the necrotic tips compared to the green tips. Similarly, the chlorine (Cl) and Ca were present to some extent in all healthy test areas both tips and middle leaves, but not in the necrotic tips. The amount of potassium (K) also decreased substantially in the necrotic tips compared to healthy test areas. For magnesium (Mg) and phosphor (P) the values varied.

4.3 Photosynthesis and Stomatal Conductance

In order to assess the physiological responses from the faba beans, the photosynthetic rate and stomatal conductance were measured.

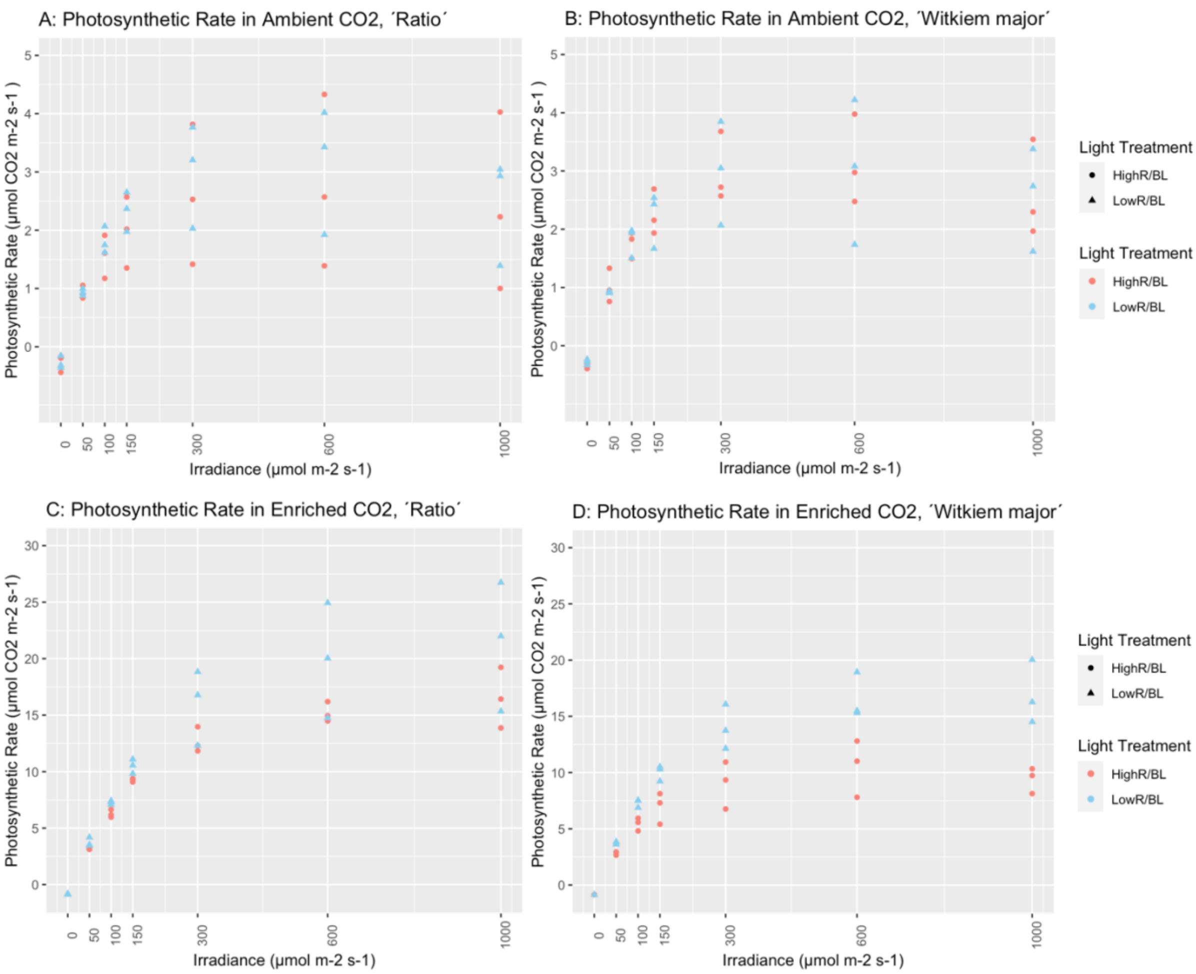


Figure 18: The effect of R/BL treatment and [CO_2] on photosynthetic rate for both ´Ratio´ (A, C) and ´Witkiem Major´ (B, D), with increasing irradiance from 0 to 1000 μmol m^{-2} s^{-1}. Comparing high (red circle) and low (blue triangle) R/BL treatment. Two-way ANOVA performed (*n*=3) for each irradiance. Note that the y-axis scale differs from A and B to C and D, due to big differences.

The photosynthetic rates increase up to approximately 300 μmol m^{-2} s^{-1} for both CO_2 concentrations and light treatments, as well as for both varieties (Fig. 18), whereafter the curves flatten, especially for a[CO_2]. There are significant differences for both varieties with regards to CO_2 concentration (p<0.05) for every irradiance tested (except 0 μmol m^{-2} s^{-1}), but no significant differences between the light treatments.

Furthermore, the chlorophyll content was registered for healthy leaves of both cultivars and all treatments. Both varieties had the lowest chlorophyll content in high R/BL ratio and e[CO_2] of values around 6 (RHE and WHE)(Fig. 19). For ´Witkiem Major´ the low R/BL with e[CO_2] treatment (WLE) gave the highest chlorophyll content, while for ´Ratio´ the highest content was found in low R/BL with a[CO_2]. When comparing the light treatments within the two [CO_2] treatments low R/BL ratio showed higher chlorophyll content for all groups, except for ´Witkiem Major´ grown in a[CO_2], where there was no difference between R/BL (WLA and WHA). Considering the effect of the [CO_2] treatments across the light quality treatments, a[CO_2] show higher levels of chlorophyll for all groups, except ´Witkiem Major´ in low R/BL (WLA and WLE) where the e[CO_2] treatment has significantly higher content than ambient concentration.

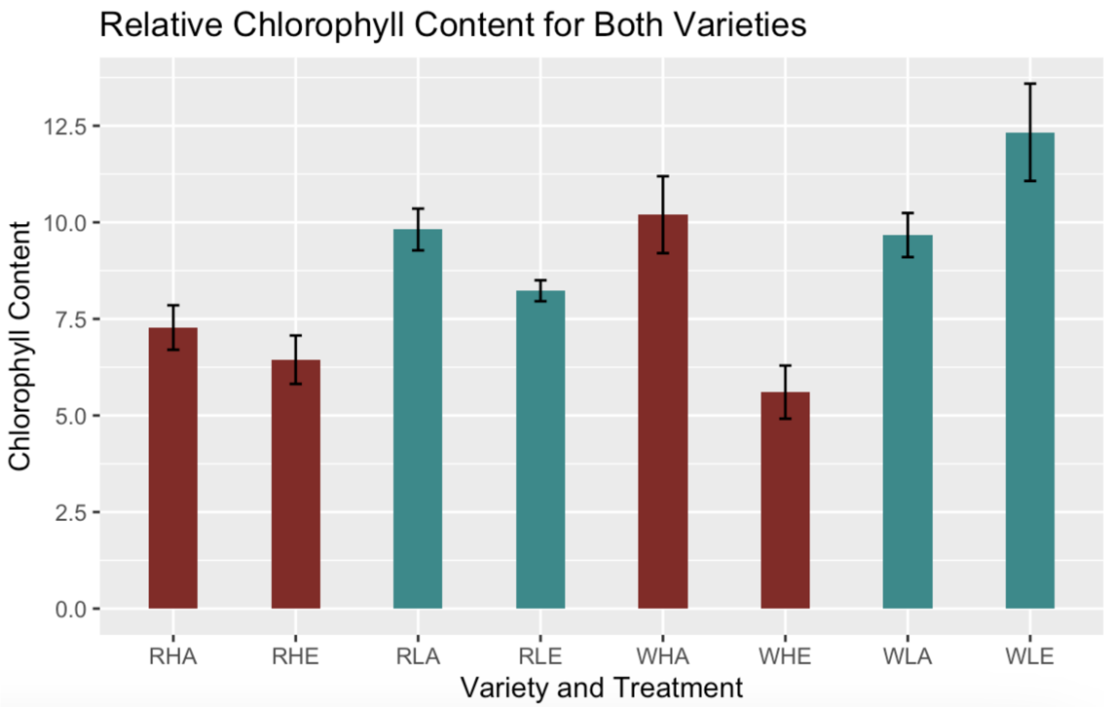


Figure 19: The effect of [CO_2] and R/BL on relative chlorophyll content, measured with a handheld LI-COR chlorophyll meter. Mean values of healthy leaves (n=3)± SE. The blue bars represent low R/BL treatment, while the red bars represent the high R/BL. Significance tested with two-way ANOVA, and post-hoc Tukey´s in RStudio. The groups are as follows, ´Ratio´ with high R/BL and a[CO_2] (RHA), ´Ratio´ with high R/BL and e[CO_2] (RHE), ´Ratio´ with low R/BL and a[CO_2] (RLA), ´Ratio´ with low R/BL and e[CO_2] (RLE), ´Witkiem major´ with high R/BL and a[CO_2] (WHA), ´Witkiem major´ with high R/BL and e[CO_2] (WHE), ´Witkiem major´ with low R/BL and a[CO_2] (WLA), and ´Witkiem major´ with low R/BL and e[CO_2] (WLE).

The same pattern can be seen for the stomatal conductance (Fig. 20) as for photosynthetic rate, a clear significant difference between the CO_2 concentrations for both varieties appeared ($p<0.05$). For the light treatments there were no difference for ´Ratio´, but for ´Witkiem Major´ on the other hand the high R/BL treatment lead to a significantly higher stomatal conductance compared to the low R/BL treatment.

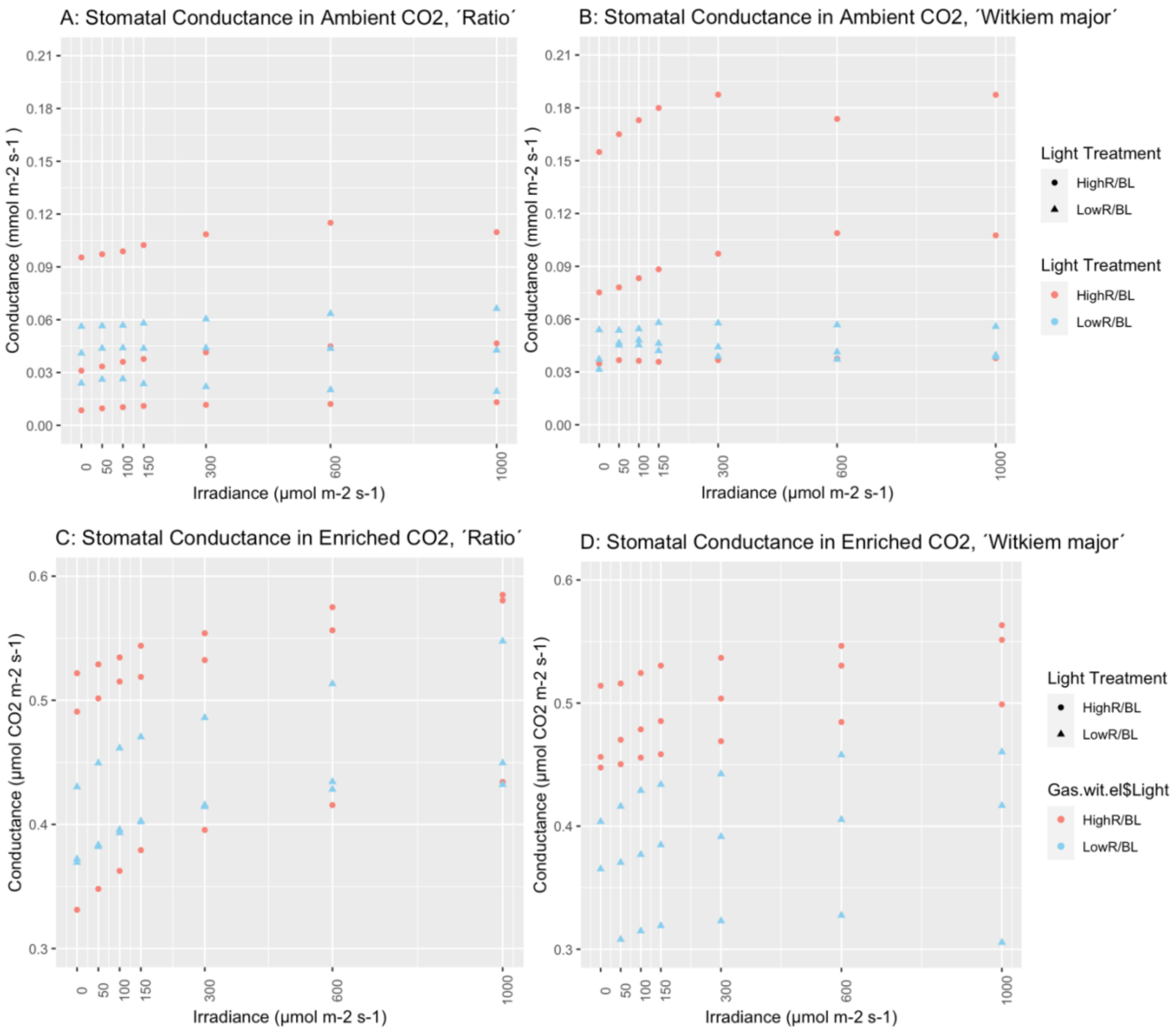


Figure 20: The effect of R/BL treatment and [CO_2] treatments on the stomatal conductance. The leaves were given a series of irradiances from 1000 to 0 μmol m^{-2} s^{-1} for ´Ratio´ (A, C) and ´Witkiem Major´ (B, D) for both high (red circles) and low (blue triangles) R/BL treatment and CO_2 concentrations. Significance tested with Kruskal-Wallis test, and pairwise Wilcox (n=6). Note that the y-axis scale differs from A and B to C and D, due to big differences.

Another aspect of the physiological response is the treatment effect on stomata density. ´Ratio´ had a significantly higher stomata density (p=0.001) than ´Witkiem Major´ (Fig. 21). No difference was found between the light treatments with regards to stomata density. However, in the high R/BL treatment of ´Witkiem Major´, the plants treated with e[CO_2] had a significantly higher stomata density compared to the a[CO_2] treatment (p=0.012). The same tendency can be seen in the difference of means for high R/BL ratio in ´Ratio´ as well, although the result is not significant.

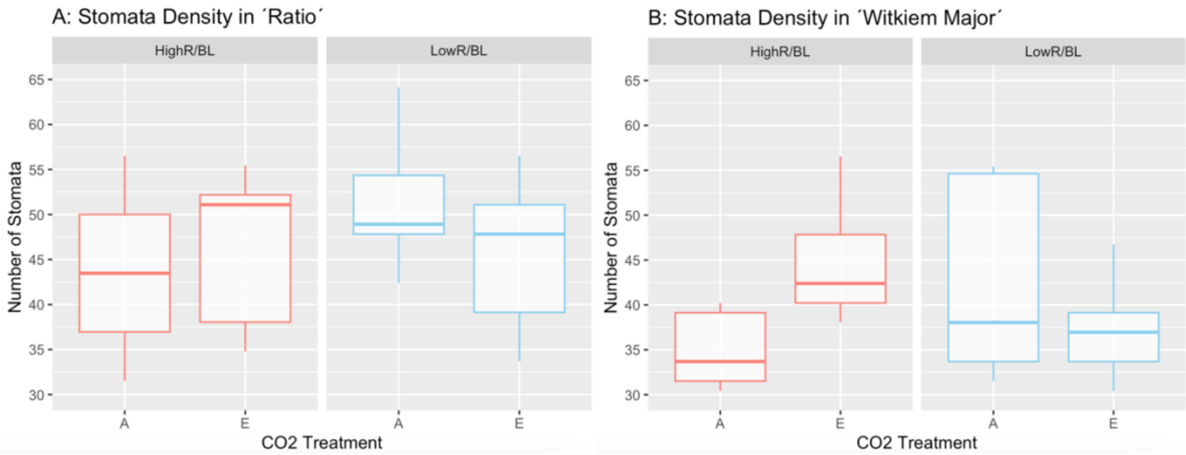


Figure 21: The effect of [CO_2] and R/BL on stomata densities (number of stomata per 0.9 mm^2) for ´Ratio´(A) and ´Witkiem major´(B). Red outline depicts the high R/BL treatment, while the blue outline represents the low R/BL treatment. Significance tested using the Kruskal-Wallis test, with the pairwise Wilcox for differences of the treatments (*n*=9).

5. Discussion

5.1 Faba Beans Grown in Controlled Environment

As expected, differences in growth and yield were found between the cultivars. ´Witkiem major´ grew generally larger and gave higher yield compared to ´Ratio´. In addition, it appeared more robust to the increase in CO_2 concentration. The genetic variance in the existing landraces and cultivars is substantial (Elshafei et al., 2019; Ibrahim, 2010; Muluálem et al., 2013), resulting in inconsistency in the actual yield. This instability has also made it difficult for growers to predict income. This emphasizes the importance of more efforts considering cultivars suitable for horticultural production. Hence, a precise breeding program aiming for horticultural implementation could help stabilize production and yield, and make faba bean a predictable crop,

When looking at new means of production of faba beans there are many possibilities to consider. This study showed that faba beans can grow with low light intensity (approx. 300 μmol m^{-2} s^{-1} at the top of the canopy). This is drastically lower intensity than the crop would experience in field conditions. Meaning that tunnel-production could be a possible choice. With tunnel-production the light intensity will be 20-30% lower than open field, as both the cover and other construction parts would intercept (Stanghellini et al., 2019). A previous study showed that faba beans grown in shade conditions led to an increase in mean grain weight and yield per unit area, most likely due to enhanced grain filling period (Nasrullahzadeh et al., 2007). This shows that faba beans have high tolerance to low irradiance. In addition, faba bean is a cool-season crop, and can withstand frost for short periods of time (Murphy-Bokern et al., 2017). However, for flowering the temperature optimum is thought to be 22/23°C (Patrick & Stoddard, 2010). This could be easier to attain in a tunnel compared to the field, although, the challenge of chocolate spot and other pests would still be a threat to yield and quality.

Furthermore, as the faba bean plant is to some extent self-pollinating (Øverland & Viken, 2020) it could be possible with even more closed production with limited pollinator access, such as greenhouses or PFALs. This study showed that both light quality and [CO_2] influenced the production, meaning that artificial lighting and the opportunity to control [CO_2] could lead to higher predictability for the crop and yield. However, more research is necessary to find the optimal conditions for the different parameters. This is particularly clear as the most robust of the two cultivars, ´Witkiem major´, showed a significant decrease in protein content in the high R/BL treatment compared to the low R/BL. In addition, to be suitable for production in PFALs the breeding of dwarf cultivars that could be applicable to vertical farming would also be necessary in order to fully utilize the indoor area.

One of the challenges that needs to be addressed with greenhouse or PFAL production is the method of harvest. Faba beans in the fields of Europe are mostly being harvested by machines (Øverland & Viken, 2020), similar to grain production, of dehydrated beans suitable for feed. However, because pods of the same plant develop at different times, harvesting fresh beans at the right time of maturation would be easier with manual harvest. In smaller cultivation areas they are mostly harvested by hand two to three times during the harvesting period (Karkanis et al., 2018). This is also the most used harvesting method in greenhouses at the moment. The manual harvest is often more labor demanding and time-consuming compared to field production. It could however be worth it if the yield was increased and with a higher quality than what has been possible to produce in the fields.

An important aspect of higher control of production is the reduction of pests and diseases. This was also evident in this experiment, as one of the most widespread fungal diseases in faba bean production, chocolate spot, was not found in any of the plants. As this disease can reduce yield and quality of the crop (Sahar et al., 2011), being able to limit the infection is imperative to future growers, and could be an incentive to invest in controlled environment. Many of the diseases and pests will spread from nearby areas, through weeds or the soil. Therefore, isolating the crop will be of great importance to secure high yield, in addition to decrease cost and time used on pest-management in the fields.

Another challenge often connected to production in controlled environment is leaf edge burn or tipburn, where necrotic lesions appear in especially young leaves (Fig. 15, right). The injury was observed in this experiment in all chambers, and has previously been observed in faba beans grown in phytotron (Bakke, 2022). There have been many different theories as of what is causing the injury; high humidity in poinsettia (*Euphorbia pulcherrima*)(Shamshiri et al., 2018), Ca deficiency in different cabbages (Kim et al., 2021), onion (*Allium cepa*) and fennel (*Foeniculum vulgare*) (Olle & Bender, 2009), associated with high levels of Mn in leaf lettuce (Bert & Honma, 1975), and with both high and low soil moisture levels (Bert & Honma, 1975). In general, tipburn is thought to occur when stress exceeds the plants´ stress tolerance (Saure, 1998), and described as a physiological disorder which is related to the environmental conditions (Bárcena et al., 2019).

High humidity have also been linked to Ca deficiency in tomatoes (Shamshiri et al., 2018). This could have been the case in the present study as high RH (80-90%) was registered, and after x-ray microanalysis the damaged leaf tips showed a significant reduction in atomic percentage of Ca. Although, Bárcena et al. (2019) found evidence that the occurrence of tipburn was independent of Ca status, it could still be possible that some of the environmental conditions leading to tipburn are also limiting Ca transport. As Ca is transported through the xylem it is dependent on water status of the plant, namely, the soil moisture conditions and the air humidity affecting stomata. It could therefore be seen as a co-occurrence rather than the cause of tipburn. If it is because of environmental conditions such as high RH this could explain why it is more common to see in greenhouses compared to field production, since air humidity is one of the most difficult parameters to decrease in controlled environment.

In contrast, the atomic percentage of Al is significantly increased in the damaged leaf tips. No Al was found in the middle parts of the leaves, and it seems as though it accumulates in the tips of both the healthy and necrotic tissue, although at a significantly higher percentage in the necrotic tips. High levels of Al could also affect the Ca uptake in the plants. Al is often found in nature as the cation Al^{3+}, and cations are known to repress the uptake and distribution of Ca (Olle & Bender, 2009). In addition, Al limits water transport capacity of the plant (Gavassi et al., 2020), which would in effect hinder the nutrient transport from roots to leaves (Legesse et al., 2017). However, this is most likely to affect the rhizosphere where Al is known to have toxicity effects, and as no soil analysis was conducted in the experiment this will only be a speculation.

The question remains why there was Al accumulation in the leaves. Previous studies have connected Al to plant defense mechanisms against fungal diseases (Banerjee et al., 2016; Satapathy et al., 2012). Satapathy et al. (2012) looked at Al conditioning of pigeon pea (*Cajanus cajan*), and found that the abiotic stress from Al significantly counteracted against *Fusarium* infections. The same response was found in wheat (Banerjee et al., 2016). In both cases the plants were treated with Al, in contrast to this study. However, Botrytis blight was present in the chambers, and it is probable that although not all plants showed signs of infection, the plant had most likely started a defense mechanism against the fungus. More research is needed to conclude on the role of Al in protection against fungal diseases.

To summarize, there is potential for production of faba beans in controlled environment. The requirement for light intensity seemed to be low to moderate, which would be easy to maintain in a tunnel or greenhouse. However, there is a need for more research on suitable cultivars, as there were big differences in the responses when comparing the two in the present study. In addition, for the species to be eligible for production in PFALs, smaller cultivars that could be suitable for vertical farming would also have to be developed. Overall, the possibility for faba beans to be grown in a greenhouse with possibility for CO_2 enrichment and temperature control could be very promising, however, the method of harvest would have to be revised, and possibly higher yield cultivars would have to be developed to make the production profitable. From a financial point of view the question will be if the reduction in costs of products and labor related to pest-management and maintenance of field-equipment will be enough to outweigh the added expenses of electricity and increased cost and time of harvest.

5.2 The Effect of Different R/BL and CO_2 Enrichment on Growth and Development

E[CO_2] was expected to increase branching, as it is a common response for numerous species (Gielen et al., 2002; Reddy et al., 1995; Sasek & Strain, 1991). The total side shoot production of ´Ratio´ was in line with this expectation, with a significant increase for both R/BL treatments, however, ´Witkiem major´ showed no difference. This emphasizes ´Witkiem major´ as the most robust cultivar of the two. It should be noted that although no difference was found in ´Witkiem major´ the number of side shoots was already high. The increase in branching in ´Ratio´ gave it similar number of side shoots as ´Witkiem major´ showed in both CO_2 conditions. Side shoot production can be of great importance to the farmer as it could affect the yield. An increase in shoots could generate the possibility of more pod bearing shoots, which could increase the yield.

However, the plant is developing based on its energy capacity. This entails that the energy the plant is spending on additional side shoots could decrease energy spent on another aspect of the plant production, such as seed filling. There was a drastic decrease in mature pods by the time of the final harvest for ´Ratio´ in the e[CO_2] chamber with low R/BL. However, the flowering occurred approximately at the same time as with ´Witkiem Major´ in the same chamber. This could imply that there was a different use of energy between flowering and seed filling. As there was a significant increase in side shoot production for ´Ratio´ after the CO_2 enrichment, this could mean that low R/BL ´Ratio´ prioritized branching rather than generating mature pods.

Time of development from the beginning of the treatments decreased significantly in chambers with e[CO_2]. The difference was approximately two days for both cultivars and light treatments when considering emergence of first flower bud and first open flower. Because of a practical issue the plants that were grown in the e[CO_2] chambers were two days older at the point of treatment start, this could therefore explain the difference as it is consistent throughout the development of first bud and first open flower.

For all treatments ´Ratio´ developed slower than ´Witkiem major´, showing that ´Witkiem major´ could be a more suitable cultivar when considering development time. The high R/BL treatment led to an earlier emergence of first pod in ´Ratio´ compared to the low R/BL for both CO_2 treatments. For ´Witkiem major´, the high R/BL gave earlier development with e[CO_2], while the opposite was true for the low R/BL. This interaction of light quality and CO_2 concentration was significant. Assuming that shorter development time is preferable it seems that when given a higher amount of R the plant can easier utilize the increased amount of available CO_2. While, when given a larger amount of BL (low R/BL) the faba bean is not able to utilize the increased CO_2 in the same way.

To explore this point further, it is possible to connect the utilization of e[CO_2] to the stomata densities (Fig. 21B). For ´Witkiem major´ the amount of stomata in the high R/BL was significantly higher in the e[CO_2] compared to the a[CO_2]. Meaning that it would possibly be easier to increase the CO_2 uptake in the leaves. However, for the plants grown in low R/BL the stomata density was highest in the a[CO_2] concentration, although not significant, which could imply that this could affect the utilization of the CO_2, as the gas exchange would be slower at lower densities.

However, this does not coincide with existing literature on CO_2 concentration´s effect on stomata density (Woodward & Kelly, 1995; Woodward et al., 2002). Woodward and Kelly (1995) tested a hundred different plant species which showed a mean reduction of 14,3% on stomata density after CO_2 enrichment. As higher CO_2 concentrations have been connected to lower stomatal densities, the lower stomata density has also been explained as part of the reason for lower conductance (Woodward et al., 2002). A recent study showed that the elevation of the CO_2 concentration from 430 to 650 ppm only reduced stomatal conductance when the plants had optimal irrigation conditions, while plants grown under water stress conditions showed almost zero effect (Alza et al., 2024).

However, as ´Ratio´ had higher stomatal density than ´Witkiem major´, this cannot be the only explanation for the difference in development, and an interaction of multiple factors is likely to lead to the final developmental rate. As the stomatal conductance showed a different pattern than the stomatal densities, more research is needed to examine the connections. In contrast, Ainsworth and Rogers (2007) did not find a reduction in stomatal density after CO_2 enrichment, although the stomatal conductance was reduced by 22%, this could indicate that the expected reduction of stomatal conductance is caused by stomatal aperture rather than stomata density.

Furthermore, the average leaf area could also affect the utilization of the light treatments, and in effect the development. The only difference found with regards to average leaf area per plant was that the plants treated with a[CO_2] had a bigger leaf area than the plants treated with e[CO_2]. This is in contrast to previous studies which have shown no difference in leaf area with regards to e[CO_2] (Lieth et al., 1986). The plants with a bigger leaf area can intercept more light, utilize more light energy into photosynthesis, and therefore more energy is available for development. However, as the opposite was observed it is likely that the plants with bigger leaf areas have used more time developing vegetative leaves, than generating pods.

To summarize, when looking at the development time from the beginning of treatments and until the emergence of first pod, the cultivar ´Witkiem major´ is faster than ´Ratio´ overall, and could be the better choice of the two. From how ´Witkiem major´ was affected by the treatments high R/BL seems to be able to utilize the increase in available carbon to a larger extent than the low R/BL, which could be connected to stomatal status. However, in order to make a recommendation of a future growth program the yield and quality needs to be examined.

5.3 The Effect of R/BL and CO_2 Enrichment on Yield and Quality

In disparity to the shorter development time in ´Witkiem major´, the cultivar still had a significantly larger average total DW per plant compared to ´Ratio´ (Fig. 11). The e[CO_2] gave significantly higher average total DW than a[CO_2]. For both ´Witkiem major´ and ´Ratio´ the high R/BL treatment led to a larger total DW compared to the low R/BL treatment, independent of CO_2 concentration, although not significant. This is in line with previous findings in lettuce (Okamoto et al., 1996).

The high R/BL treatment in ´Witkiem major´ gave also higher total seed FW compared to low R/BL, however, the protein content (%) in seeds of the high R/BL plants was reduced from 30% to approximately 26%, in both CO_2 concentrations. Similar results have been found for Chinese kale, where low R/BL led to decrease in dry matter while promoting the accumulation of soluble protein in flower stalks (Zhang et al., 2020). This is a challenge as producers would want an increase in yield without the cost of product quality. As all plants were grown in the same sized pots it is possible that the plants grown in the high R/BL treatment would have needed more water in order to maximize their yield potential, and therefore experienced a reduction in quality compared to the low R/BL treatment. There have been reports showing a reduction in seed N concentration after e[CO_2], however, some of the reduction has been observed under drought conditions (Parvin et al., 2019), which could justify examining this point further.

In addition, Zheng et al. (2020) found that young seeds of soybean (*Glycine max*) had higher protein content, thus it could be that the seed filling had been faster in the high R/BL treatment and therefore, more mature seeds led to lower protein content (Zheng et al., 2020). Although, there are no indication that the time of emergence of the first pod corelates to the amount of protein present in the beans at the day of harvest. In contrast, no significant difference was found with regards to seed protein content for ´Ratio´ with regards to the R/BL ratios, further emphasizing the genetic variation that will be important in future studies.

Another assumption that has been made is the connection between N content and stomatal conductance. The conductance was expected to decrease after e[CO_2] (Bævre & Gislerod, 1999), and many studies conducted outside have shown this trend (Ainsworth & Rogers, 2007; Taub, 2010). As the stomata can have smaller apertures and still transport sufficient CO_2 to the photosynthetic apparatus, the reduced aperture will most likely limit nutrient transport through the plant, causing a reduction in N. However, in this experiment in the chambers with e[CO_2] the high R/BL for ´Witkiem major´ had the highest stomatal conductance rates, while still having significantly lower protein content in seeds and N content in leaves. As the plants treated with e[CO_2] got higher nutrient concentration during watering, it is possible that this measure diminished the effect the e[CO_2] had on reduced transport through the plant. The effect of the light treatments seems to be more relevant, and there is a need for more research on the topic to be able to increase both yield and quality of the final product.

Going further, the total N content of the leaves were measured. The leaves chosen were expected to have source leaf status for assimilates, and it was hypothesized that their N status could shed light on the protein content of the beans. The N that accumulates in the beans during seed filling is translocated from other parts of the plant (Xing et al., 2019). However, during seed filling the N is not exclusively transported from the leaves, as experiments done on peas (*Pisum sativum*) have shown that approximately 30% of seed N can be traced back to source leaves, while 20% is translocated from the adjacent pod wall, and 11% and 10% from roots and stem respectively (Schiltz et al., 2005). Therefore, considering translocation from the leaves would only partially explain the N content. Moreover, there was significantly higher N content in the leaves treated with low R/BL compared to high R/BL for both cultivars. Since ´Ratio´ showed no differences for the protein content of the seeds between any of the treatments, it is possible that the difference found between high and low R/BL in ´Witkiem major´ is caused by something else.

Previous studies suggest that total N is increased in rice leaves (*Oryza sativa*) when parts of the R is substituted with BL during vegetative growth (Ohashi et al., 2005). However, what is found is that the combination of R and BL is beneficial compared to monochromatic R or BL, and different ratios were not tested. The BL effect on N assimilation or uptake is suspected to be related to the activity of the enzyme NADH nitrate reductase (NR). In rice seedlings treated with monochromatic R and BL for different durations, R increased NR activity after 5 minutes illumination, however, for longer illumination (up to 12h) the BL led to higher NR activity (Sasakawa & Yamamoto, 1979). In the present faba bean experiment the low R/BL treatment had a larger portion of green light than the high R/BL. Green light supplementation has been found to further rise the NR activity in lettuce (Bian et al., 2018), which means that the green addition in the low R/BL treatment could also affect the increase in N content in both leaves and seeds.

Furthermore, the chambers with e[CO_2] for ´Witkiem major´ showed a significantly higher photosynthetic rate in the low R/BL. In ´Ratio´ the low R/BL also showed somewhat higher rates of photosynthesis, although not significant. This is supported by a previous study on faba beans where BL had a positive effect on the net photosynthetic rate (Huang et al., 2020). In addition, a study done on *Arabidopsis thaliana* examined the effect of BL by the use of phototropin mutants, which showed that BL led to increased stomatal aperture, resulting in more effective CO_2 assimilation, which enhanced the transpiration rate and gave higher photosynthetic rate (Takemiya et al., 2005). This is only partly in line with our findings, as the ´Witkiem major´ grown in e[CO_2] chamber with low R/BL was the only treatment that gave a significant difference on transpiration rate based on the light conditions, and it showed a decrease in transpiration rate compared to high R/BL. However, Takemiya et al. (2005) looked at low photosynthetic radiation conditions, meaning it could be a difference based on the combination of light intensity and quality.

Moreover, the plants treated with low R/BL appears to be more affected by an increase in light intensity, as the photosynthetic rate reaches plateau phase later than the high R/BL ratio treatment. This is supported by early findings on peas grown in low to moderate light (20-200 μmol m^{-2} s^{-1}) by Anderson et al. (1995). The study showed that an increase in fluence of white blue-enriched light led to enhanced Chl *a/b* ratio, where levels of RuBisCO and Cytochrome *f* also doubled in response to increased BL (Anderson et al., 1995). This increase in photosystem components could be a stress response as more of the energy in the BL is lost when its transferred from the carotenoids to the reaction center of the photosystem. This inefficiency of energy consumption could lead to the plant needing a higher amount of photosystems in order to obtain enough light energy at the same irradiance as that of R. Furthermore, this could explain that the low R/BL treatment had higher response to increased irradiances in the light response curves, as they had a higher amount of photosystems that could be saturated compared to the plants in the high R/BL treatment. This coincides with the findings of the low R/BL treated plants having generally higher chlorophyll content than their high R/BL counterpart (Fig. 19).

The photosynthetic rate significantly increased for both varieties with e[CO_2]. This was expected as it has been observed in previous studies of legumes in growth chamber conditions (Zheng et al., 2020). Wu and Wang (2000) found that in favorable soil conditions, meaning non-drought, photosynthetic rate increased from 5.53 μmol m^{-2} s^{-1} to 25.3 μmol m^{-2} s^{-1} when concentration of CO_2 was doubled. While the effect of doubling the CO_2 in drought conditions showed that the photosynthetic rate only approximately doubled, from 6.50 μmol m^{-2} s^{-1} to 12.6 μmol m^{-2} s^{-1}. At the same time the transpiration rate (mmol m^{-2} s^{-1}) decreased from 4.24 to 1.43 (Wu & Wang, 2000). However, the average RH in their experiment was at nearly 35% which was drastically lower than what the faba beans in the NMBU growth chamber experiment experienced. As high RH is also expected to increase stomatal opening, and since it was around 80% when the plants were grown, it is possible that the high RH mitigated the effect of e[CO_2] on the stomatal conductance, while not affecting the increase in photosynthetic rate.

To summarize, ´Witkiem major´ gave higher yield compared to ´Ratio´ in the time given. The high R/BL treatment led to higher total DW, and increased total seed FW compared to low R/BL, however, with lower protein content for ´Witkiem major´. This could be because of light induced NR activity. In contrast to earlier findings, the e[CO_2] treatment did not affect nitrogen content. Two reasons for this could be; the increase in fertilizer EC that diminished the expected reduction in conductance, and the high RH which could have led to a larger stomatal aperture, and therefore not affecting the nutrient transport through the plant. As expected, increased CO_2 concentration gave higher photosynthetic rates for both light treatments.

6. Conclusion

The objective of this study was to examine the potential for production of faba bean as a vegetable in controlled environment. Furthermore, how different R/BL and CO_2 concentrations would affect development, photosynthesis, yield and quality. The results show that it is possible to grow faba beans in more controlled environment, and under the right environmental conditions, development, yield and quality can benefit.

Walk-in tunnel production of faba beans could be beneficial in order to prolong growth season and keep higher temperatures, as the faba beans can grow well with decreased light intensity. However, as the potential of increasing yield without compromising the plant quality after elevating CO_2 in a greenhouse shows promise, this could be an investment that would lead to more predictable and increased yield and revenue for further production. In addition, the benefit of isolating the crop from pests and fungal diseases would decrease time and cost of field management, as well as securing a bigger part of the yield. At this point, production in PFAL would not be recommended, as new cultivars suited for that type of production would need to be developed first.

The cultivar ´Witkiem major´ generally grew larger and gave higher yield than ´Ratio´. While ´Witkiem major´ developed earlier, it also gave higher yield in e[CO_2], while the e[CO_2] in ´Ratio´ led to more vegetative production in the form of side shoots, and would have needed more time for seed-filling in order to attain a sufficient yield. The high R/BL treatment increased yield in ´Witkiem major´, however, it also reduced seed protein content to approximately 26%, compared to low R/BL at 30%.

More research is needed in order to find the optimal R/BL that will give high yield without a reduction in the nutritional quality. Furthermore, to test different ratios for different developmental stages could also be interesting in order to optimize lighting for each stage. It would also be beneficial to test different cultivars of faba beans. These tests indicate that both the growth, yield and influence of the treatments varied between ´Ratio´ and ´Witkiem major´. Therefore, before further optimization of the climate conditions it could be wise to find a cultivar with the best fitted traits suitable for controlled environment, such as bigger seeds, more seeds per pod, or reduced plant size.

7. References

Aamot, H. U., Simonsen, S. K., Skårn, M. N., Nielsen, K. A. G., Henriksen, B., & Brodal, G. (2023). Funn av soppmiddelresistent Botrytis i åkerbønne. *Jord-og Plantekultur 2023 Forsøk i korn, olje-og belgvekster, engfrøavl og potet 2022*.

Abrahamsen, U., Uhlen, A. K., Waalen, W., & Stabbetorp, H. (2019). Muligheter for økt proteinproduksjon på kornarealene. *Jord-og Plantekultur 2019. Forsøk i korn, olje-og proteinvekster, engfrøavl og potet 2018*.

Ainsworth, E. A., & Long, S. P. (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*(1), 27-49.

Ainsworth, E. A., & Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO2]: mechanisms and environmental interactions. *Plant, cell & environment*, *30*(3), 258-270.

Alharbi, N. H., & Adhikari, K. N. (2020). Factors of yield determination in faba bean (Vicia faba). *Crop and Pasture Science*, *71*(4), 305-321.

Alrajhi, A. A., Alsahli, A. S., Alhelal, I. M., Rihan, H. Z., Fuller, M. P., Alsadon, A. A., & Ibrahim, A. A. (2023). The effect of LED light spectra on the growth, yield and nutritional value of red and green lettuce (Lactuca sativa). *Plants*, *12*(3), 463.

Alza, J. O., Gutiérrez, N., Cabezas, J. M., Torres, A. M., & Lorite, I. J. (2024). Interactive effects of atmospheric CO2 concentration and water stress on the phenology and physiology of faba bean (Vicia faba L.). *Environmental and Experimental Botany*, *218*, 105571.

Anderson, J. M., Chow, W. S., & Park, Y.-I. (1995). The grand design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues. *Photosynthesis research*, *46*, 129-139.

Bævre, O., & Gislerod, H. (1999). Plantedyrking i regulert klima. I,. In (pp. pp. 70-77, 148-150). Landbruksforlaget.

Bakke, A. H. (2022). *Dyrking av bondebønne (Vicia faba L. cv Witkiem major) som grønnsak under ulike temperaturregimer i kontrollert klima* Norwegian University of Life Sciences, Ås].

Bakken, A. K., & Mittenzwei, K. (2023). Produksjonspotensial i jordbruket og nasjonal sjølforsyning med mat. Utredning for Klimautvalget 2050. *NIBIO Rapport*.

Banerjee, A., Das, A., & Mittra, B. (2016). Aluminium pre-treatment induces activation of defense responses against Fusarium infection in Triticum aestivum. *Russian Journal of Plant Physiology*, *63*, 483-489.

Bárcena, A., Graciano, C., Luca, T., Guiamet, J. J., & Costa, L. (2019). Shade cloths and polyethylene covers have opposite effects on tipburn development in greenhouse grown lettuce. *Scientia Horticulturae*, *249*, 93-99.

Bert, J. S., & Honma, S. (1975). Effect of Soil Moisture and Irrigation Method on Tipburn and Edgeburn Severity in Greenhouse Lettuce1. *Journal of the American Society for Horticultural Science*, *100*(3), 278-282.

Bian, Z., Cheng, R., Wang, Y., Yang, Q., & Lu, C. (2018). Effect of green light on nitrate reduction and edible quality of hydroponically grown lettuce (Lactuca sativa L.) under short-term continuous light from red and blue light-emitting diodes. *Environmental and Experimental Botany*, *153*, 63-71.

Bloom, A. J., Burger, M., Asensio, J. S. R., & Cousins, A. B. (2010). Carbon Dioxide Enrichment Inhibits Nitrate Assimilation in Wheat and <i>Arabidopsis</i>. *Science*, *328*(5980), 899-903. https://doi.org/doi:10.1126/science.1186440

Cernay, C., Ben-Ari, T., Pelzer, E., Meynard, J.-M., & Makowski, D. (2015). Estimating variability in grain legume yields across Europe and the Americas. *Scientific reports*, *5*(1), 11171.

Chen, M., & Chory, J. (2011). Phytochrome signaling mechanisms and the control of plant development. *Trends in cell biology*, *21*(11), 664-671.

Cicero. (2021). *VOM - Virkemidler for Omstilling av Matsystemet*. https://cicero.oslo.no/no/prosjekter/vom-virkemidler-for-omstilling-av-matsystemet (read 20.02.2024)

Confalone, A., Lizaso, J. I., Ruiz-Nogueira, B., López-Cedrón, F.-X., & Sau, F. (2010). Growth, PAR use efficiency, and yield components of field-grown Vicia faba L. under different temperature and photoperiod regimes. *Field crops research*, *115*(2), 140-148.

Cotrufo, M. F., Ineson, P., & Scott, A. (1998). Elevated CO2 reduces the nitrogen concentration of plant tissues. *Global Change Biology*, *4*(1), 43-54.

Crépon, K., Marget, P., Peyronnet, C., Carrouée, B., Arese, P., & Duc, G. (2010). Nutritional value of faba bean (Vicia faba L.) seeds for feed and food. *Field crops research*, *115*(3), 329-339.

Cubero, J. I., & Nadal, S. (2005). Faba bean (Vicia faba L.). *Genetic Resources, Chromosome Engineering and Crop Improvement Grain Legumes. Series II-Grain Legumes. CRC, Boca Raton, FL, USA*, 163-186.

Demotes-Mainard, S., Péron, T., Corot, A., Bertheloot, J., Le Gourrierec, J., Pelleschi-Travier, S., Crespel, L., Morel, P., Huché-Thélier, L., & Boumaza, R. (2016). Plant responses to red and far-red lights, applications in horticulture. *Environmental and Experimental Botany*, *121*, 4-21.

Den Magiske Fabrikken. https://denmagiskefabrikken.no/den-magiske-fabrikken/ (Read 20.02.2024)

Dutta Gupta, S., & Agarwal, A. (2017). Artificial lighting system for plant growth and development: Chronological advancement, working principles, and comparative assessment. *Light emitting diodes for agriculture: smart lighting*, 1-25.

Elshafei, A. A. M., Amer, M. A. E., Elenany, M. A. M., & Helal, A. G. A. E. (2019). Evaluation of the genetic variability of faba bean (Vicia faba L.) genotypes using agronomic traits and molecular markers. *Bulletin of the National Research Centre*, *43*(1), 1-10.

Farchi, S., De Sario, M., Lapucci, E., Davoli, M., & Michelozzi, P. (2017). Meat consumption reduction in Italian regions: health co-benefits and decreases in GHG emissions. *PloS one*, *12*(8), e0182960.

Foyer, C. H., Lam, H.-M., Nguyen, H. T., Siddique, K. H., Varshney, R. K., Colmer, T. D., Cowling, W., Bramley, H., Mori, T. A., & Hodgson, J. M. (2016). Neglecting legumes has compromised human health and sustainable food production. *Nature plants*, *2*(8), 1-10.

Frechilla, S., Talbott, L. D., & Zeiger, E. (2004). The blue light-specific response of Vicia faba stomata acclimates to growth environment. *Plant and Cell physiology*, *45*(11), 1709-1714.

Galmés, J., Aranjuelo, I., Medrano, H., & Flexas, J. (2013). Variation in Rubisco content and activity under variable climatic factors. *Photosynthesis research*, *117*, 73-90.

Gavassi, M. A., Dodd, I. C., Puértolas, J., Silva, G. S., Carvalho, R. F., & Habermann, G. (2020). Aluminum-induced stomatal closure is related to low root hydraulic conductance and high ABA accumulation. *Environmental and Experimental Botany*, *179*, 104233.

Gielen, B., Calfapietra, C., Claus, A., Sabatti, M., & Ceulemans, R. (2002). Crown architecture of Populus spp. is differentially modified by free-air CO2 enrichment (POPFACE). *New phytologist*, *153*(1), 91-99.

Gomez-Zavaglia, A., Mejuto, J. C., & Simal-Gandara, J. (2020). Mitigation of emerging implications of climate change on food production systems. *Food Research International*, *134*, 109256.

González, N., Marquès, M., Nadal, M., & Domingo, J. L. (2020). Meat consumption: Which are the current global risks? A review of recent (2010–2020) evidences. *Food Research International*, *137*, 109341.

GreenCap Solutions. *Agricultural Solutions*. https://greencap-solutions.com/agricultural-solutions/ (Read 22.02.2024)

Grieu, C., Abrahamsen, U., & Waalen, W. (2021). Sortsforsøk i åkerbønne. *Jord-og plantekultur 2021 Forsøk i korn, olje-og belgvekster, engfrøavl og potet 2020*.

Hatfield, J. L., & Prueger, J. H. (2015). Temperature extremes: Effect on plant growth and development. *Weather and climate extremes*, *10*, 4-10.

Huang, L., Xiao, Y., Ran, J., Wei, L., Li, Z., Li, Y., Zhang, X., Liao, L., Wang, D., & Zhao, X. (2020). Drought tolerance of faba bean (Vicia faba L.) can be improved by specific LED light wavelengths. *Photosynthetica*, *58*(4).

Ibrahim, H. M. (2010). Heterosis, combining ability and components of genetic variance in faba bean (Vicia faba L.). *Meteorology, Environment and Arid Land Agriculture Sciences*, *21*(1), 35-50.

Intergovernmental Panel on Climate Change, I. (2023). Food, Fibre and Other Ecosystem Products. In I. P. o. C. Change (Ed.), *Climate Change 2022 – Impacts, Adaptation and Vulnerability: Working Group II Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 713-906). Cambridge University Press. https://doi.org/DOI: 10.1017/9781009325844.007

Jensen, E. S., Peoples, M. B., & Hauggaard-Nielsen, H. (2010). Faba bean in cropping systems. *Field crops research*, *115*(3), 203-216.

Jung, S., Rickert, D., Deak, N., Aldin, E., Recknor, J., Johnson, L., & Murphy, P. (2003). Comparison of Kjeldahl and Dumas methods for determining protein contents of soybean products. *Journal of the American Oil Chemists' Society*, *80*, 1169-1173.

Kant, S., Seneweera, S., Rodin, J., Materne, M., Burch, D., Rothstein, S. J., & Spangenberg, G. (2012). Improving yield potential in crops under elevated CO2: integrating the photosynthetic and nitrogen utilization efficiencies. *Frontiers in Plant Science*, *3*, 162.

Karkanis, A., Ntatsi, G., Lepse, L., Fernández, J. A., Vågen, I. M., Rewald, B., Alsiņa, I., Kronberga, A., Balliu, A., & Olle, M. (2018). Faba bean cultivation–revealing novel managing practices for more sustainable and competitive European cropping systems. *Frontiers in Plant Science*, 1115.

Kasperbauer, M., & Loughrin, J. (2004). Butterbean seed yield, color, and protein content are affected by photomorphogenesis. *Crop Science*, *44*(6), 2123-2126.

Khazaei, H., Hawkins, G., & Vandenberg, A. (2021). Historical review of faba bean improvement in western Canada. *Legume Science*, *3*(4), e92.

Khazaei, H., Wach, D., Pecio, A., Vandenberg, A., & Stoddard, F. L. (2019). Genetic analysis of photosynthesis-related traits in faba bean (Vicia faba) for crop improvement. *Plant Breeding*, *138*(6), 761-769.

Kim, K.-D., Suh, J.-T., Kim, C.-S., & Jee, S.-N. (2021). Tipburn occurrence according to soil moisture, calcium deficiency, and day/night relative humidity regime in highland Kimchi cabbage. *Horticultural Science and Technology*, *39*(6), 738-749.

Landbruksdirektoratet. (2022). *Nasjonalt miljøprogram 2023-2026* (Nasjonale miljømål og virkemidler for miljø- og klimaarbeidet i jordbruket, Issue 33/2022). https://www.landbruksdirektoratet.no/nb/filarkiv/rapporter/Nasjonalt%20Miljøprogram%202023-2026_Rapport%20nr.%2033.2022.pdf/_/attachment/inline/4ad2cb64-81de-4126-b33c-17fb2713e105:c04a0f68cc20d72b45375a0285f881b4f30ec033/Nasjonalt%20Miljøprogram%202023-2026_Rapport%20nr.%2033.2022.pdf (Read 20.02.2024)

Landbruksdirektoratet. (2023). *Markedsrapport 2022* (Markeds- og prisvurderinger av sentrale norske landbruksvarer og RÅK-varer, Issue 6/2023). https://www.landbruksdirektoratet.no/nb/filarkiv/rapporter/Markedsrapport%202022%20Rapport%206%202023.pdf/_/attachment/inline/1a23a968-2b7b-479c-98af-9615fa5c481a:ba0e79b5b0c7f0386bc0dfdcda4b1bcf0eeb3f79/Markedsrapport%202022%20Rapport%206%202023.pdf (Read 17.01.2024)

Legesse, H., Dechassa, N., Gebeyehu, S., Bultosa, G., & Mekbib, F. (2017). Yield and leaf nutrient concentrations of common bean genotypes as influenced by aluminium toxicity under acidic soils. *J. Biosci. Agric. Res*, *12*(01), 1003-1015.

Lieth, J. H., Reynolds, J. F., & Rogers, H. H. (1986). Estimation of leaf area of soybeans grown under elevated carbon dioxide levels. *Field crops research*, *13*, 193-203.

López-Bellido, F., López-Bellido, L., & López-Bellido, R. (2005). Competition, growth and yield of faba bean (Vicia faba L.). *European Journal of Agronomy*, *23*(4), 359-378.

Mathan, J., Bhattacharya, J., & Ranjan, A. (2016). Enhancing crop yield by optimizing plant developmental features. *Development*, *143*(18), 3283-3294.

Matthews, J. S., Vialet-Chabrand, S., & Lawson, T. (2020). Role of blue and red light in stomatal dynamic behaviour. *Journal of Experimental Botany*, *71*(7), 2253-2269.

Miao, Y., Chen, Q., Qu, M., Gao, L., & Hou, L. (2019). Blue light alleviates 'red light syndrome'by regulating chloroplast ultrastructure, photosynthetic traits and nutrient accumulation in cucumber plants. *Scientia Horticulturae*, *257*, 108680.

Mitchell, C. A., Dzakovich, M. P., Gomez, C., Lopez, R., Burr, J. F., Hernández, R., Kubota, C., Currey, C. J., Meng, Q., & Runkle, E. S. (2015). Light-Emitting Diodes in Horticulture. *Horticultural Reviews: Volume 43*, 1-88.

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in plant science*, *11*(1), 15-19.

Moe, R., Grimstad, S. O., & Gislerod, H. (2005). The use of artificial light in year round production of greenhouse crops in Norway. V International Symposium on Artificial Lighting in Horticulture 711,

Möglich, A., Yang, X., Ayers, R. A., & Moffat, K. (2010). Structure and function of plant photoreceptors. *Annual review of plant biology*, *61*, 21-47.

Müller, J. (2017). Dumas or Kjeldahl for reference analysis? Comparison and considerations for Nitrogen/Protein analysis of food and feed. *FOSS Analytics*.

Mulualem, T., Dessalegn, T., & Dessalegn, Y. (2013). Genetic variability, heritability and correlation in some faba bean genotypes (Vicia faba L.) grown in Northwestern Ethiopia. *International Journal of Genetics and Molecular Biology*, *5*(1), 8-12.

Murphy-Bokern, D., Stoddard, F. L., & Watson, C. A. (2017). *Legumes in cropping systems*. CABI.

Nanya, K., Ishigami, Y., Hikosaka, S., & Goto, E. (2012). Effects of blue and red light on stem elongation and flowering of tomato seedlings. VII International Symposium on Light in Horticultural Systems 956,

Nasrullahzadeh, S., Ghassemi-Golezani, K., Javanshir, A., Valizade, M., & Shakiba, M. (2007). Effects of shade stress on ground cover and grain yield of faba bean (Vicia faba L.). *Journal of Food Agriculture and Environment*, *5*(1), 337.

Naznin, M. T., Lefsrud, M., Gravel, V., & Azad, M. O. K. (2019). Blue light added with red LEDs enhance growth characteristics, pigments content, and antioxidant capacity in lettuce, spinach, kale, basil, and sweet pepper in a controlled environment. *Plants*, *8*(4), 93.

Nemali, K. (2022). History of controlled environment horticulture: Greenhouses. *HortScience*, *57*(2), 239-246.

Ohashi, K., Goji, K., Matsuda, R., Fujiwara, K., & Kurata, K. (2005). Effects of blue light supplementation to red light on nitrate reductase activity in leaves of rice seedlings. V International Symposium on Artificial Lighting in Horticulture 711,

Okamoto, K., Yanagi, T., & Kondo, S. (1996). Growth and morphogenesis of lettuce seedlings raised under different combinations of red and blue light. II Workshop on Environmental Regulation of Plant Morphogenesis 435,

Olle, M., & Bender, I. (2009). Causes and control of calcium deficiency disorders in vegetables: A review. *The Journal of Horticultural Science and Biotechnology*, *84*(6), 577-584.

Øverland, J. I., & Viken, N. (2020). Dyrkingsveiledning for åkerbønner. *Norsk landbruksrådgiving Viken*.

Parvin, S., Uddin, S., Tausz-Posch, S., Fitzgerald, G., Armstrong, R., & Tausz, M. (2019). Elevated CO2 improves yield and N2 fixation but not grain N concentration of faba bean (Vicia faba L.) subjected to terminal drought. *Environmental and Experimental Botany*, *165*, 161-173.

Patrick, J., & Stoddard, F. (2010). Physiology of flowering and grain filling in faba bean. *Field crops research*, *115*(3), 234-242.

Piovene, C., Orsini, F., Bosi, S., Sanoubar, R., Bregola, V., Dinelli, G., & Gianquinto, G. (2015). Optimal red: blue ratio in led lighting for nutraceutical indoor horticulture. *Scientia Horticulturae*, *193*, 202-208.

Reddy, K. R., Hodges, H. F., & McKinion, J. M. (1995). Cotton crop responses to a changing environment. *Climate change and agriculture: Analysis of potential international impacts*, *59*, 3-30.

Sahar, A., El-Shennawy, R. Z., & Ismail, A. (2011). Fungicidal management of chocolate spot of faba bean and assessment of yield losses due to the disease. *Annals of Agricultural Sciences*, *56*(1), 27-35.

Sahile, S., Ahmed, S., Fininsa, C., Abang, M. M., & Sakhuja, P. K. (2008). Survey of chocolate spot (Botrytis fabae) disease of faba bean (Vicia faba L.) and assessment of factors influencing disease epidemics in northern Ethiopia. *Crop protection*, *27*(11), 1457-1463.

Samuolienė, G., Brazaitytė, A., Urbonavičiūtė, A., Šabajevienė, G., & Duchovskis, P. (2010). The effect of red and blue light component on the growth and development of frigo strawberries. *Zemdirbyste-Agriculture*, *97*(2), 99-104.

Sasakawa, H., & Yamamoto, Y. (1979). Effects of red, far red, and blue light on enhancement of nitrate reductase activity and on nitrate uptake in etiolated rice seedlings. *Plant Physiology*, *63*(6), 1098-1101.

Sasek, T. W., & Strain, B. R. (1991). Effects of CO2 enrichment on the growth and morphology of a native and an introduced honeysuckle vine. *American Journal of Botany*, *78*(1), 69-75.

Satapathy, P., Achary, V. M. M., & Panda, B. B. (2012). Aluminum-induced abiotic stress counteracts Fusarium infection in Cajanus cajan (L.) Millsp. *Journal of Plant Interactions*, *7*(2), 121-128.

Saure, M. (1998). Causes of the tipburn disorder in leaves of vegetables. *Scientia Horticulturae*, *76*(3-4), 131-147.

Schiltz, S., Munier-Jolain, N., Jeudy, C., Burstin, J., & Salon, C. (2005). Dynamics of exogenous nitrogen partitioning and nitrogen remobilization from vegetative organs in pea revealed by 15N in vivo labeling throughout seed filling. *Plant Physiology*, *137*(4), 1463-1473.

Shamshiri, R. R., Jones, J. W., Thorp, K. R., Ahmad, D., Man, H. C., & Taheri, S. (2018). Review of optimum temperature, humidity, and vapour pressure deficit for microclimate evaluation and control in greenhouse cultivation of tomato: a review. *International agrophysics*, *32*(2), 287-302.

Stanghellini, C., Van't Ooster, B., & Heuvelink, E. (2019). Greenhouse horticulture: Technology for optimal crop production. In *Greenhouse horticulture*. Wageningen Academic.

Staniak, M., Księżak, J., & Bojarszczuk, J. (2014). Mixtures of legumes with cereals as a source of feed for animals. *Organic agriculture towards sustainability*(6), 123-145.

Stoddard, F., Nicholas, A. H., Rubiales, D., Thomas, J., & Villegas-Fernández, A. (2010). Integrated pest management in faba bean. *Field crops research*, *115*(3), 308-318.

Sun, J., Nishio, J. N., & Vogelmann, T. C. (1998). Green light drives CO2 fixation deep within leaves. *Plant and Cell physiology*, *39*(10), 1020-1026.

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant physiology and development*. Sinauer Associates Incorporated.

Takemiya, A., Inoue, S.-i., Doi, M., Kinoshita, T., & Shimazaki, K.-i. (2005). Phototropins promote plant growth in response to blue light in low light environments. *The Plant Cell*, *17*(4), 1120-1127.

Taub, D. (2010). Effects of rising atmospheric concentrations of carbon dioxide on plants. *Nature Education Knowledge*, *1*(8).

Terashima, I., Fujita, T., Inoue, T., Chow, W. S., & Oguchi, R. (2009). Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. *Plant and Cell physiology*, *50*(4), 684-697.

Verheul, M., & Maessen, H. (2021). Verification of the GreenCap Solutions AS Environmental Control System. *NIBIO Rapport*.

Wang, H., & Deng, X. W. (2004). Phytochrome signaling mechanism. *The Arabidopsis Book/American Society of Plant Biologists*, *3*.

Wang, X., Xu, X., & Cui, J. (2015). The importance of blue light for leaf area expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of Cucumis sativus grown under weak light. *Photosynthetica*, *53*(2), 213-222.

Willey, N. (2018). *Environmental plant physiology*. Garland Science.

Woodward, F., & Kelly, C. (1995). The influence of CO2 concentration on stomatal density. *New phytologist*, *131*(3), 311-327.

Woodward, F., Lake, J., & Quick, W. (2002). Stomatal development and CO 2: ecological consequences. *New phytologist*, 477-484.

Wu, D.-X., & Wang, G.-X. (2000). Interaction of CO2 enrichment and drought on growth, water use, and yield of broad bean (Vicia faba). *Environmental and Experimental Botany*, *43*(2), 131-139.

Xing, Y., Jiang, W., He, X., Fiaz, S., Ahmad, S., Lei, X., Wang, W., Wang, Y., & Wang, X. (2019). A review of nitrogen translocation and nitrogen-use efficiency. *Journal of Plant Nutrition*, *42*(19), 2624-2641.

Yitayih, G., & Azmeraw, Y. (2018). Evaluation of faba bean varieties against chocolate spot (Botrytis fabae Sard) disease at Farta, south gondar, ethiopia. *Journal of Crop Science and Biotechnology*, *21*(1), 35-41.

Zha, L., & Liu, W. (2018). Effects of light quality, light intensity, and photoperiod on growth and yield of cherry radish grown under red plus blue LEDs. *Horticulture, Environment, and Biotechnology*, *59*, 511-518.

Zhang, Y., Ji, J., Song, S., Su, W., & Liu, H. (2020). Growth, nutritional quality and health-promoting compounds in Chinese Kale grown under different ratios of red: blue LED lights. *Agronomy*, *10*(9), 1248.

Zheng, G., Chen, J., & Li, W. (2020). Impacts of CO2 elevation on the physiology and seed quality of soybean. *Plant diversity*, *42*(1), 44-51.

Zong, X., Yang, T., & Liu, R. (2019). Faba bean (Vicia faba L.) breeding. *Advances in Plant Breeding Strategies: Legumes: Volume 7*, 245-286.

8. Sources of Error

There were some challenges during the experimental period. Firstly, difficulty with watering because of small pots and amount of soil compared to the size of the plants for the last two or three weeks, this was especially prevalent in the ambient chambers. Therefore some of the plants were sometimes a bit dry in the morning, which could affect processes as the faba beans are prone to drought conditions, with emphasis on the seed-filling stage.

Furthermore, bacterial growth was discovered on the wick of the PRIVA-boxes which has affected the RH measurements. This was not noticed until late in the enriched CO_2 experiment, which mean that the accurate RH was not measured. However, after cleaning of the wick and the PRIVA-box the measurements showed approximately 80% RH the following period. The dip in RH after cleaning the wick can be seen in Figure 3. This could indicate that the RH has been varying between 60-90% during the experiment, but it will be no way of knowing for sure.

In addition, the ideal RH would have been 60% throughout the experiment. However, this is a parameter which is very difficult to control in small growth chambers when the biomass reach a certain size, as the transpiration from the large leaf area will continuously increase the levels of H_2O in the surrounding air. It is generally difficult to control RH in controlled environment, especially with added CO_2. As increased air flow to decrease RH would also decrease the CO_2 concentration, in addition to possibly affect the temperature. As CO_2 was one of the desired controlled conditions in this experiment, and as temperature is viewed as an important factor regarding growth, development and yield, it was decided that it would be of greater importance to keep temperature and CO_2 stable, compared to RH.

There was a malfunction in the IRGA when light response curves were to be taken in the enriched chambers. As we had to wait for repair a different leaf was chosen for the e[CO_2] plants, as the leaf that was chosen for the a[CO_2] was already too old for measurements. A leaf of approximately the same height but slightly younger was chosen for comparison, as it would be the closest comparable leaf available. However, this means that the effect of e[CO_2] on photosynthetic rate and stomatal conductance is not directly comparable.

9. Appendix

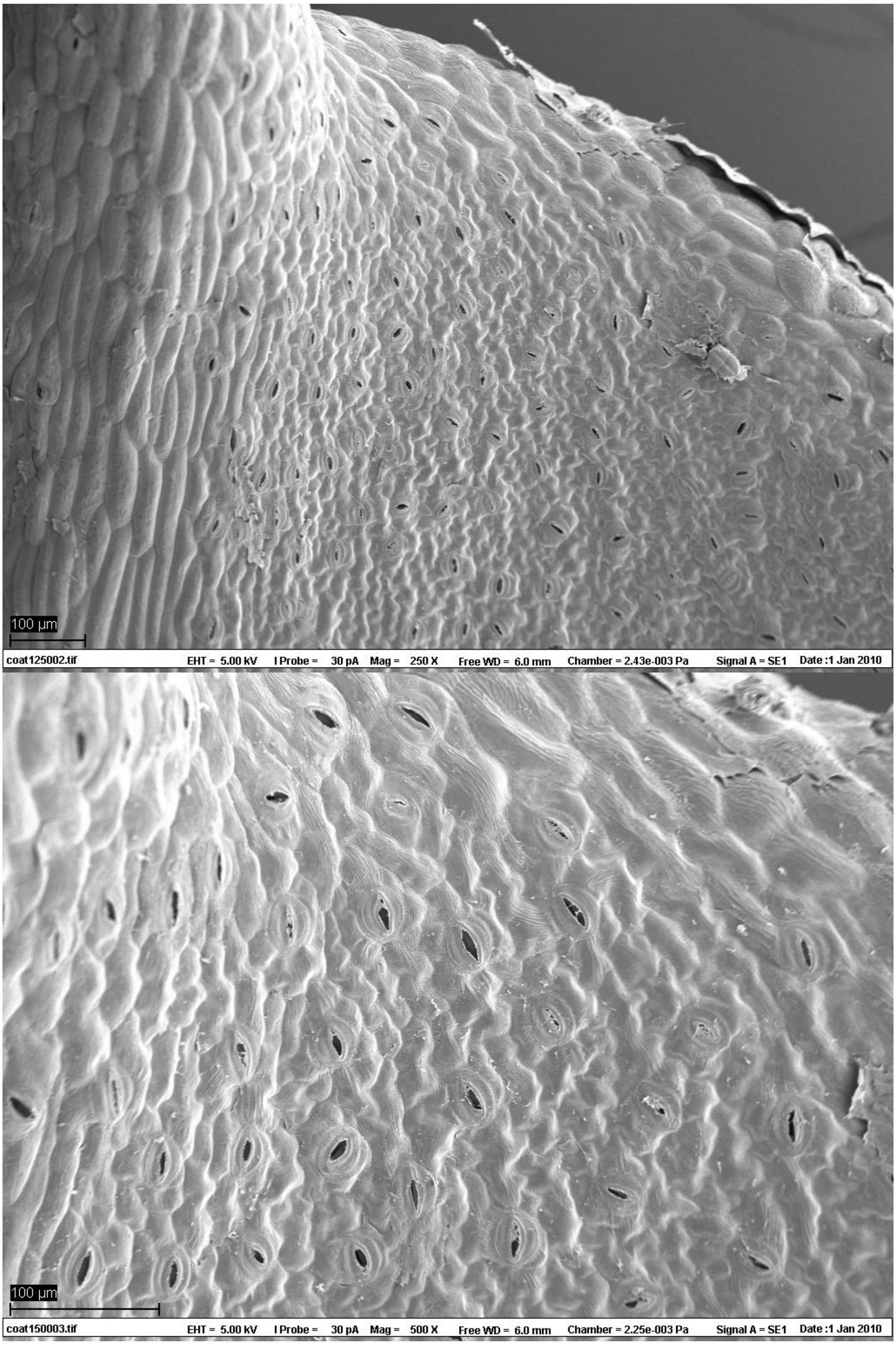


Figure 22: SEM image of healthy leaf tip from ´Ratio´ treated with high R/BL and e[CO_2] concentration, at 250X (Upper), and 250 X (Lower) magnification.

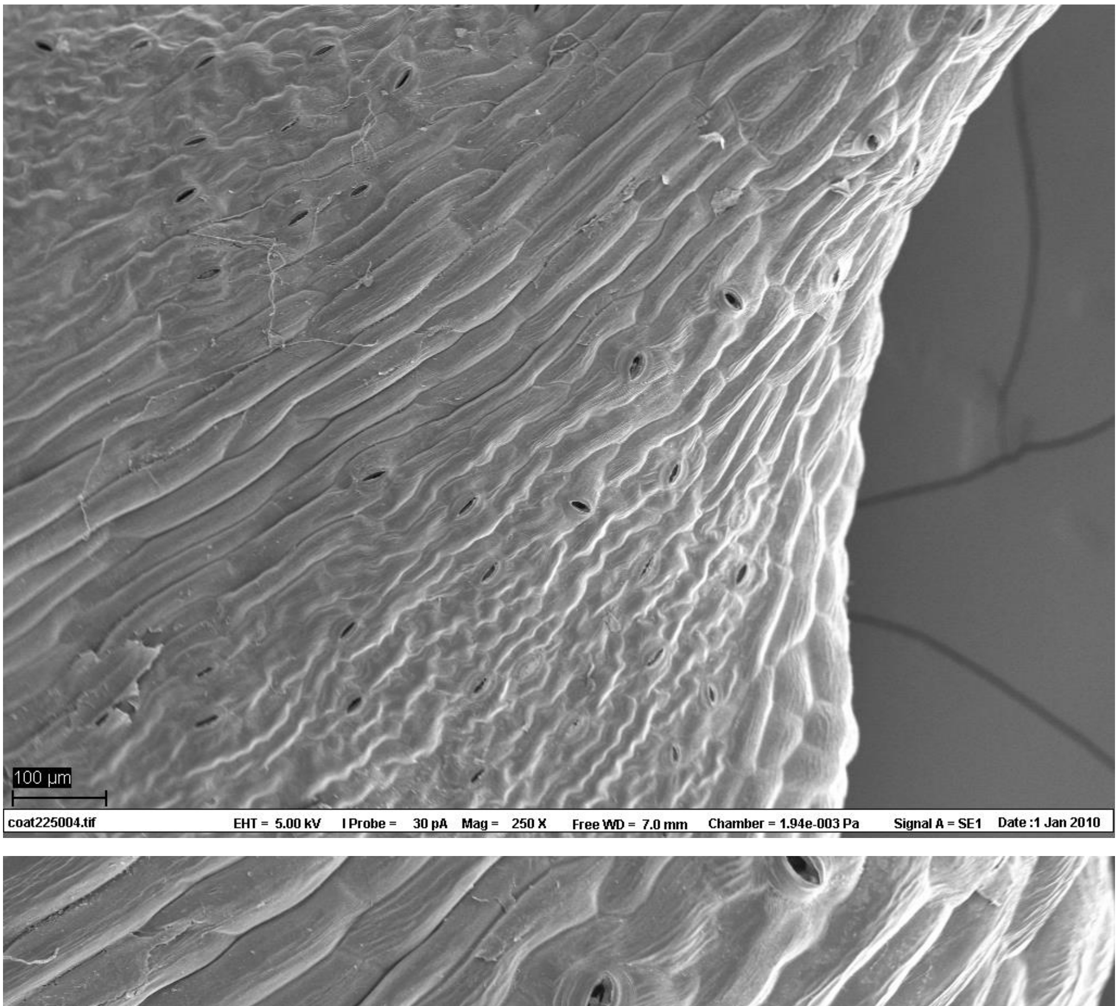


Figure 22: SEM image of healthy leaf tip from ´Ratio´ treated with high R/BL and e[CO_2] concentration., at 250 X (upper) and 500 X (lower) magnification

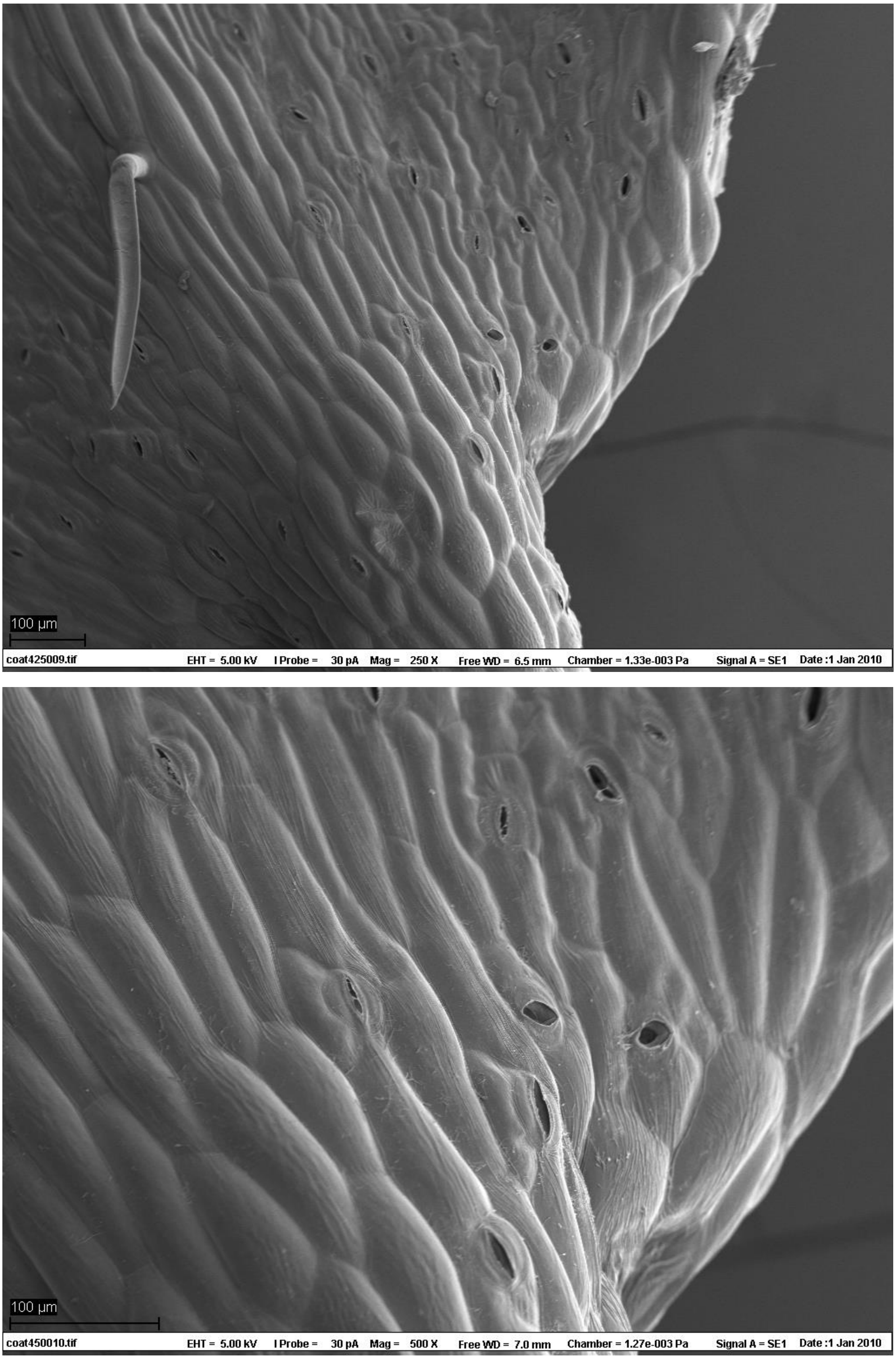


Figure 23: SEM image of damaged leaf tip from ´Ratio´ treated with high R/BL and e[CO_2] concentration., at 250 X (upper) and 500 X (lower) magnification.

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway