The Effect of Iron Deficiency on Photosynthesis in Soybean

Master's Thesis

By Chris Jungerling
F140315 or 6039618 (UU)
c.j.m.jungerling@students.uu.nl

Sustainable Development, Track Environmental Change and Ecosystems
45 ECTS

Supervised by: Dr Hugo de Boer (UU)

Utrecht University

The Effect of Iron Deficiency on Photosynthesis in Soybean

By

Chris Jungerling
F140315 or 6039618 (UU)
c.j.m.jungerling@students.uu.nl

Master's Thesis
Sustainable Development, Track Environmental Change and Ecosystems
45 ECTS

Supervised by: Dr Hugo de Boer (UU)

September 2019

Utrecht University

Abstract

Sustainable development Goal 2 (zero hunger) composed by the United Nations focuses on improving agriculture to increase food security. One of the potential improvements to agriculture is specialised fertiliser rich in micronutrients, which are essential elements necessary for the healthy development of plants. Iron (Fe) deficiency is a known problem in crop production. A promising new development to limit iron deficiency is the use of the iron fertiliser N,N'-bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (FeHBED). FeHBED is an organic molecule able to bind iron ions, increasing available iron. Most agricultural systems in developing countries, however, are unable to use fertiliser. These countries suffer most from Fe deficiency and gain the most from an improved understanding of plant processes. The literature is lacking in data on photosynthesis parameters Jmax and Vcmax. This thesis therefore aims to answer the following question: What is the effect of iron deficiency on photosynthesis parameters (Vcmax and Jmax) in soybean (Glycine max) under different iron and light conditions over time?

Our experiment used two climate cabinets with different light intensities (140–240 µmol/m2/s) and iron conditions. Four soybean populations of 14 plants apiece were grown over a period of 37 days. Two destruction events took place: one at 18 days after the start of the experiment and one at 37 days. Measured were the maximum electron transport rate (Jmax) and the maximum carboxylation rate (Vcmax), which are critical photosynthesis parameters. Other data collected was the total leaf area, stem length and dry matter used for above- and below-ground measurements of 24 plants. The same occurred on Day 37, when all 30 remaining plants were destroyed. A database was compiled and analysed through MATLAB, R and ImageJ.

Our results showed that Fe fertiliser HBED had a significant effect on biomass at both destructions, while Vcmax was not significantly impacted. Jmax was negatively impacted at the second destruction, indicating that non-FeHBED plants under low light intensity have a higher Jmax , which could be a stress reaction to iron deficiency. This thesis provides useful insights into previous experiments, notably the behaviour exhibited by Jmax and Vcmax as a result of FeHBED in soybean. The resulting database adds to the current understanding of the effects of FeHBED in plants.

Developing countries suffer from Fe deficiency. A society aware of the behaviour of plant processes under this stress can detect and take sustainable intensification measures using FeHBED to prevent further Fe deficiency in crops.

Table of contents

1 Introduction

1.1 Societal background

Sustainable development Goal 2 (zero hunger) focuses on improving agriculture to increase food security (Grebmer et al., 2016). Sustainable intensification (SI) in crops is necessary to mitigate food shortages (Godfray et al., 2010; Kromdijk & Long, 2016). SI is defined by Godfray et al. (2014) as arguing: ''(i) that increased production must play at least some role in meeting the food security challenge of the next fifty years; (ii) that the vast majority of this increase must come from existing agricultural land; (iii) that increasing the sustainability of food production is of equal importance; and (iv) that we must consider a broad range of tools and production methods to achieve these goals''.

One potential improvement to agriculture is specialised fertiliser focused on micronutrients (Malakouti, 2008). Micronutrients are essential elements necessary in small amounts for the healthy development of plants, animals and humans (Alloway, 2008; Briat et al., 2015). Essential micronutrients for plants include boron (B), chloride (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), nickel (Ni) and cobalt (Co) (Rashid & Ryan, 2004; Welch & Shuman, 1995). Our current fertiliser largely contains macronutrients, shifting the nutritional limiting factor for plant growth towards micronutrients (Malakouti, 2008). The use of fertiliser aimed at micronutrients has been shown to lessen the limiting factor and increase crop yields (Fageria e.a., 2002).

While all of the aforementioned micronutrients are important, one of the more common problems in crop production is iron (Fe) deficiency; a lack of this element results in both lower quantity and quality of crops (Mortvedt, 1991; Schenkeveld, 2010). Fe deficiency has limiting effects on photosynthesis, directly limiting production, which is the primary problem researched in this thesis. Developing countries often suffer from Fe deficiency manifested as anaemia (Figure 1.1), mainly because their diet is lacking Fe (Benoist e.a., 2008; Nestel, Bouis, Meenakshi & Pfeiffer, 2006) due to the lower quality of food produced by Fe-deficient plants.

As fertiliser is expensive, most agricultural systems in developing countries are unable to use it (Gilbert, 2014; Kijima, Otsuka & Sserunkuuma, 2011). It is these countries, however, that would gain the most from a better understanding of plants' photosynthetic biochemistry adaptation to Fe deficiency. Understanding the effects of Fe deficiency on photosynthesis is therefore an essential piece of the puzzle.

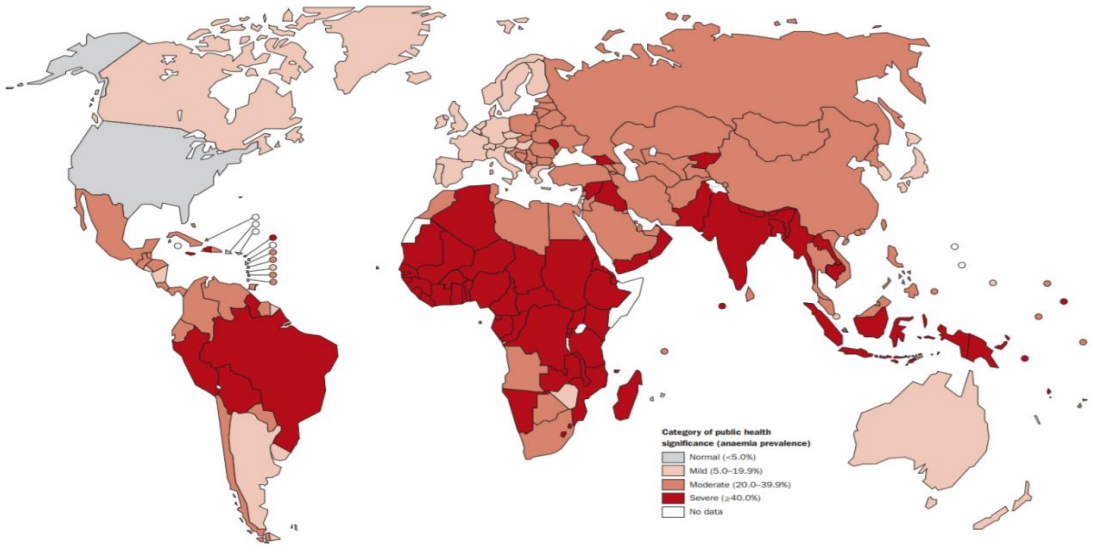

Figure 1.1: Anaemia as a public health problem by country: Preschool-age children. Source: (Benoist, e.a., 2008)

1.2 Scientific background

Plants face two challenges in their Fe uptake: the fact that the physiological pH range of the soil limits the solubility of Fe, and Fe's toxicity. To counter Fe's lack of solubility, plants use reduction systems at the root surface and deficiency-inducible chelation. The toxicity is overcome by binding Fe to specialised proteins or organic acids (primarily nicotianamine) before entering the plant (Hell & Stephan, 2003).

The Fe content in green plant tissue averages 50–100 mg per kg of dry weight (Mengel & Kirkby, 2001). For a micronutrient, this amount is quite high, revealing that Fe is the most prevalent micronutrient in plants (Bauer & Hell, 2006). Fe is essential in many metabolic processes, most crucially photosynthesis and cell respiration (Morrissey & Guerinot, 2009). Green leaves contain up to 80% of the plant's Fe, where it is located in the chloroplasts responsible for photosynthesis (Schenkeveld, 2010).

Within plants, two strategies for iron uptake can be observed. Strategy 1, seen in most non-gramineous species, consists of increased activity of plasma membrane-bound reductase, enhancing Fe^{+3} reduction to increase the plant's Fe^{+2} uptake (Murata e.a., 2006). Strategy 2 is only observed in grasses; they employ phytosiderophores (Greek for 'plant iron-carrier'). Phytosiderophores mobilise Fe^{+3} and allow for its uptake, whereupon the plant transforms the Fe^{+3} to Fe^{+2}, enabling it to satisfy the Fe demand. Apart from Fe mobilisation, phytosiderophores mobilise Zn, Mn and Cu (Araki e.a., 2015; Marschner, Römheld & Kissel, 1986; Römheld, 1991).

Fe deficiency visibly manifests itself as chlorosis (Figure 1.4), which can be observed as discolouration of the leaves due to chlorophyll deterioration. One of the methods to counteract Fe deficiency is Fe fertilisation. The most frequently used methods for iron fertilisation are trunk injection, foliar application and soil application. Soil application is the most common technique for soil-grown crops (Lucena, 2006), as trunk injection is only effective for trees and foliar application is only partly successful. A combination of soil and foliar application has been argued to be the most successful approach (Álvarez-Fernández, e.a., 2004). Since Fe fertiliser is well established, this research uses the N,N'-bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid chelate (FeHBED) (López-Rayo, Hernández & Lucena, 2009) specific to Strategy 1 plants.

Figure 1.4: Iron chlorosis in leaves resulting in yellowing of the chlorophyll pigment Source: (Iron Powder Corporation, 2018)

Plants rely on pigments for their ability to absorb light at specific wavelengths (Figure 1.2); in plants, the most common is the greenish pigment chlorophyll, containing a porphyrin ring. This porphyrin ring is made of electrons and enables electrons to migrate freely. The ring can potentially gain or lose electrons when charged by photons. Within chlorophyll, a distinction can be made between chlorophyll a and chlorophyll b. Chlorophyll a can lose its energised electron to a specific protein function as a mobile electron carrier. The carrier starts the electron's journey along the electron transport chain (ETC)(Figure 1.3). Chlorophyll, having lost its electron, takes an electron from H_20 in the cell, splitting the molecule. The splitting causes the freeing of the oxygen, creating the air we breathe and two protons (H^+). The electron in the ETC is transported to the cytochrome complex, which uses a bit of the electron's energy to transfer another proton (H^+) to the thylakoid; this process

creates an electrochemical proton gradient, which drives the enzyme ATP synthase. The ATP synthase enzyme produces adenosine triphosphate (ATP), which is used for intercellular energy transfer (Knowles, 1980; Reece, e.a., 2011). The described process represents Photosystem 2.

Fe deficiency interferes in this process by limiting the amount of chlorophyll a and chlorophyll b content per chloroplast. Fe deficiency causes a reduction in the number of grana and stromal lamellae per chloroplast and the number of thylakoids per granum (Spiller & Terry, 1980; Terry, 1980).

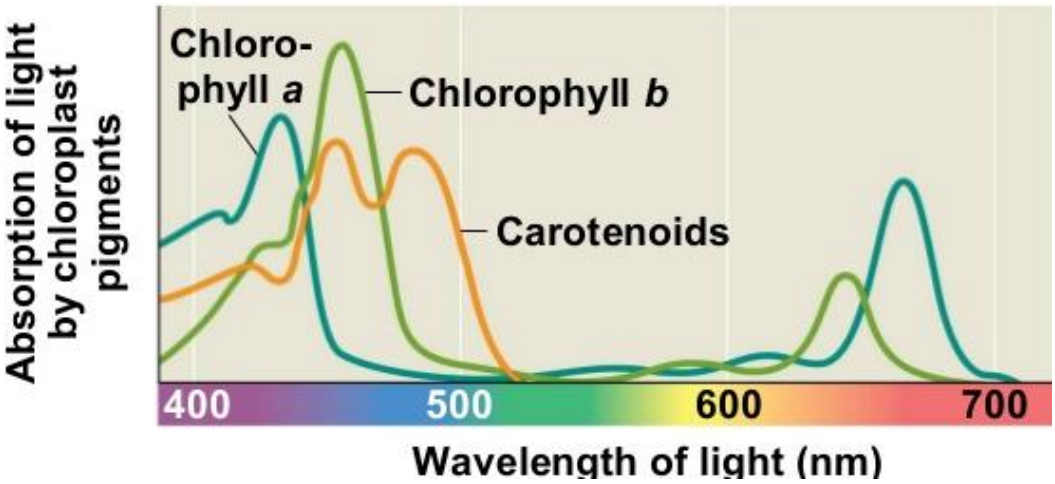

Figure 1.2: The absorption spectrum of the chlorophyll a and chlorophyll b pigments. Source: (Reece, e.a., 2011)

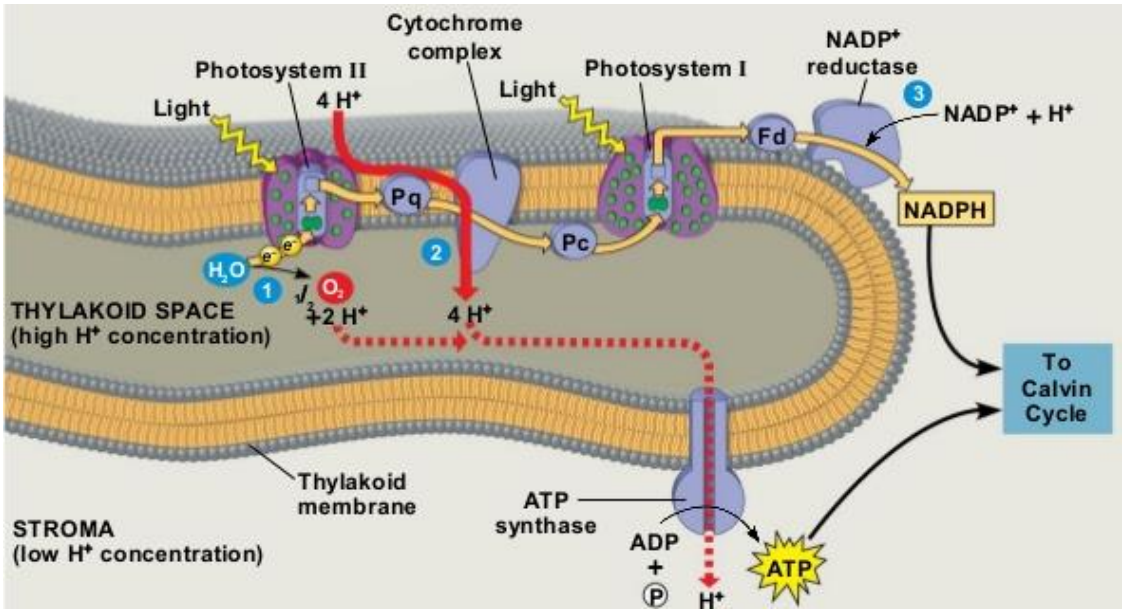

Figure 1.3: Photosystems 1 and 2, shown are the light reactions and chemiosmosis within the thylakoid membrane. Source: (Reece, e.a.,2011)

Fe deficiency reduces the ability of plants to absorb light through reduction of the photosynthetic machinery (specifically thylakoids, granam and stomal lamellae), as well as a reduction of the electron transport (Siedlecka & BaszyńAski, 1993). The electron transport reduction manifests itself in the maximum rate of photosynthetic electron transport (J_{max})(Grassi, e.a., 2002). J_{max} is generally limited by the availability of light and its intensity; due to Fe deficiency, however, the amount of photosynthetic apparatus is reduced, resulting in a reduction of absorbed light. Photosystem 2

produces ATP. This process powers several plant processes, notably atmospheric carbon fixation through the Rubisco enzyme (Reece, e.a., 2011). The fixation by Rubisco is measured through the maximum rate of Rubisco carboxylase activity (V_{cmax}). V_{cmax} is dependent on ATP; changes in ATP supply affect Rubisco and thereby the Calvin cycle . Fe deficiency limits ATP production, thus limiting V_{cmax}. V_{cmax} and J_{max} limit photosynthetic capacity (A_{max}), or the maximum rate of carbon fixation by the leaves (Walker e.a., 2014). Both V_{cmax} and J_{max} represent the leaf's nitrogen investment and are in balance to optimise photosynthetic nitrogen use efficiency (Kromdijk & Long, 2016). Fe deficiency negatively affects the balance between V_{cmax} and J_{max}, thus impacting A_{max} even more negatively because both V_{cmax} and J_{max} limit A_{max}.

1.3 Knowledge gap

Although much is known about photosynthesis, the literature is lacking in information regarding the functional responses of plants to iron deficiency, and specifically how photosynthetic biochemistry adapts to these conditions. Literature about improving Fe deficiency through Fe fertilisation and the effect of this fertilisation on photosynthesis is also lacking. Analysing the effect of Fe deficiency on biochemical activity through the parameters J_{max} and V_{cmax} may prove useful. Although the relationship between these parameters has been studied (Walker e.a., 2014), the effect of Fe deficiency and FeHBED fertiliser on these parameters—and thereby photosynthesis—is still unknown. The adaptability of soybean under Fe deficiency is also unknown; this was tested by exposing soybean to different light conditions. A literature review showed that different tools are used to collect data about photosynthetic parameters; comparisons between these tools are seldom seen and would add validity to research conducted using these devices.

1.4 Research aim and Summary of Methods

This thesis focuses on linking Fe deficiency in the soil to limitations relating specifically to photosynthesis occurring on the leaf level. The effect of Fe deficiency in plants was measured through photosynthesis measurements. The study used a C3 plant, *Glycine max* (soybean), in an experimental setting. Soybean was found, through the literature, to react quickly to iron deficiency. Different light conditions were used to measure the adaption ability of soybean when under Fe deficiency. The experiment consisted of two parts: one focusing on the plant's reaction to different light conditions under low Fe availability, and the other measuring the effect on soybean with a Fe chelate (FeHBED) added to the soil. Both experiments measured V_{cmax} and J_{max} on leaf level, as they are the most critical photosynthesis parameters. Additionally, the thesis compares photosynthetic data collected by different devices (SPAD and LI-COR). SPAD data focused on chlorophyll in leaves, using colour as an indicator. The LI-COR measured other photosynthetic parameters, one of which is J_{max}. The relationship between J_{max} and chlorophyll was then analysed.

1.5 Research questions

The main research question 9addressed by the thesis is as follows:
What is the effect of iron deficiency on photosynthesis parameters (V_{cmax} and J_{max}) in soybean (Glycine max) under different iron and light conditions?

The main research question aims to assess how this effect is affected by Fe deficiency and FeHBED-fertilised soil conditions. Additionally, different light conditions were introduced to investigate how these affected the soybean under different conditions and if there were any interaction effects between the two.

Sub-research question 1:
How do photosynthesis parameters (V_{cmax} and J_{max}) react in soybean (Glycine max) under different iron conditions?

Sub-research question 2:
How do photosynthesis parameters (V_{cmax} and J_{max}) react in soybean (Glycine max) under different light conditions?

Sub-research question 3:
How does the biomass in soybean (Glycine max) react to different light and iron conditions?

Sub-research question 4:
How do the data collected from a SPAD meter compare to data collected from a LI-COR meter in soybean (Glycine max) over time?

1.6 Hypotheses

Based on the current literature and the knowledge gap concerning the interaction of Fe deficiency with photosynthetic parameters (V_{cmax} and J_{max}), the following hypotheses were formed. As the thesis consists of multiple research questions, multiple hypotheses were tested.

H1: The photosynthetic parameters V_{cmax} and J_{max} in soybean (Glycine max) will show lower values, as they are limited by Fe deficiency, showing as lower biochemistry within the plant. J_{max} is directly impacted by a lack of iron; FeHBED would therefore be a significant factor in J_{max}. The parameter V_{cmax} has an optimal balance with J_{max} a change in one parameter results in an adjustment from the other parameter.

H2: Under higher light conditions, soybean will show higher photosynthetic activity, as the Fe deficiency is more pronounced under low light conditions.

H3: The population with FeHBED added to their soil will show the most growth. The cabinet with higher light intensity will show more growth compared to the low light intensity chamber. An interaction effect between FeHBED and light is expected. The combined stress of low light and Fe deficiency will cause that specific soybean population to have significantly lower parameter measurements.

H4: The data comparison between data collected using SPAD and LI-COR will show the same trends when comparing photosynthetic activity (A), J_{max} and the SPAD data. The literature showed chlorophyll to be a valid indicator for A. Where J_{max} refers to electron transport, SPAD refers to the chlorophyll level in the leaves; chlorophyll and J_{max} are strictly related, which would manifest in the same trend among both datasets.

1.7 Thesis relevance

This thesis illuminates the relationship between Fe deficiency and photosynthesis, specifically the effect of Fe deficiency on chlorophyll and the photosynthetic parameters V_{cmax} and J_{max}. The research provides a dataset of the Fe deficiency effect on soybean under different light conditions with and without Fe fertiliser (FeHBED). Soybean is a C3 and Strategy 1 plant; the thesis offers insights that may be comparable to other plants in the same category. The thesis can explain the adverse effects of Fe deficiency and showcase the impact of FeHBED fertilisation. Moreover, literature is lacking in species-specific information, and the effect of Fe deficiency on photosynthesis. The data comparison between SPAD and LI-COR adds to the literature, as the output could support the use of both tools.

The measurements under different light intensities provide agricultural stakeholders with information about growth and favourable conditions, potentially helping them to overcome or determine Fe deficiency in their crops. This may be especially helpful in developing countries, where Fe deficiency is a human health problem. The data from the screening can also be used by fellow researchers interested in iron deficiency, HBED's usage as a possible method of Fe uptake enhancement, or the adaptability of soybean exposed to Fe deficiency and a 'low light' stress factor.

2 Theory

The research was built upon several established theories and concepts: photosynthesis, Strategy 1 and 2 plants, and the FeHBED fertiliser. The latter two concepts are explained in this chapter.

2.1 Strategy 1 and 2 plants

Fe deficiency causes a root response that aims to enhance Fe mobilisation in the rhizosphere (figure 2.1). Two different strategies to cope with Fe deficiency have been identified. Through the use of the AHA2 gene, Strategy 1 plants exclude H^+ ions into the rhizosphere, which causes local acidification (lower Ph) and increases the solubility of Fe^{3+}. The FRO2 gene encodes Fe^{3+} to Fe^{2+}; with this Fe^{2+} now available, the IRT1 gene begins the Fe^{2+} uptake, IRT1 being its major transporter (Connolly, Campbell, Grotz, Prichard & Guerinot, 2003; Marschner, Römheld & Kissel, 1986; Verbon, Trapet, Stringlis & Kruijs, 2017). Strategy 2 plants, consisting of gramineous species, show a different response in the rhizosphere. Fe stress activates the biosynthesis of nicotianamine (NAAT), which activates Deoxymugineic Acid Synthase (DMAS). DMAS then begins producing iron-chelating phytosiderophores; these are transported to the rhizosphere through the TOM1 transporter and chelate Fe^{3+} in the rhizosphere (Willey, 2015; Bashir & Nishizawa, 2006). The Fe^{3+} is then absorbed in its chelated form by the YS1/YSL transporter into the plant, where it is encoded to Fe^{2+}.

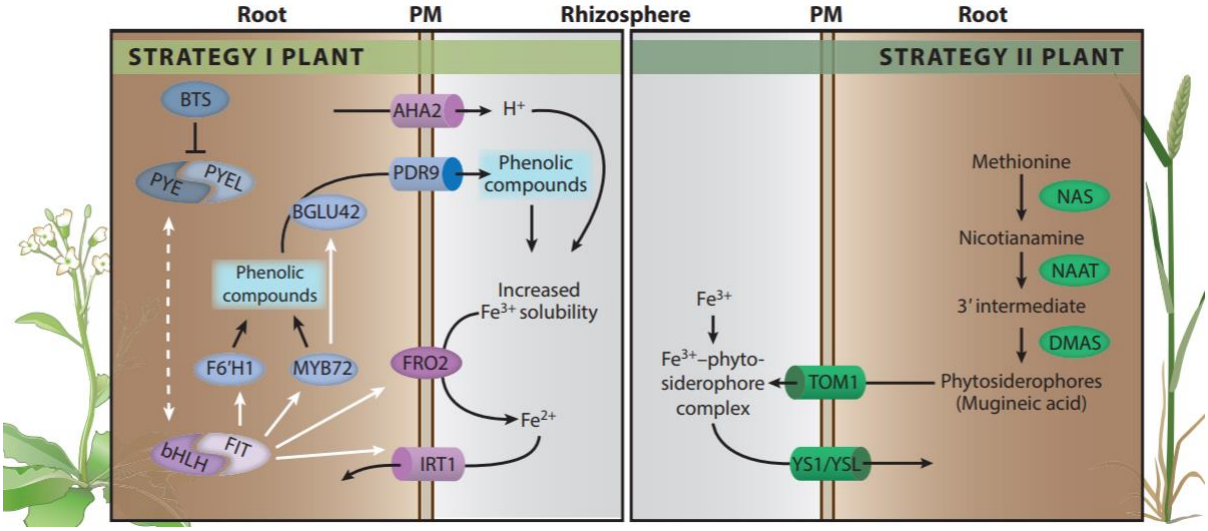

Figure 2.1: Schematic presentation of the phytosiderophores (PS) system in Gramineae.
Source: (Verbon, e.a., 2017).

2.2 Fe fertiliser HBED

Chlorosis can be prevented using a Fe fertiliser. These consist of iron chelating agents or steel slag and are applied directly to the soil (Lucena, 2006)(Wang & Cai, 2006); a solution of these chelates can also be sprayed directly onto the leaves. For Strategy 1 plants, the iron chelate used in soybean fertilisation experiments is *N,N'*-bis(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (FeHBED) (Nadal et al., 2012; Gonzalo et al., 2013). FeHBED is an organic molecule (ligand), which means that i binds itself to a central metal atom. Different HBED ligands are available; the one used in this research is FeHBED, which specifically binds to Fe^{3+}. The ligand forms a coating surrounding the Fe, protecting it from the surrounding OH- ions. The bonded Fe^{3+} is still available for absorption by the plant. FeHBED is known to be a strong chelator and stable in soil pH up to 12 (Gonzalo et al., 2013). The theory of iron fertilisation to improve the available iron in the soil, specifically with the use of Fe^{3+}-HBED, is tested in this research, as well as FeHBED effect on the photosynthesis parameters in comparison to low Fe conditions.

3 Methods

3.1 Experimental setup

These sub-chapters explain the steps taken during the experiment and provide a guide should the experiment be repeated.

3.1.1 Germination phase

The experiment consists of two populations in different growth cabinets. Both are pot trial experiments, where plants are grown in pots in controlled conditions. The size of the pots (11x11x12cm) was chosen to offer sufficient space for root growth. Each pot contains one germinated soybean seed; this was done to prevent interference via contact between plants. The pots were square with holes in the bottom to facilitate water flow out of the soil and prevent anaerobe conditions at the bottom of the soil. At the bottom of each pot, a piece of root cloth was placed that prevents the outflow of soil but allows water to flow through. Each individual sample had an individual tray to catch any outflow of water. If water was left in the tray, it was returned to the topsoil to prevent possible nutrient loss. When the soybean plant stems grew beyond 15 centimetres, wooden support sticks were added, as the stem could not support the weight of the leaves. The sticks were only needed in the low light cabinet. A total of 56 plants were used in the experiment. Two growth chambers were used with 28 samples each; of these 28, 14 in each group contained FeHBED while the other group contained nothing. The growth chambers had different light conditions: 140 µmol/m^2/s and 240 µmol/m^2/s.

For the experiment, a soybean variety was chosen that was similar to that used in Schenkeveld, e.a. (2010). Schenkeveld, e.a.'s (2010) research focused on the use of FeEDDHA chelate to reduce iron deficiency in soybeans. As this variety had already been researched and shown to be vulnerable to iron deficiency, it was also used in this experiment to determine the effect of Fe deficiency on photosynthesis.

The soybean experiment began with the seed germination, which was done using sand-filled trays (10 kg) with demineralised water (1.2 litres) (Figure 3.2). Each seed was submerged in water for 10 minutes to activate dormant growing mechanisms, after which they were wiped dry, placed in the tray and covered with six centimetres of sand. The tray was covered with a plastic cover, creating a higher humidity within. The number of seeds in the germination phase was twice as large as the number of seeds required. The germination phase lasted three days, after which the next phase began. Following the germination phase, the most comparable and successful seeds were chosen for the pot trial. The criteria for the chosen seed were a flat growth rate (both below and above ground) compared to the other seeds of the same variety. Seeds that were not yet germinated were excluded through this procedure, as well as seeds that showed exceptional growth. After the selection, the remaining seeds were planted in the prepared soil and placed in the growth chambers.

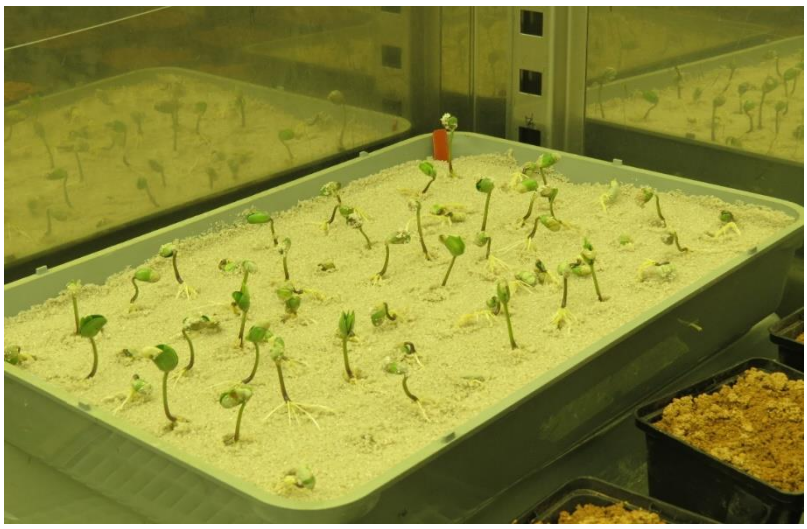

Figure 3.2: Sand-filled tray used for soybean (*Glycine* Max) germination phase three days after planting.

3.1.2 Growth cabinets

The growth cabinets were used to regulate certain environmental conditions. The parameters were temperature (15–30 degrees), light intensity (maximum 140 and 240 µmol/m^2/s), length of day/night (12/12), humidity. The level of CO_2 could not be adjusted in these cabinets. The other parameters were adjusted accordingly to simulate the same conditions, with light intensity as the one changing parameter. The growth cabinets had different surface areas; both cabinets, however, contained 28 soybean samples.

Both populations of 28 samples had the following environmental parameters in their growth cabinets, with only the light intensity varying. The temperature was set at 22 degrees (this will slightly vary across the experiment due to the transition from day to night, which causes a small fluctuation). To compensate for the low light intensity of the cabinets (compared to direct sunlight 1200 µmol/m^2/s), a more extended day/night cycle was chosen, which consisted of 16 hours of day and eight hours of night. The start times of the day cycles were set two hours apart from one another. This allowed for more accessible measurements in the future, as measuring each chamber takes time. For the chambers to be comparable, however, the measurements occurred at the same time in the day cycle, one hour after the beginning of the cycle. Humidity was set at 70%.

3.2 Soil characteristics and preparation

To understand how FeHBED impacts the growth of soybean, iron-deficient soil was needed. Based on available soil and the literature, we chose to use silica sand and Santomera soil. The Santomera soil has been documented and was used for an experiment concerning iron deficiency in soybean (Schenkeveld, e.a., 2014). The Santomera soil was mixed with silica sand at a ratio of 1:1. The soil characteristics (Table 3.1), gained from a DTPA extraction using Quevauviller (1996) protocol, showed the amount available of each element per mg of kg of soil (Appendix 6). This indicated iron-deficient soil.

Table 3.1: Soil characteristics from DTPA extraction; grey values are untrustworthy, blue values may have a 10% error, black values are trustworthy.

	Co	Cu	Fe	Mn	Ni	Zn
Sample	**(Mg Kg^{-1})**					
Blank 1	*-0,001*	0,004	0,019	0,001	0,004	0,005
Blank 2	*-0,001*	0,004	0,016	0,000	0,002	0,007
Quartzsand 1	*-0,001*	0,039	0,041	0,005	0,001	0,018
Quartzsand 2	*-0,001*	0,034	0,051	0,006	*0,001*	0,016
Santomera 1	0,004	0,159	0,496	0,861	0,041	0,071
Santomera 2	0,004	0,177	0,507	0,952	0,045	0,053
Mix 1	0,005	0,268	0,607	1,512	0,077	0,075
Mix 2	0,005	0,274	0,608	1,509	0,076	0,082
Spoel	*-0,001*	*0,000*	0,002	*0,000*	*0,000*	0,002

Based on this knowledge and a comparative study on soybean by Walter (2010), a nutrient solution was prepared to be added at the start of the experiment (Table 3.2). Apart from these nutrients, FeHBED was made using the protocol by Hernández-Apaolaza, Lourdes and Lucena (2011) and added to the first 14 samples of each chamber for a total of 28 samples. The nutrient solution was added at the start of the experiment while mixing the soil in preparation for moving the plants from the tray to the pot. The samples were watered three times per week; the amount of demineralised water was determined by soil weight (roughly 1kg), where 120ml of water were added per 1kg (Table 3.3). At the start of the experiment, the weight of both water and soil were measured (starting weight). During the experiment, demineralised water was added until this 'starting weight' was matched (Appendix 1).

Table 3.2: Nutrient solution added to soybean sample at the start of the experiment, based on Walter (2010).

Molecule name	Chemical formula	Molmass in Lab (g/mol)	Gram/pot
Ammonimiumnitraat	NH4NO3	80,49	0,27
Kaliumwaterstoffosfaat	K2HPO4	174,18	0,36
Calciumchloride	CACl2	110,98	0,18
Magnesiumsulfaat	MgSO4.7H2O	246,48	0,21
Boorzuur	H3BO3	61,83	2,58*10-3
Ammonium molybdate tetrahydrate	(NH4)6Mo7O24	1235,86	3,86*10-4

Table 3.3: Saturation per soil type; the soybean experiment used 120ml per 1kg soil as watering.

Soiltype	Saturation in ml measured at 100 gram soil	Amount of water (60% saturation) per 1 kg soil
Santomera	29,8	178,8
Quartzsand	27,8	166,8
Mix Santomera+Quartzsand	20	**120**

3.3 Measurement Tools

3.3.1 LI-COR

The measurement tool used in all of our experiments was LI-COR 6400XT (Figure 3.3); this device allows for measuring photosynthetic activity by simulating set conditions in the sensor head. The device was operated using instruction manual version 6 (LI-COR, 2018). The device allows the 6400-40 LCF (Figure 3.3) to be placed upon the leaf area, preferably in the middle and covering as much of the leaf area as possible. The sensor head/IRGA (Figure 3.3) regulates light intensity, water vapour concentration, and atmospheric CO_2, among other conditions. These parameters were put into the console (Figure 3.3) and set to the parameter settings in the growth cabinets where the experiments took place.

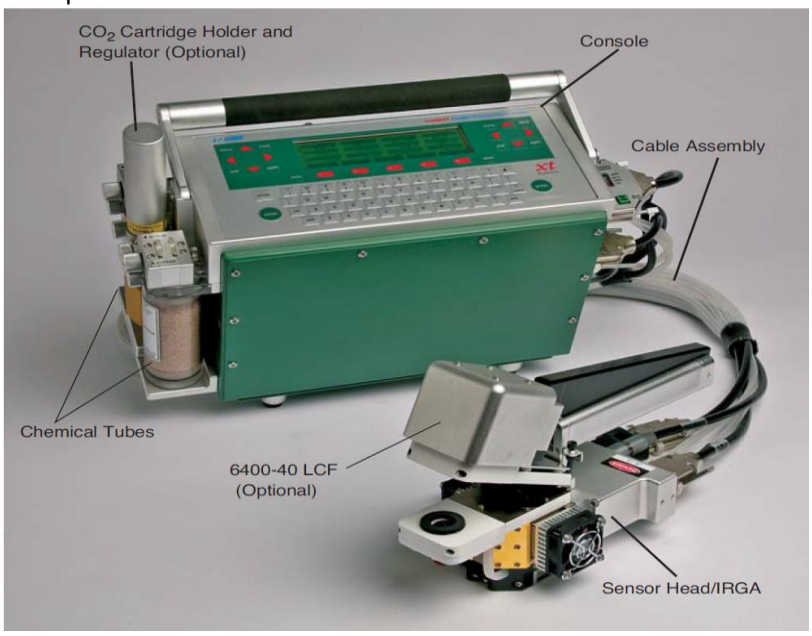

Figure 3.3: LI-COR 6400XT, portable photosynthetic measurements tool (LI-COR, 2018).

The LI-COR was used to gather photosynthesis measurements through, A/Ci curves and weekly leaf measurements. These tests provided the variables A_{max}, gs, V_{cmax}, J_{max}, Cit and Ci/Ca (Appendix 5).
V_{cmax}: maximum rate of carboxylation (µmol m^{-2} s^{-1})
J_{max}: maximum rate of electron transport (µmol m^{-2} s^{-1})
A_{max}: Photosynthetic capacity, amount of carbon dioxide fixed per meter squared per second
gs: Stomatal conductance
Cit: The intersection of J_{max} and V_{cmax} lines in an A-Ci curve
Ci/Ca ratio: Intercellular CO_2/ ambient CO_2 measured as ratio from two CO_2 (ppmV) values
A ambient: Ambient carbon dioxide content

The above data were measured on the device and could be accessed using a network port located on the console. Three tests were run using the LI-COR, as explained in the protocol by Evans and Santiago (2014). One of these was a light-response test; the two others used in the thesis are:

Week-to-week samples of photosynthesis using ambient conditions
These samples were taken starting in Week 3 of the experiment, as at that point, the leaves were large enough for the LCF head. One leaf from each sample was placed inside the chamber, and the chamber was set at the ambient conditions of the growth cabinet. After the leaf parameters were stabilised for roughly two minutes, five measurements were taken one second apart from each other. These were later averaged to provide a week-by-week output showing photosynthesis in ambient conditions.

A-Ci curves

The A-Ci curves were conducted twice during the experiment, once at the halfway point and once at the end. The test consisted of placing a leaf in the LI-COR chamber, setting the light settings on 1500 µmol/m2/s and setting the ambient temperature based on the growth cabinet's conditions. The leaf was then exposed to different CO_2 ppms (50, 100, 150, 250, 350, 500, 700, 900, 1200, 400); the leaf parameters were then stabilised (roughly 2–3 minutes) and measured. The purpose of this test is to compare the four groups; by setting the light to a maximum level, the CO_2 assimilation by plants was tested, resulting in saturation curves that can be compared. These curves were also used to determine the V_{cmax} and J_{max}, which are needed to answer the research question.

3.3.2 SPAD Meter

The second measurement tool at our disposal was the Minolta-502 SPAD Meter. The measurement tool has been used in comparable research about photosynthesis parameters and is used to measure chlorophyll (Schenkeveld, 2010). The tool provides an indication of the chlorophyll (µmol/m^2) content of plant leaves (Alvarez-Fernandez et al., 2004; Banuls et al., 2003). The measurements were taken three times weekly, with a day without measurements in between (Appendix 7).
When the leaves were large enough, the tool was used to measure the two youngest leaves and the two second-youngest leaves. Over time, the measured leaves shifted as the plants grew new foliates. The youngest leaves were chosen because chlorosis (iron deficiency) is more evident in them (Zhou et al., 2013). When a newly developed leaf was large enough, the SPAD meter was shifted to it. The device was placed at the midpoint of the leaf, between the leaf edge and the central vein (Figure 3.4). A small circular sensor in the tool takes a measurement that is an indication of chlorophyll content. Each measurement took 10–15 seconds, or around one minute to sample each plant. The measurements of the youngest and second-youngest leaves were then averaged for each pot. Compared to the LI-COR, this method is faster but provides less in-depth measurements.

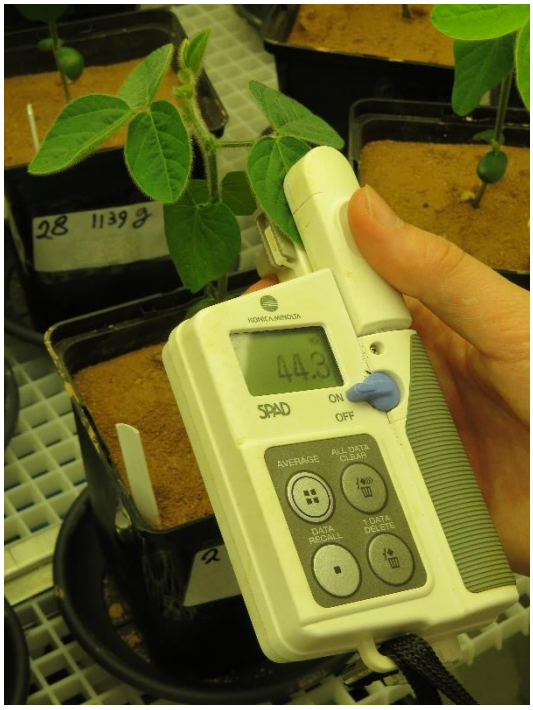

Figure 3.4: SPAD meter used in soybean experiment.

3.4 Parameters from the R program

The three most important parameters measured were V_{cmax}, J_{max} and A_{max}. These can be extracted from an A-Ci curve. The LI-COR calculated the rates of CO_2 assimilation and transpiration, stomatal conductance and the concentration of CO_2 in the intercellular airspaces within the leaf (Evans & Santiago, 2014). The data collected using the LI-COR were extracted as Excel files. These files were then used by the program MATLAB, which averages the measurements collected. These were saved as a text file, which the program R used for further analysis. In R, the Excel data can be analysed via the 'plantecophys' package (a premade program created by Duursma(2015)). R linked the measurements to corresponding models and fit the data into a graph (Figure 3.5). These graphs are A-Ci curves, from which V_{cmax}, J_{max} and A_{max} can be determined. These models include the Ball-Berry models of stomatal conductance; the Farquhar-von Caemmerer-Berry (FvCB) model of leaf photosynthesis; and the coupled leaf gas exchange model, which combines supply and demand.

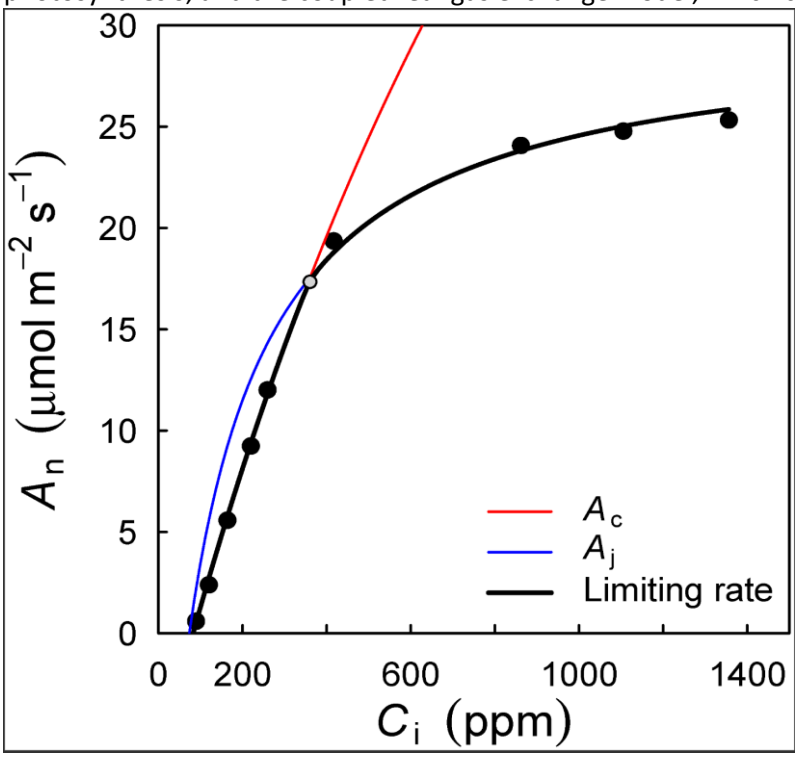

Figure 3.5: Standard output from the fitaci function in R; An is the net photosynthetic rate, Ci the intercellular CO_2 concentration. Symbols are measurements; the black line is the fitted FvCB model of photosynthesis. Coloured lines indicate the two photosynthesis rates in the FvCB model. In default mode, the fitaci function estimates V_{cmax}, J_{max} from the fitted curve. The intersection point between the blue and red line is called Cit Source: (Duursma, 2015).

3.5 Plant weight and ImageJ

During the experiment, there were two destruction events. The first destruction was performed with the 24 plants for which we had the A-Ci curves, at 18 days after the start of the experiment following germination. The second destruction was done with the other 32 plants, 24 of which had A-Ci curves at 37 days. The destruction gave us access to the plant material. The plant material was divided into five separate measurements: All leaves, Leaf A-Ci, Stem, Beans and Roots. Before these measurements were taken, the leaf area was determined using a scanner and the ImageJ program. ImageJ is an image processing program designed for multidimensional scientific images. Following a protocol for leaf area analysis, it allows photos (Figure 3.6) of the leaves to be analysed to determine the total leaf area (Glozer, 2008).

Figure 3.6: Photo of all leaves from plant sample used in ImageJ.

The separate parts were placed in different marked envelopes and into the oven for three days at 70 degrees Celsius. The plant material, now without the water weight (dry matter), was then removed from the envelopes and measured. Measurement was done after both destructions, allowing for all of the plant material to be measured (Appendix 2). The beans of the soybean plants developed around the fourth to fifth weeks and were only available for the second destruction.

3.6 MATLAB

The data collected from the experiment were visualised in graphs and boxplots using MATLAB as a medium (Appendix 3). After the visualisation, the data were further analysed through the two-way ANOVA test (Appendix 4). The two-way ANOVA test is an extension of the ANOVA test. The ANOVA tests the effect of independent variables on the dependent variable between groups. The two-way ANOVA can test for multiple independent variables and can provide measurements of their relation. The data consisted mostly of four different groups (soybean in Chambers 1 and 2 with different light conditions and with and without HBED), and the dependent variable was scale-based (J_{max}, V_{cmax}, Plant weight, Chlorophyll, Leaf area, Plant weight and Leaf area ratio (LAR)). This way, the relationship between the four groups could be analysed to find possible interaction effects, which were then used to answer the research questions.

The data in MATLAB were split into four different groups containing the four different soybean populations (Table 3.4). Additionally, most of the results consisted of two data sets, which were created in order to compare the difference between groups over time. The first dataset was taken 18 days after the start of the experiment (the first destruction), and the second dataset was taken after 37 days, at the end of the experiment (the second destruction).

Table 3.4: Overview of experimental conditions and acronyms used in soybean experiment.

Groups	Description group
HLNH	**H**igh **L**ight **N**o **H**BED, this group was in the high light cabinet without FeHBED added to the soil
HLYH	**H**igh **L**ight **Y**es **H**BED, this group was in the the high light cabinet with FeHBED added to the soil
LLNH	**L**ow **L**ight **N**o **H**BED, this group was in the the low light cabinet without FeHBED added to the soil
LLYH	**L**ow **L**ight **Y**es **H**BED, this group was in the the low light cabinet with FeHBED added to the soil

4 Results

This chapter shows the found results, consisting of several graphs, boxplots and statistical tests.

4.1 SPAD data and Photosynthetic efficiency

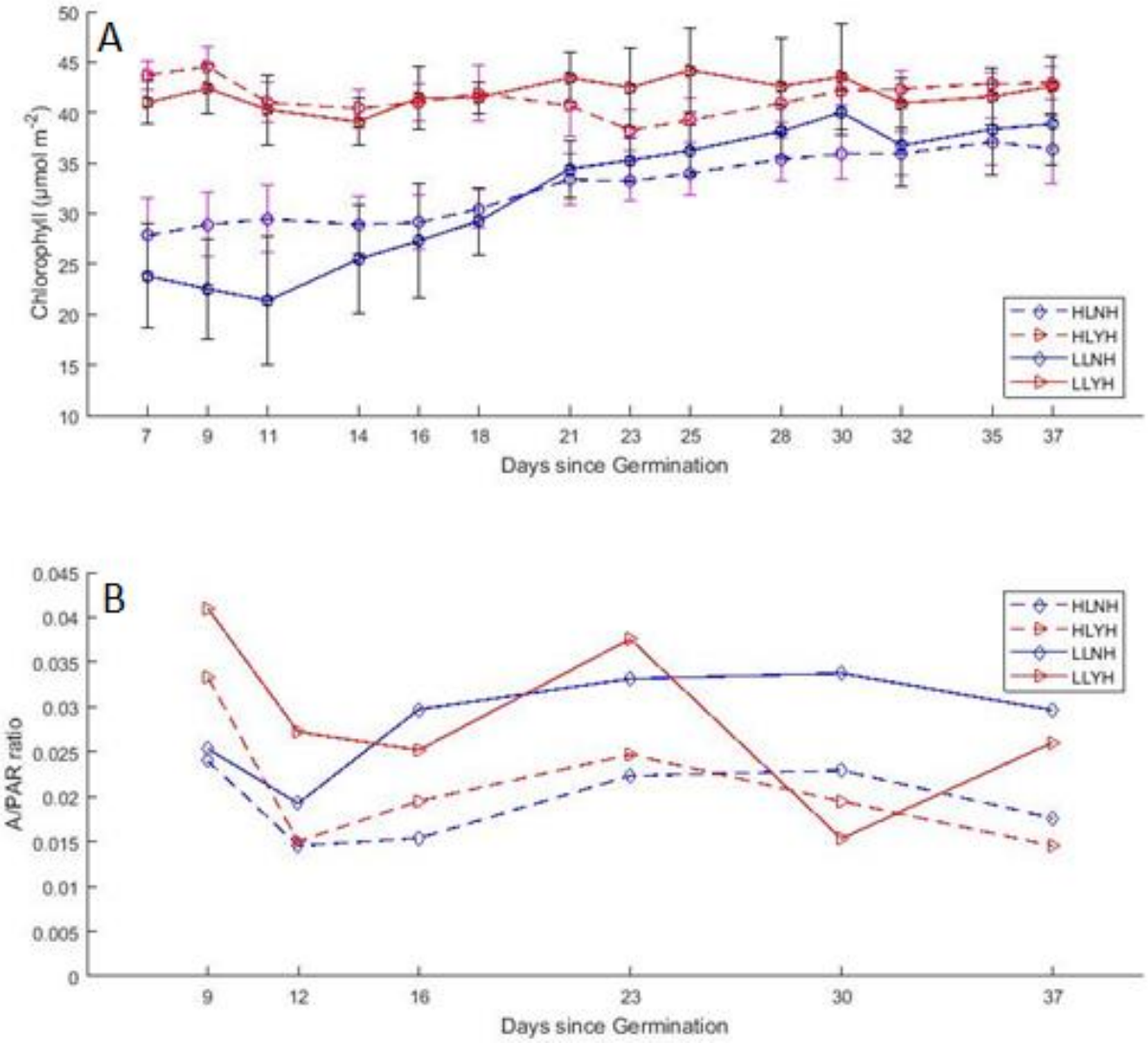

Figure 4.1: Graph A expresses the measured chlorophyll content using a SPAD meter taken 14 times during the experiment, showing the chlorophyll content of the youngest leaves in the four groups. Error bars denote standard error. Graph B refers to the photosynthetic (A) efficiency, A divided by PAR (photosynthetically active radiation), HL was 240 PAR and LL was 140 PAR; these were measured six times during the experiment and coincide with the SPAD measurements above.

Our results indicate that the iron-deficient plants had lower chlorophyll content than the plants receiving additional FeHBED (Figure 4.1) (Figure 4.3). This effect was most pronounced during the first phase of the experiment, and gradually diminished after 18 days; however, it remained significant at 37 days. The results showed that the different light treatment caused the low light populations to have less chlorophyll compared to their counterparts. There was no significant interaction effect observed in the chlorophyll measurements (Table 4.1).

Table 4.1: Summary of the two-way ANOVA test focusing on Light, FeHBED and their interaction. NS is not significant, MS is marginally significant, * is $p < 0.05$, ** is $p < 0.01$, *** is $p < 0.001$.

Treatments	Jmax	Vcmax	Chloropyhll	Stem	LAR	Leaf area	Below ground	Above ground
Light_1	NS	NS	*	*	***	NS	NS	NS
Light_2	*	NS	NS	*	***	NS	***	**
FeHBED_1	NS	NS	***	NS	*	*	**	***
FeHBED_2	*	NS	***	NS	***	NS	**	*
Interaction_1	NS	NS	NS	MS	**	NS	NS	NS
Interaction_2	NS	NS	NS	NS	NS	MS	NS	MS

This transition was also visible at the phenotype (Figure 4.2), which showed discolouration of the leaves, a symptom of iron chlorosis. Graph B (Figure 4.1) shows that the low light populations were more productive with the available light.

Figure 4.2: Photo of Soybean (*Glycine max*) 17 days after start of the experiment, population from the low light chamber.

4.2 J_{max}, V_{cmax} and Chlorophyll

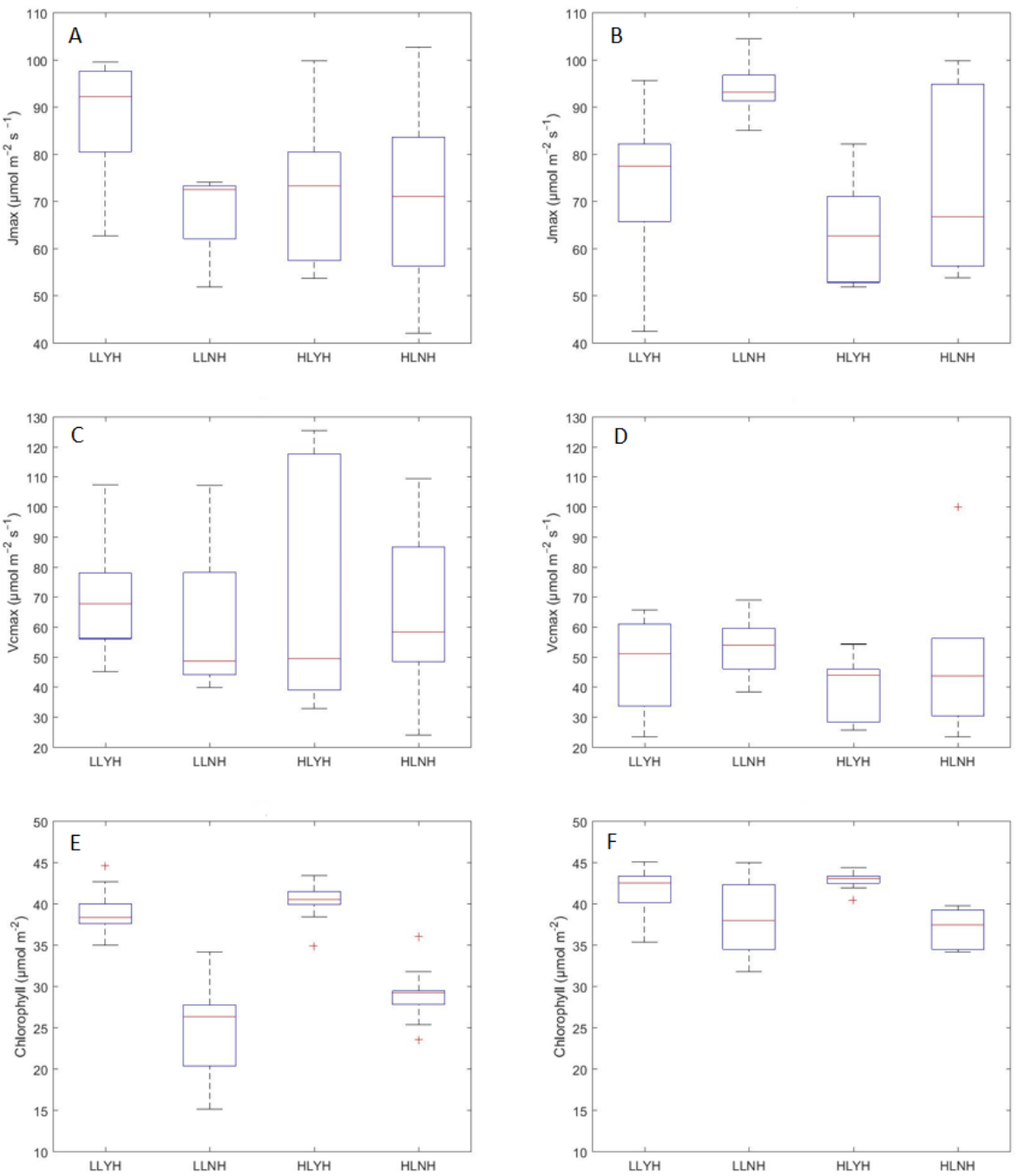

Figure 4.3: Boxplot A and B represent the J_{max} photosynthetic parameter measured at 18 and 37 days after the start of the experiment. Boxplot C and D represent the V_{cmax} photosynthetic parameter measured at 18 and 37 days after the start of the experiment. Boxplot E and F represent the Chlorophyll amount measured at 18 and 37 days after the start of the experiment.

The J_{max} (Figure 4.3) in soybean was not significantly impacted by FeHBED or light at the first destruction. A higher J_{max} was noticeable at the second destruction in iron-deficient plants. The light factor caused populations exposed to a lower light intensity to have a significantly higher J_{max}. The V_{cmax} parameter does not show a significant impact of FeHBED or light at either destruction. No interaction effects between FeHBED and light were observed in either J_{max} and V_{cmax}.

4.5 Stem length and seeds

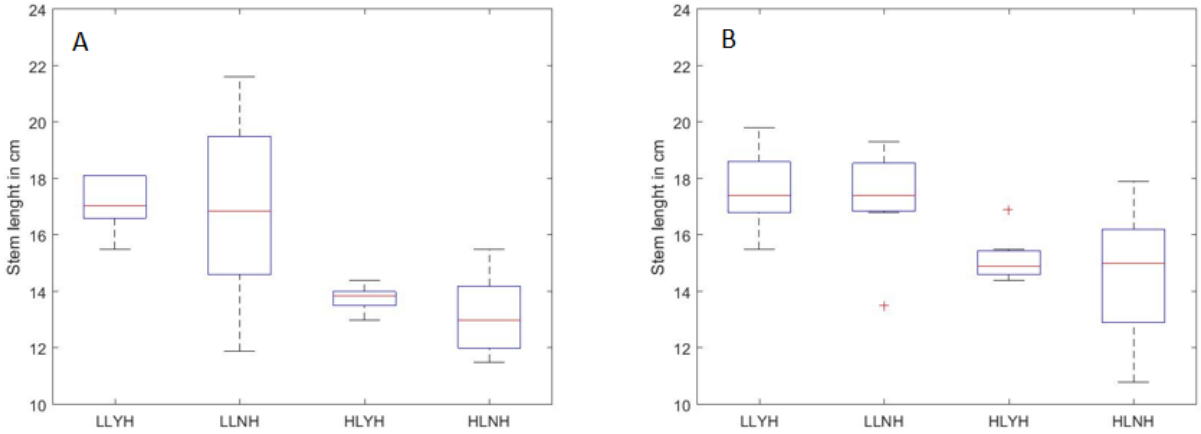

Figure 4.4: Boxplots A and B represent the stem length of the soybean in cm measured at 18 and 37 days after the start of the experiment.

Plants exposed to a lower light intensity had longer stems at both destructions (Figure 4.4). Different iron conditions had no significant effect on stem length. A marginally significant interaction effect was observed at the second destruction.

4.3 LAR and Leaf area

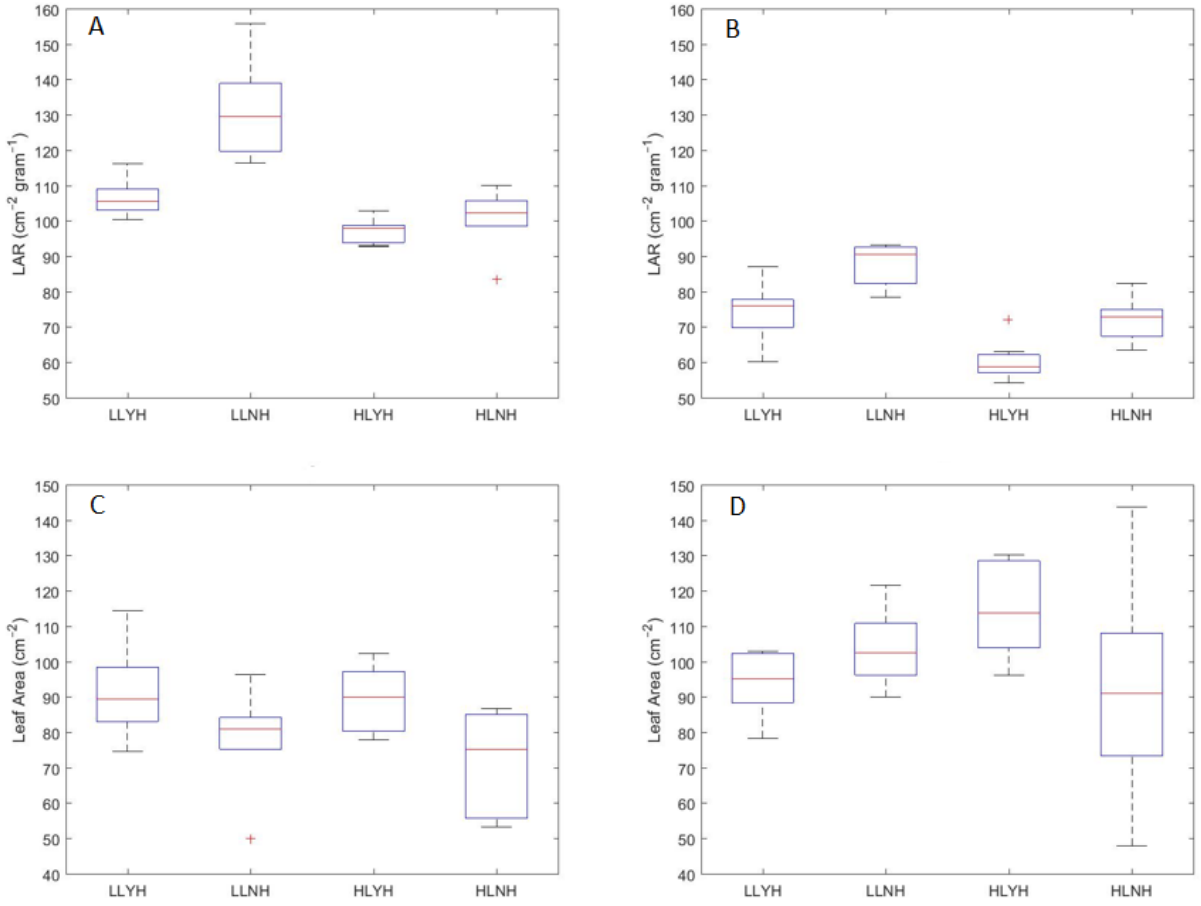

Figure 4.5: Boxplots A and B represent the Leaf area ratio (LAR), measured at 18 and 37 days after the start of the experiment using data collected from Leaf area and Plant weight. Boxplots C and D represent the Leaf area measured at 18 and 37 days after the start of the experiment using data collected from ImageJ.

The results (Figure 4.5) suggest that iron-deficient plants had a higher LAR ratio at both destructions. The populations exposed to a higher light intensity had a significantly lower LAR. During the first phase, an interaction effect was observed, negatively impacting the plants that received FeHBED and grew under a higher light intensity. This effect was not detectable during the second phase. Plants with FeHBED had a larger leaf area in the first phase. Iron-deficient populations did not differ significantly in the second phase. The light intensity had no effect on leaf area in either the first and second phase.

4.4 Plant weight

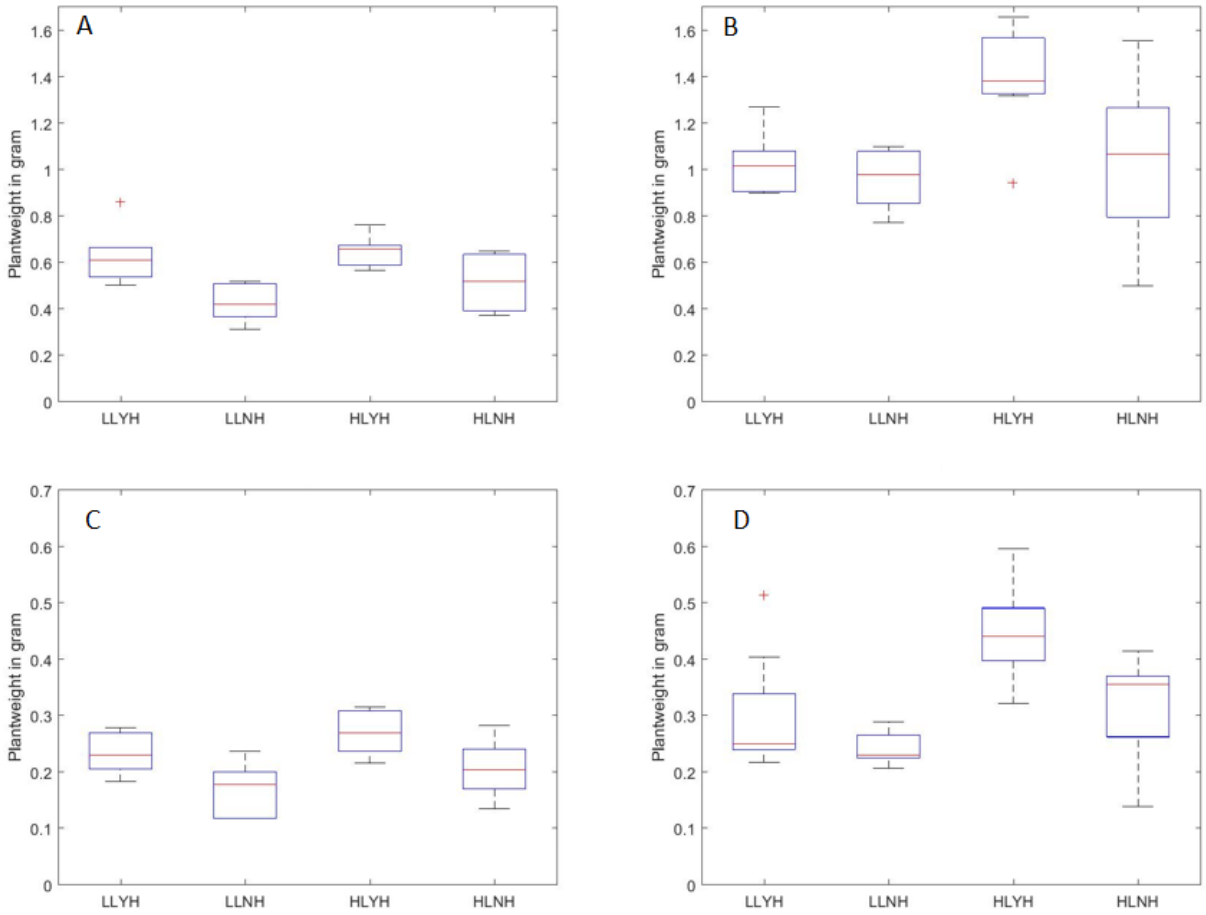

Figure 4.6: Boxplots A and B show the Above-ground measurements taken at 18 and 37 days after the start of the experiment. Boxplots C and D show the Below-ground measurements taken at 18 and 37 days after the start of the experiment.

Our results (Figure 4.6) indicate that iron-deficient plants had a pronounced lower plant weight at both destructions. Populations exposed to a higher light intensity had a significantly higher plant weight at the second phase. A marginal interaction effect was seen at the below-ground plant weight during the second phase of the experiment.

5 Discussion

This chapter answers the four sub-research questions and discusses the broader implications of the results and how they relate to previous literature.

Sub-research question 1: *How do photosynthesis parameters (V_{cmax} and J_{max}) react in soybean (Glycine max) under different iron conditions?* Although Winder and Nishio (1995) found that a significant consequence of iron deficiency was reduced V_{cmax} activity, our study contradicted these findings. It was expected that, like J_{max}, V_{cmax} would be impacted by FeHBED. The experiment showed iron deficiency in the non FeHBED treated plants. The V_{cmax} data, however, showed that FeHBED did not make a significant difference in V_{cmax} in soybean. The J_{max} data showed the non-FeHBED populations surpassing their FeHBED counterparts. Particularly in the LLNH group, it seems that the stress response to iron deficiency increased the J_{max} parameter. Kromdijk and Long (2016) found that V_{cmax} and J_{max} exist in an optimal balance. Iron deficiency may have offset the balance between the two parameters. The unexpected stress behaviour between V_{cmax} and J_{max} warrants more extensive research.

J_{max} was gradually affected by FeHBED, showing a pronounced negative effect at the second destruction. The long-term effect of FeHBED was also observed by Nadal et al. (2013), who tested different synthetic Fe chelates and determined through SPAD measurements that FeHBED prevents chlorosis. The same trend is visible in our chlorophyll data. Bin et al. (2016) showed the diminishing effect of FeHBED over time, with a comparable trend seen in our SPAD index. FeHBED seems to improve long-term performance, as suggested by Nadal et al. (2013), who attributed the long-term effect to FeHBED's stability and ability to maintain Fe in solution longer compared to other Fe-chelating substances such as FeEDDHA. Several studies (Andaluz et al*., 2006;* Timperio et al*., 2007;* Laganowsky et al., 2009) have found that the protein contents in the electron chain components (responsible for J_{max}) decrease under Fe deficiency. The expected decrease ran counter to our results, which show that iron-deficient plants have a noticeably higher J_{max}.

J_{max} and V_{cmax} were based on data measured by the LI-COR, while measuring the parameters in the IRGA (Figure 3.3) the controlling the reference CO_2 levels could not be set to zero. It was manifesting itself in the data as small irregularities. These irregularities are corrected using empty chamber measurements before and after a leaf measurement. This problem was mainly present at the start of the experiment and caused some of the first A-Ci curves to be excluded from the data analysis. Additionally, a single A-Ci curve took roughly 40 minutes for the LI-COR, making it impossible to measure multiple plants at the same time of day.

Sub-research question 2: *How do photosynthesis parameters (V_{cmax} and J_{max}) react in soybean (Glycine max) under different light conditions?*
The light factor proved to have a significant impact on J_{max} over time, supported by the significant effects at the second destruction. The low light populations performed better over time and were more efficient with their light use, developing higher stems, which increased their light capture. The same effect was observed by Zhang et al. (2011), who detected elongated soybean stems as a result of shade treatment.

The low light intensity cabinet showed faster development compared to the high light intensity cabinet, as demonstrated by the earlier seed development in the low light cabinet. Roughly the same number of seeds were developed (29 by low light and 33 by high light), but the seeds' development was visible a week earlier in the low light population. The iron condition did not have a noticeable effect on seed development. Under stress, soybean begins its seed development phase earlier. Kumar et al. (2012) confirm that plants exposed to abiotic stresses can regulate the extent and

pattern of development. Plants adapt based on the stress experienced to increase survival and reproductive success.
Different light conditions did not affect the seed amount harvested.

Further research into the harvested seeds could provide the seeds' chemical composition. This would enable us to research the effects of different light or iron conditions on the offspring.
A limitation regarding this research question was the level of light intensity available. The growth cabinets used a low light intensity (maximum 140 and 240 µmol/m^2/s) compared to natural sunlight (1200 µmol/m^2/s); although this was mitigated by adjusting the day/night cycle (16 hours of day/eight hours of night), using a more considerable distinction between light intensities is advised for future research.

Sub-research question 3: *How does the biomass in soybean (Glycine max) react to different light and iron conditions?*
Iron-deficient plants had noticeably lower above- and below-ground biomass at both the first and second destructions. A study by Caliskan et al. (2008) confirms increased biomass and yields as a result of iron fertilisation of soybean. Plants exposed to lower light intensity showed lower biomass at the second destruction; plants under lower light and iron-deficient conditions, however, had a significantly higher LAR in both destruction phases. During the first phase, an interaction effect was observed, negatively impacting the plants that received FeHBED and were under a higher light intensity. Iron-deficient plants exposed to low light were least impacted by the interaction effect and showed a detectable higher LAR compared to the other groups. A possible explanation for this comes from Kumar et al. (2012), who in their research on physiology and morphology found that plants adapt to abiotic stresses by changing their patterned genetic development. The soybean plants under iron deficiency and low light adapted by becoming more stress-tolerant.

Sub-research question 4: *How do the data collected from a SPAD meter compare to data collected from a LI-COR meter in soybean (Glycine max) over time?*
SPAD and the LI-COR measure different plant properties and do not support the hypothesis that the same trends can be seen between photosynthetic activity and chlorophyll. The SPAD data show the long-term effect of FeHBED, which was more pronounced at the start of the experiment and diminished over time. The same trend was not observed in the LI-COR data. Chlorophyll, J_{max} and V_{cmax} are indicative plant parameters, and SPAD measurements are more widely used in literature than the LI-COR. Using both tools is recommended. Several studies (Ma et al., 1995; Netto et al., 2005) have concluded that SPAD data were positively correlated with leaf photosynthesis in soybean. A possible explanation for the difference is the light intensity used in the experiment, which was low (140 and 240 µmol/m^2/s). The difference in photosynthetic activity (A) measured by the LI-COR may be too small, prohibiting a comparison between SPAD and A.

6 Conclusion

This chapter answers the main research question and highlights some of the key findings.

What is the effect of iron deficiency on photosynthesis parameters (Vcmax and Jmax) in Soybean (Glycine max) under different iron and light conditions?

The soybean populations exposed to different iron and light conditions were measured using the LI-COR apparatus. These measurements were taken before destruction events at 18 and 37 days after the start of the experiment. We measured the photosynthetic parameter values of V_{cmax} and J_{max}. V_{cmax} was not significantly impacted by the different iron and light conditions. Soybean with iron deficiency and placed under low light intensity conditions showed a significantly higher J_{max} compared to the other groups. This was only visible at the second destruction. A previous study by Kromdijk and Long (2016) found that V_{cmax} and J_{max} were in optimal balance with each other. Based on our contradicting results we propose that iron deficiency could be responsible for the offset of the balance between the two parameters. This unexpected behaviour warrants additional research.

Other key findings included the LI-COR and SPAD data comparison. The literature has suggested that chlorophyll is a proven indicator for photosynthetic activity (A). It was hypothesised that the same trends would be visible between A, J_{max} and chlorophyll. The electron transport (J_{max}) is closely related to chlorophyll. The study found no clear evidence to confirm this hypothesis. The absence of a thorough literature-established trend may be explained by the small difference in photosynthetic activity due to the low amount of light availability.

Additionally, during the last two weeks of the experiment, a difference in seed development was observed in both climate cabinets. Soybean in the low light intensity group began seed development one week before the soybean population in the high light intensity cabinet. Although the yields were comparable, the abiotic stress caused by low light availability made the soybean adapt by starting its seed phase earlier.

7 References

Alloway, B.J. *Micronutrient Deficiencies in Global Crop Production*. Springer Science & Business Media, 2008.

Álvarez-Fernández, A., P. García-Laviña, C. Fidalgo, J. Abadía, en A. Abadía. "Foliar Fertilization to Control Iron Chlorosis in Pear (Pyrus Communis L.) Trees". *Plant and Soil* 263, nr. 1 (1 juni 2004): 5–15. https://doi.org/10.1023/B:PLSO.0000047717.97167.d4.

Andaluz, Sofía, Ana-Flor López-Millán, Javier De las Rivas, Eva-Mari Aro, Javier Abadía, en Anunciación Abadía. "Proteomic Profiles of Thylakoid Membranes and Changes in Response to Iron Deficiency". *Photosynthesis Research* 89, nr. 2 (1 september 2006): 141–55. https://doi.org/10.1007/s11120-006-9092-6.

Bañuls, Josefina, Ana Quiñones, Bernardo Martín, Eduardo Primo-Millo, en Francisco Legaz. "Effects of the Frequency of Iron Chelate Supply by Fertigation on Iron Chlorosis in Citrus". *Journal of Plant Nutrition* 26, nr. 10–11 (1 september 2003): 1985–96. https://doi.org/10.1081/PLN-120024258.

Bashir, Khurram, en Naoko K Nishizawa. "Deoxymugineic Acid Synthase". *Plant Signaling & Behavior* 1, nr. 6 (2006): 290–92.

Bauer, Petra, en Rüdiger Hell. "Translocation of Iron in Plant Tissues". In *Iron Nutrition in Plants and Rhizospheric Microorganisms*, onder redactie van Larry L. Barton en Javier Abadia, 279–88. Dordrecht: Springer Netherlands, 2006. https://doi.org/10.1007/1-4020-4743-6_13.

Benoist, B. de, McLean, E., Egll, I., en Cogswell, M. "Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia.", 2008. https://www.cabdirect.org/cabdirect/abstract/20093013528.

Bin, Levi M., Liping Weng, en Marcel H. J. Bugter. "Effectiveness of FeEDDHA, FeEDDHMA, and FeHBED in Preventing Iron-Deficiency Chlorosis in Soybean". *Journal of Agricultural and Food Chemistry* 64, nr. 44 (9 november 2016): 8273–81. https://doi.org/10.1021/acs.jafc.6b01382.

Briat, Jean-François, Christian Dubos, en Frédéric Gaymard. "Iron nutrition, biomass production, and plant product quality". *Trends in Plant Science* 20, nr. 1 (1 januari 2015): 33–40. https://doi.org/10.1016/j.tplants.2014.07.005.

Caliskan, S., I. Ozkaya, M. E. Caliskan, en M. Arslan. "The effects of nitrogen and iron fertilization on growth, yield and fertilizer use efficiency of soybean in a Mediterranean-type soil". *Field Crops Research* 108, nr. 2 (23 augustus 2008): 126–32. https://doi.org/10.1016/j.fcr.2008.04.005.

Connolly, Erin L., Nathan H. Campbell, Natasha Grotz, Charis L. Prichard, en Mary Lou Guerinot. "Overexpression of the FRO2 Ferric Chelate Reductase Confers Tolerance to Growth on Low Iron and Uncovers Posttranscriptional Control". *Plant Physiology* 133, nr. 3 (november 2003): 1102–10. https://doi.org/10.1104/pp.103.025122.

Duursma, Remko A. "Plantecophys - An R Package for Analysing and Modelling Leaf Gas Exchange Data". *PLOS ONE* 10, nr. 11 (18 november 2015): e0143346. https://doi.org/10.1371/journal.pone.0143346.

Evans, John R., en Louis S. Santiago. "PrometheusWiki Gold Leaf Protocol: Gas Exchange Using LI-COR 6400". *Functional Plant Biology* 41, nr. 3 (4 maart 2014): 223–26. https://doi.org/10.1071/FP10900.

Evans, John R., en Louis S. Santiago. "PrometheusWiki Gold Leaf Protocol: Gas Exchange Using LI-COR 6400". *Functional Plant Biology* 41, nr. 3 (4 maart 2014): 223–26. https://doi.org/10.1071/FP10900.

Fageria, N. K., V. C. Baligar, en R. B. Clark. "Micronutrients in Crop Production". In *Advances in Agronomy*, onder redactie van Donald L. Sparks, 77:185–268. Advances in Agronomy. Academic Press, 2002. https://doi.org/10.1016/S0065-2113(02)77015-6.

FAO. "FAO Cereal Supply and Demand Brief | World Food Situation | Food and Agriculture Organization of the United Nations", 2018. http://www.fao.org/worldfoodsituation/csdb/en/.

Gilbert, Natasha. "Costly Fertiliser Holds Back a Green Revolution in Africa | Natasha Gilbert". *The Guardian*, 5 december 2014, sec. Global development. https://www.theguardian.com/global-development/2014/dec/05/costly-fertiliser-holds-back-a-green-revolution-in-africa.

Glozer, Kitren. "Protocol for Leaf Image Analysis – Surface Area (Although Other Measures Could Also Be Made)", 2008, 8. https://ucanr.edu/sites/fruittree/files/49325.pdf

Godfray H. Charles J., en Garnett Tara. “Food security and sustainable intensification”. *Philosophical Transactions of the Royal Society B: Biological Sciences* 369, nr. 1639 (5 april 2014): 20120273. https://doi.org/10.1098/rstb.2012.0273.

Godfray, H. Charles J., John R. Beddington, Ian R. Crute, Lawrence Haddad, David Lawrence, James F. Muir, Jules Pretty, Sherman Robinson, Sandy M. Thomas, en Camilla Toulmin. “Food Security: The Challenge of Feeding 9 Billion People”. *Science* 327, nr. 5967 (12 februari 2010): 812–18. https://doi.org/10.1126/science.1185383.

Gonzalo, M. J., Lucena, J. J., en Hernández-Apaolaza, L. “Effect of silicon addition on soybean (Glycine max) and cucumber (Cucumis sativus) plants grown under iron deficiency - ScienceDirect”, 2013. https://www.sciencedirect.com/science/article/abs/pii/S0981942813002386.

Grassi, G., P. Meir, R. Cromer, D. Tompkins, en P. G. Jarvis. “Photosynthetic Parameters in Seedlings of Eucalyptus Grandis as Affected by Rate of Nitrogen Supply”. *Plant, Cell & Environment* 25, nr. 12 (2002): 1677–88. https://doi.org/10.1046/j.1365-3040.2002.00946.x.

Grebmer, Klaus, von, Jill Bernstein, Nabarro David, Nilam Prasai, Amin Shazia, Yohannes Yisehac, Sonntag Andrea, Patterson Fraser, Towey Olive, en Thompson Jennifer. *2016 Global Hunger Index: Getting to Zero Hunger*. Intl Food Policy Res Inst, 2016.

Hell, Rüdiger, en Udo W. Stephan. “Iron Uptake, Trafficking and Homeostasis in Plants”. *Planta* 216, nr. 4 (1 februari 2003): 541–51. https://doi.org/10.1007/s00425-002-0920-4.

Hernández-Apaolaza, Lourdes, en Juan J. Lucena. “Influence of the Soil/Solution Ratio, Interaction Time, and Extractant on the Evaluation of Iron Chelate Sorption/Desorption by Soils”. *Journal of Agricultural and Food Chemistry* 59, nr. 6 (23 maart 2011): 2493–2500. https://doi.org/10.1021/jf104120e.

Kromdijk Johannes, en Long Stephen P. “One crop breeding cycle from starvation? How engineering crop photosynthesis for rising CO2 and temperature could be one important route to alleviation”. *Proceedings of the Royal Society B: Biological Sciences* 283, nr. 1826 (16 maart 2016): 20152578. https://doi.org/10.1098/rspb.2015.2578.

Kumar, Ajay, Priyanka Mishra, K. Kamala Suganya Kumari, en Kishore C. S. Panigrahi. “Environmental stress influencing plant development and flowering.” *Frontiers in bioscience* 4 (2012): 1315–24. https://doi.org/10.2741/s333.

Laganowsky, Arthur, Stephen M. Gómez, Julian P. Whitelegge, en John N. Nishio. “Hydroponics on a chip: Analysis of the Fe deficient Arabidopsis thylakoid membrane proteome”. *Journal of Proteomics*, Plant Proteomics, 72, nr. 3 (13 april 2009): 397–415. https://doi.org/10.1016/j.jprot.2009.01.024.

LI-COR. “LI-6400/XT | Instruction Manuals”, 2018. https://www.licor.com/env/support/LI-6400/manuals.html.

López-Rayo, Sandra, Diana Hernández, en Juan J. Lucena. “Chemical Evaluation of HBED/Fe(3+) and the Novel HJB/Fe(3+) Chelates as Fertilizers to Alleviate Iron Chlorosis”. *Journal of Agricultural and Food Chemistry* 57, nr. 18 (23 september 2009): 8504–13. https://doi.org/10.1021/jf9019147.

Lu, Yoko, Keijiro Otsuka, en Dick Sserunkuuma. “An Inquiry into Constraints on a Green Revolution in Sub-Saharan Africa: The Case of NERICA Rice in Uganda”. *World Development* 39, nr. 1 (1 januari 2011): 77–86. https://doi.org/10.1016/j.worlddev.2010.06.010.

Lucena, Juan J. “Synthetic Iron Chelates to Correct Iron Deficiency in Plants”. In *Iron Nutrition in Plants and Rhizospheric Microorganisms*, onder redactie van Larry L. Barton en Javier Abadia, 103–28. Dordrecht: Springer Netherlands, 2006. https://doi.org/10.1007/1-4020-4743-6_5.

Ma, B. L., M. J. Morrison, en H. D. Voldeng. “Leaf Greenness and Photosynthetic Rates in Soybean”. *Crop Science* 35, nr. 5 (10/01 1995): 1411–14. https://doi.org/10.2135/cropsci1995.0011183X003500050025x.

Malakouti, Mohammad Jafar. “The Effect of Micronutrients in Ensuring Efficient Use of Macronutrients”. *TURKISH JOURNAL OF AGRICULTURE AND FORESTRY* 32, nr. 3 (28 april 2008): 215–20.

Marschner, H., V. Römheld, en M. Kissel. “Different strategies in higher plants in mobilization and uptake of iron”. *Journal of Plant Nutrition* 9, nr. 3–7 (1 maart 1986): 695–713. https://doi.org/10.1080/01904168609363475.

Mengel, Konrad, en Ernest A. Kirkby. *Principles of Plant Nutrition*. 5de dr. Springer Netherlands, 2001. //www.springer.com/la/book/9780792371502.

Morrissey, Joe, en Mary Lou Guerinot. "Iron uptake and transport in plants: The good, the bad, and the ionome". *Chemical reviews* 109, nr. 10 (oktober 2009): 4553–67. https://doi.org/10.1021/cr900112r.

Mortvedt, J. J. "Correcting Iron Deficiencies in Annual and Perennial Plants: Present Technologies and Future Prospects". *Plant and Soil* 130, nr. 1 (1 januari 1991): 273–79. https://doi.org/10.1007/BF00011883.

Murata, Yoshiko, Jian Feng Ma, Naoki Yamaji, Daisei Ueno, Kyosuke Nomoto, en Takashi Iwashita. "A Specific Transporter for Iron(III)–Phytosiderophore in Barley Roots". *The Plant Journal* 46, nr. 4 (2006): 563–72. https://doi.org/10.1111/j.1365-313X.2006.02714.x.

Nadal, P., García-Delgado, C., Hernández, D., López-Rayo, S., en Lucena, J. J. "Evaluation of Fe-N,N'-Bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetate (HBED/Fe3+) as Fe carrier for soybean (Glycine max) plants grown in calcareous soil | SpringerLink", 2012. https://link.springer.com/article/10.1007/s11104-012-1246-z.

Nadal, Paloma, Sandra López-Rayo, Javier Loren, en Juan J. Lucena. "Efficacy of HBED/Fe3+ at supplying iron to Prunus persica in calcareous soils". *European Journal of Agronomy* 45 (1 februari 2013): 105–13. https://doi.org/10.1016/j.eja.2012.11.003.

Nestel, Penelope, Howarth E. Bouis, J. V. Meenakshi, en Wolfgang Pfeiffer. "Biofortification of Staple Food Crops". *The Journal of Nutrition* 136, nr. 4 (1 april 2006): 1064–67. https://doi.org/10.1093/jn/136.4.1064.

Netto, Alena Torres, Eliemar Campostrini, Jurandi Gonçalves de Oliveira, en Ricardo Enrique Bressan-Smith. "Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves". *Scientia Horticulturae* 104, nr. 2 (30 maart 2005): 199–209. https://doi.org/10.1016/j.scienta.2004.08.013.

Quevauviller, Ph., M. Lachica, E. Barahona, G. Rauret, A. Ure, A. Gomez, en H. Muntau. "Interlaboratory comparison of EDTA and DTPA procedures prior to certification of extractable trace elements in calcareous soil". *Science of The Total Environment*, Harmonization of Leaching/Extraction Tests for Environmental Risk Assessment, 178, nr. 1 (19 januari 1996): 127–32. https://doi.org/10.1016/0048-9697(95)04804-9.

Rashid, A., en J. Ryan. "Micronutrient Constraints to Crop Production in Soils with Mediterranean-Type Characteristics: A Review". *Journal of Plant Nutrition* 27, nr. 6 (27 december 2004): 959–75. https://doi.org/10.1081/PLN-120037530.

Reece, J.B., Urry, L.A., Cain, M.L., Wasserman, S.A., Minorsky, P.V., en Jackson, R.B. "Campbell Biology, 9th Edition", 2011. https://www.pearson.com/us/higher-education/product/Reece-Campbell-Biology-9th-Edition/9780321558237.html.

Rochaix, Jean-David. "Regulation of photosynthetic electron transport". *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 1807, nr. 3 (1 maart 2011): 375–83. https://doi.org/10.1016/j.bbabio.2010.11.010.

Römheld, V. "The Role of Phytosiderophores in Acquisition of Iron and Other Micronutrients in Graminaceous Species: An Ecological Approach". In *Iron Nutrition and Interactions in Plants: "Proceedings of the Fifth International Symposium on Iron Nutrition and Interactions in Plants", 11–17 June 1989, Jerusalem, Israel, 1989*, onder redactie van Y. Chen en Y. Hadar, 159–66. Developments in Plant and Soil Sciences. Dordrecht: Springer Netherlands, 1991. https://doi.org/10.1007/978-94-011-3294-7_21.

Schenkeveld W. D. C. "Iron Fertilization with FeEDDHA : The Fate and Effectiveness of FeEDDHA Chelates in Soil-Plant Systems". S.n., 2010. https://library.wur.nl/WebQuery/wurpubs/397666.

Schenkeveld, W.D.C., Schindlegger, Y., Oburger, E., Puschenreiter, M, Hann, S., en Kraemer, S. M. "Geochemical Processes Constraining Iron Uptake in Strategy II Fe Acquisition - Environmental Science & Technology (ACS Publications)", 2014. https://pubs.acs.org/doi/abs/10.1021/es5031728.

Siedlecka, Anna, en Tadeusz BaszyńAski. "Inhibition of Electron Flow around Photosystem I in Chloroplasts of Cd-Treated Maize Plants Is Due to Cd-Induced Iron Deficiency". *Physiologia Plantarum* 87, nr. 2 (1993): 199–202. https://doi.org/10.1111/j.1399-3054.1993.tb00142.x.

Spiller, Susan, en Norman Terry. “Limiting Factors in Photosynthesis: II. IRON STRESS DIMINISHES PHOTOCHEMICAL CAPACITY BY REDUCING THE NUMBER OF PHOTOSYNTHETIC UNITS”. *Plant Physiology* 65, nr. 1 (1 januari 1980): 121–25. https://doi.org/10.1104/pp.65.1.121.

Terry, Norman. “Limiting Factors in Photosynthesis: I. USE OF IRON STRESS TO CONTROL PHOTOCHEMICAL CAPACITY IN VIVO”. *Plant Physiology* 65, nr. 1 (1 januari 1980): 114–20. https://doi.org/10.1104/pp.65.1.114.

Timperio, Anna Maria, Gian Maria D’Amici, Csengele Barta, Francesco Loreto, en Lello Zolla. “Proteomics, Pigment Composition, and Organization of Thylakoid Membranes in Iron-Deficient Spinach Leaves”. *Journal of Experimental Botany* 58, nr. 13 (1 oktober 2007): 3695–3710. https://doi.org/10.1093/jxb/erm219.

Verbon, Eline H., Pauline L. Trapet, Ioannis A. Stringlis, Sophie Kruijs, Peter A. H. M. Bakker, en Corné M. J. Pieterse. “Iron and Immunity”. *Annual Review of Phytopathology* 55 (04 2017): 355–75. https://doi.org/10.1146/annurev-phyto-080516-035537.

Walker, Anthony P., Andrew P. Beckerman, Lianhong Gu, Jens Kattge, Lucas A. Cernusak, Tomas F. Domingues, Joanna C. Scales, Georg Wohlfahrt, Stan D. Wullschleger, en F. Ian Woodward. “The Relationship of Leaf Photosynthetic Traits – Vcmax and Jmax – to Leaf Nitrogen, Leaf Phosphorus, and Specific Leaf Area: A Meta-Analysis and Modeling Study”. *Ecology and Evolution* 4, nr. 16 (2014): 3218–35. https://doi.org/10.1002/ece3.1173.

Wang, Xian, en Qing-Sheng Cai. “Steel Slag as an Iron Fertilizer for Corn Growth and Soil Improvement in a Pot Experiment11Project supported by the National Natural Science Foundation of China (No. 30270800).” *Pedosphere* 16, nr. 4 (1 augustus 2006): 519–24. https://doi.org/10.1016/S1002-0160(06)60083-0.

Welch, R. M., Shuman, L. “Micronutrient Nutrition of Plants: Critical Reviews in Plant Sciences: Vol 14, No 1”, 1995. https://www.tandfonline.com/doi/abs/10.1080/07352689509701922.

Winder, T. L., en J. N. Nishio. “Early Iron Deficiency Stress Response in Leaves of Sugar Beet”. *Plant Physiology* 108, nr. 4 (1 augustus 1995): 1487–94. https://doi.org/10.1104/pp.108.4.1487.

Zhang, J., D. L. Smith, W. Liu, X. Chen, en W. Yang. “Effects of Shade and Drought Stress on Soybean Hormones and Yield of Main-Stem and Branch”. *African Journal of Biotechnology* 10, nr. 65 (1 januari 2011): 14392-14398–14398. https://doi.org/10.5897/AJB11.2143.

Zhou, Kunneng, Yulong Ren, Jia Lv, Yihua Wang, Feng Liu, Feng Zhou, Shaolu Zhao, e.a. “Young Leaf Chlorosis 1, a Chloroplast-Localized Gene Required for Chlorophyll and Lutein Accumulation during Early Leaf Development in Rice”. *Planta* 237, nr. 1 (1 januari 2013): 279–92. https://doi.org/10.1007/s00425-012-1756-1.

Zhu, Xin-Guang, Rafael Alba, en Eric de Sturler. “A Simple Model of the Calvin Cycle Has Only One Physiologically Feasible Steady State under the Same External Conditions”. *Nonlinear Analysis: Real World Applications* 10, nr. 3 (juni 2009): 1490–99. https://doi.org/10.1016/j.nonrwa.2008.01.021.

Appendix 1 Watering weight

Sample	Light	FeHBED (Yes/No)	Start weight grams	Week 1			Week 2:		Week 3:				Week 4:				Week 5:				Week 6:			Week 7:	
			28-2-2019	4-3-2019	6-3-2019	8-3-2019	11-3-2019	13-3-2019	15-3-2019	18-3-2019	19-3-2019	20-3-2019	22-3-2019	23-3-2019	25-3-2019	26-3-2019	27-3-2019	29-3-2019	1-4-2019	2-4-2019	3-4-2019	5-4-2019	8-4-2019	9-4-2019	10-4-2019	12-4-2019	15-4-2019	16-4-2019	17-4-2019
1	High	No	1144	1101	1105	1105	1102	1099	1130	1101	1120	1133	1118	1123	1123	1109	1131	1130	1111	1130	1140	1123	1109	1132	1124	1134	1140	1128	
2	High	No	1154	1110	1121	1110	1119	1115	1133	1109	1033	1142	1128	1135	1125	1130	1130	1130	1107	1126	1131	1119	1103	1117	1117	1126	1101	1131	1127
3	High	No	1143	1101	1107	1108	1102	1107	1108	1095	1122	1129	1126	1119	1113	1133	1119	1115	1107	1128	1136	1123	1105	1132	1121	1125	1111	1132	1124
4	High	No	1149	1110	1118	1100	1108	1112	1123	1102	1125	1134	1124	1133	1122	1133	1126	1128	1107	1128	1136	1123	1105	1132	1121	1125	1111	1132	1124
5	High	No	1159	1118	1121	1117	1110	1126	1130	1113	1138	1138	1133	1126	1135	1141	1133	1132	1108	1139	1141	1128	1112	1134	1132	1136	1119	1147	1142
6	High	No	1160	1108	1122	1119	1111	1123	1134	1111	1136	1143	1138	1133	1129	1144	1133	1129	1112	1133	1135	1130	1107	1130	1128	1131	1121	1140	1128
7	High	No	1150	1112	1115	1118	1105	1118	1115	1100	1129	1143	1127	1127	1126	1136	1130	1129	1112	1133	1135	1130	1107	1130	1128	1131	1121	1140	1128
8	High	No	1147	1118	1118	1111	1116	1111	1119	1111	1111	1139	1122	1124	1113	1135	1129	1124	1113	1125	1130	1126	1110	1127	1122	1126	1111	1137	1128
9	High	No	1146	1116	1118	1112	1110	1115	1126	1113	1122	1140	1114	1135	1124	1133	1130	1124	1113	1125	1130	1126	1110	1127	1122	1126	1111	1137	1128
10	High	No	1156	1115	1121	1121	1126	1115	1127	1112	1136	1148	1138	1127	1126	1127	1143	1127	1114	1132	1132	1132	1110	1134	1124	1131	1120	1139	1127
11	High	No	1152	1119	1123	1109	1116	1112	1134	1111	1130	1142	1129	1122	1128	1140	1132	1127	1114	1132	1132	1132	1110	1134	1124	1131	1120	1139	1127
12	High	No	1156	1114	1123	1113	1118	1125	1115	1108	1138	1141	1139	1128	1128	1143	1134	1128	1119	1133	1140	1130	1115	1135	1129	1133	1117	1145	1133
13	High	No	1146	1102	1115	1106	1096	1119	1120	1104	1131	1132	1123	1124	1121	1129	1126												
14	High	No	1161	1122	1126	1119	1118	1126	1139	1111	1139	1152	1141	1146	1135	1148	1136												
15	High	Yes	1152	1112	1118	1109	1113	1112	1124	1106	1129	1148	1132	1120	1129	1141	1125	1129	1109	1124	1144	1131	1109	1135	1125	1136	1112	1135	1133
16	High	Yes	1153	1110	1119	1115	1110	1106	1124	1103	1131	1145	1121	1123	1123	1124	1145												
17	High	Yes	1149	1107	1120	1107	1107	1125	1126	1106	1123	1129	1121	1131	1122	1140	1128	1103	1107	1129	1141	1123	1099	1129	1124	1126	1113	1137	1125
18	High	Yes	1153	1114	1119	1112	1106	1105	1132	1106	1135	1134	1133	1129	1126	1138	1127	1121	1108	1132	1135	1130	1105	1138	1122	1130	1117	1143	1134
19	High	Yes	1153	1111	1117	1119	1108	1113	1132	1106	1129	1137	1126	1125	1117	1114	1133												
20	High	Yes	1152	1110	1120	1105	1117	1118	1115	1102	1128	1136	1130	1125	1131	1142	1130	1131	1115	1110	1136	1124	1106	1134	1125	1126	1119	1135	1128
21	High	Yes	1157	1105	1124	1113	1118	1115	1124	1097	1135	1144	1136	1131	1131	1141	1136	1130	1120	1138	1142	1126	1108	1138	1130	1130	1124	1140	1127
22	High	Yes	1154	1100	1122	1114	1109	1105	1128	1099	1131	1139	1120	1127	1124	1125	1144												
23	High	Yes	1151	1103	1118	1099	1118	1107	1129	1103	1130	1137	1128	1127	1122	1140	1125	1127	1107	1130	1130	1120	1105	1130	1124	1127	1116	1142	1128
24	High	Yes	1155	1105	1120	1113	1119	1116	1110	1106	1130	1142	1131	1126	1130	1135	1135												
25	High	Yes	1138	1086	1100	1105	1097	1101	1108	1090	1121	1121	1117	1111	1109	1126	1112	1110	1094	1116	1126	1109	1090	1122	1113	1113	1095	1125	1112
26	High	Yes	1143	1094	1107	1104	1094	1103	1119	1098	1115	1135	1119	1117	1113	1130	1123												
27	High	Yes	1156	1111	1114	1119	1117	1127	1125	1115	1131	1148	1133	1133	1131	1129	1127												
28	High	Yes	1139	1093	1108	1097	1101	1100	1120	1080	1121	1133	1119	1106	1112	1128	1116	1112	1090	1126	1118	1112	1094	1121	1103	1116	1098	1123	1114

Sample	Light	FeHBED (Yes/No)	Start weight grams	Week 1			Week 2:		Week 3:				Week 4:				Week 5:				Week 6:			Week 7:	
			28-2-2019	4-3-2019	6-3-2019	8-3-2019	11-3-2019	13-3-2019	15-3-2019	18-3-2019	19-3-2019	20-3-2019	22-3-2019	23-3-2019	25-3-2019	26-3-2019	27-3-2019	29-3-2019	1-4-2019	2-4-2019	3-4-2019	5-4-2019	8-4-2019	9-4-2019	10-4-2019	12-4-2019	15-4-2019	16-4-2019	17-4-2019
29	Low	No	1141	1113	1113	1114	1097	1112	1108	1106	1119	1125	1121	1130	1113	1118	1126												
30	Low	No	1140	1104	1107	1109	1095	1105	1109	1099	1116	1123	1113	1124	1108	1129	1112												
31	Low	No	1145	1110	1099	1123	1102	1110	1117	1108	1124	1120	1120	1131	1112	1136	1121	1130	1103	1135	1132	1123	1110	1127	1126	1126	1116	1133	1124
32	Low	No	1162	1117	1115	1134	1111	1130	1119	1116	1149	1150	1133	1137	1134	1149	1139	1138	1117	1137	1157	1142	1124	1138	1144	1139	1130	1146	1142
33	Low	No	1158	1115	1118	1122	1094	1126	1128	1108	1149	1130	1124	1142	1123	1149	1129	1134	1106	1136	1143	1129	1117	1139	1129	1140	1119	1143	1129
34	Low	No	1157	1107	1124	1124	1099	1125	1129	1111	1137	1147	1125	1141	1130	1147	1129												
35	Low	No	1152	1105	1114	1123	1102	1113	1118	1107	1137	1135	1119	1137	1117	1146	1124	1131	1107	1125	1149	1128	1112	1126	1133	1132	1127	1140	1128
36	Low	No	1159	1114	1121	1128	1099	1131	1129	1109	1135	1145	1141	1142	1129	1156	1137	1144	1115	1138	1142	1137	1120	1134	1140	1134	1128	1145	1134
37	Low	No	1162	1112	1121	1134	1117	1122	1138	1114	1135	1139	1137	1148	1131	1153	1143	1143	1115	1136	1155	1136	1119	1138	1143	1140	1139	1149	1134
38	Low	No	1156	1112	1118	1128	1105	1121	1132	1108	1143	1147	1127	1137	1125	1138	1146	1125	1127	1107	1130	1130	1120	1105	1130	1124	1127	1116	1142	1128
39	Low	No	1151	1107	1105	1117	1105	1115	1113	1106	1137	1127	1125	1141	1113	1146	1125	1127	1113	1132	1139	1131	1108	1126	1134	1132	1110	1136	1129
40	Low	No	1149	1106	1108	1114	1101	1109	1103	1106	1126	1123	1130	1126	1124	1138	1146												
41	Low	No	1154	1105	1120	1120	1100	1115	1129	1104	1134	1139	1130	1141	1117	1146	1128												
42	Low	No	1156	1098	1117	1130	1108	1115	1128	1105	1131	1140	1137	1146	1123	1146	1124	1130	1106	1140	1139	1130	1114	1136	1135	1124	1133	1139	1133
43	Low	Yes	1145	1098	1094	1124	1090	1119	1110	1096	1128	1133	1120	1132	1118	1130	1116	1126	1096	1120	1135	1121	1104	1120	1130	1125	1113	1130	1116
44	Low	Yes	1144	1100	1114	1102	1105	1105	1110	1108	1111	1127	1116	1130	1113	1132	1123												
45	Low	Yes	1141	1101	1098	1118	1086	1116	1096	1104	1123	1125	1114	1123	1113	1131	1116												
46	Low	Yes	1148	1110	1103	1123	1102	1109	1116	1096	1136	1122	1123	1136	1112	1125	1136												
47	Low	Yes	1143	1103	1107	1099	1105	1116	1098	1110	1116	1130	1115	1131	1110	1134	1117	1118	1104	1123	1131	1127	1101	1120	1124	1111	1121	1132	1117
48	Low	Yes	1150	1110	1109	1128	1102	1117	1122	1105	1135	1135	1117	1132	1122	1141	1127	1130	1099	1132	1138	1124	1112	1128	1127	1128	1115	1139	1124
49	Low	Yes	1145	1102	1113	1123	1093	1112	1113	1101	1121	1141	1110	1128	1116	1132	1118	1119	1097	1128	1135	1123	1109	1123	1129	1130	1115	1130	1121
50	Low	Yes	1154	1114	1109	1129	1113	1123	1123	1107	1140	1135	1126	1141	1123	1132	1145												
51	Low	Yes	1155	1112	1103	1130	1093	1122	1119	1111	1129	1133	1130	1129	1123	1140	1130												
52	Low	Yes	1155	1114	1109	1129	1107	1136	1119	1112	1139	1135	1130	1136	1125	1146	1128	1125	1109	1133	1140	1131	1113	1132	1135	1135	1125	1142	1132
53	Low	Yes	1160	1119	1112	1137	1111	1117	1122	1112	1150	1143	1125	1151	1128	1134	1130												
54	Low	Yes	1156	1116	1108	1122	1105	1120	1118	1106	1150	1136	1127	1135	1123	1151	1136	1135	1113	1144	1137	1131	1114	1131	1143	1127	1131	1147	1129
55	Low	Yes	1155	1122	1111	1129	1113	1114	1131	1117	1132	1142	1120	1135	1129	1138	1138	1128	1114	1130	1142	1130	1114	1135	1135	1132	1126	1139	1131
56	Low	Yes	1158	1117	1110	1125	1120	1130	1135	1121	1136	1136	1137	1134	1125	1150	1132	1137	1107	1137	1151	1128	1109	1133	1138	1139	1116	1141	1134

Appendix 2 Plant Dry matter

Drymatter, Measured at 7-5-2019													
Plant sample	FeHBED	Light condition	Harvest	All Leaves (g)	Leaf A/Ci (g)	Stem (g)	Roots (g)	Beans (g)	Above ground (g)	Below ground (g)	Plant weight (g)	Total leaf area (cm^2)	LAR
1	No	High	1	0,3590	0,0379	0,1734	0,2030	0,0000	0,5703	0,2030	0,7733	85,221	110,2043
2	No	High	2	0,7666	0,1049	0,3195	0,4146	0,1564	1,3474	0,4146	1,7620	128,787	73,09137
3	No	High	2	0,5775	0,0863	0,2951	0,3232	0,0228	0,9817	0,3232	1,3049	86,658	66,40969
4	No	High	2	0,6130	0,0960	0,2984	0,3620	0,1436	1,1510	0,3620	1,5130	96,408	63,71976
5	No	High	2	0,9020	0,1102	0,4342	0,3682	0,1085	1,5549	0,3682	1,9231	143,98	74,8687
6	No	High	1	0,3001	0,0557	0,1134	0,2830	0,0000	0,4692	0,2830	0,7522	79,369	105,5158
7	No	High	2	0,4082	0,0000	0,1730	0,2017	0,0557	0,6369	0,2017	0,8386	69,169	82,48152
8	No	High	1	0,2361	0,0364	0,0990	0,1701	0,0000	0,3715	0,1701	0,5416	53,472	98,72969
9	No	High	2	0,3310	0,0000	0,1461	0,1396	0,0216	0,4987	0,1396	0,6383	48,11	75,37208
10	No	High	1	0,3062	0,0263	0,3148	0,2067	0,0000	0,6473	0,2067	0,8540	71,525	83,75293
11	No	High	2	0,7430	0,0952	0,3142	0,3497	0,0316	1,1840	0,3497	1,5337	112,157	73,12838
12	No	High	2	0,4934	0,0740	0,2536	0,3722	0,1329	0,9539	0,3722	1,3261	91,19	68,76555
13	No	High	1	0,2377	0,0484	0,1064	0,1351	0,0000	0,3925	0,1351	0,5276	55,877	105,9079
14	No	High	1	0,4055	0,0677	0,1620	0,2414	0,0000	0,6352	0,2414	0,8766	86,96	99,20146
15	Yes	High	2	0,5152	0,0984	0,2485	0,3217	0,0815	0,9436	0,3217	1,2653	91,364	72,20738
16	Yes	High	1	0,4177	0,0794	0,1772	0,3089	0,0000	0,6743	0,3089	0,9832	97,347	99,01037
17	Yes	High	2	0,8649	0,1162	0,3180	0,5967	0,1187	1,4178	0,5967	2,0145	114,991	57,08166
18	Yes	High	2	1,1892	0,0000	0,2460	0,4558	0,1987	1,6339	0,4558	2,0897	128,552	61,51696
19	Yes	High	1	0,5100	0,0710	0,1805	0,2885	0,0000	0,7615	0,2885	1,0500	102,414	97,53714
20	Yes	High	2	0,7218	0,1243	0,3140	0,4048	0,1834	1,3435	0,4048	1,7483	100,282	57,35972
21	Yes	High	2	0,7936	0,0953	0,3208	0,3899	0,1096	1,3193	0,3899	1,7092	108,042	63,21203
22	Yes	High	1	0,4081	0,0850	0,1807	0,2370	0,0000	0,6738	0,2370	0,9108	89,935	98,74286
23	Yes	High	2	1,0104	0,0000	0,3512	0,4455	0,1401	1,5017	0,4455	1,9472	112,968	58,01561
24	Yes	High	1	0,4254	0,0478	0,1705	0,3153	0,0000	0,6437	0,3153	0,9590	90,241	94,09906
25	Yes	High	2	1,0141	0,1233	0,3435	0,5263	0,1757	1,6566	0,5263	2,1829	130,457	59,76316
26	Yes	High	1	0,3866	0,0506	0,1498	0,2517	0,0000	0,5870	0,2517	0,8387	78,073	93,08811
27	Yes	High	1	0,3817	0,0578	0,1250	0,2160	0,0000	0,5645	0,2160	0,7805	80,482	103,116
28	Yes	High	2	0,7578	0,1037	0,2829	0,4351	0,1915	1,3359	0,4351	1,7710	96,335	54,39582
29	No	Low	1	0,1758	0,0485	0,0876	0,1176	0,0000	0,3119	0,1176	0,4295	50,118	116,6892
30	No	Low	1	0,2074	0,0461	0,1138	0,2003	0,0000	0,3673	0,2003	0,5676	78,957	139,1068
31	No	Low	2	0,4536	0,0000	0,2342	0,2220	0,2022	0,8900	0,2220	1,1120	103,122	92,73561
32	No	Low	2	0,5302	0,0650	0,2253	0,2898	0,2787	1,0992	0,2898	1,3890	115,519	83,16703
33	No	Low	2	0,4634	0,0827	0,2463	0,2280	0,2503	1,0427	0,2280	1,2707	103,779	81,67073
34	No	Low	1	0,2872	0,0331	0,1506	0,1702	0,0000	0,4709	0,1702	0,6411	83,271	129,8877
35	No	Low	2	0,4635	0,0609	0,2485	0,2301	0,1440	0,9169	0,2301	1,1470	106,578	92,91892
36	No	Low	2	0,4125	0,0496	0,2188	0,2676	0,1370	0,8179	0,2676	1,0855	101,355	93,37172
37	No	Low	2	0,4322	0,0525	0,2005	0,2072	0,0860	0,7712	0,2072	0,9784	90,117	92,1065
38	No	Low	1	0,2168	0,0195	0,1292	0,1177	0,0000	0,3655	0,1177	0,4832	75,427	156,0989
39	No	Low	2	0,6584	0,0000	0,3912	0,2639	0,0474	1,0970	0,2639	1,3609	121,713	89,43567
40	No	Low	1	0,3110	0,0467	0,1509	0,2370	0,0000	0,5086	0,2370	0,7456	96,548	129,4903
41	No	Low	1	0,3031	0,0522	0,1623	0,1858	0,0000	0,5176	0,1858	0,7034	84,377	119,9559
42	No	Low	2	0,4962	0,0677	0,2926	0,2318	0,2050	1,0615	0,2318	1,2933	101,706	78,64069
43	Yes	Low	2	0,6411	0,0881	0,3136	0,5138	0,2274	1,2702	0,5138	1,7840		0
44	Yes	Low	1	0,4048	0,0797	0,1777	0,2382	0,0000	0,6622	0,2382	0,9004	95,459	106,0184
45	Yes	Low	1	0,3265	0,0573	0,1527	0,1831	0,0000	0,5365	0,1831	0,7196	83,715	116,3355
46	Yes	Low	1	0,4216	0,0642	0,1789	0,2700	0,0000	0,6647	0,2700	0,9347	98,643	105,5344
47	Yes	Low	2	0,5566	0,0000	0,2627	0,2460	0,0803	0,8996	0,2460	1,1456	78,56	68,57542
48	Yes	Low	2	0,4756	0,0699	0,2347	0,2424	0,2289	1,0091	0,2424	1,2515	93,115	74,40272
49	Yes	Low	2	0,5175	0,0662	0,2495	0,2376	0,2550	1,0882	0,2376	1,3258	102,336	77,18811
50	Yes	Low	1	0,3273	0,0690	0,1605	0,2051	0,0000	0,5568	0,2051	0,7619	83,277	109,3017
51	Yes	Low	1	0,5280	0,0618	0,2715	0,2791	0,0000	0,8613	0,2791	1,1404	114,669	100,5516
52	Yes	Low	2	0,5951	0,0000	0,2722	0,2745	0,2057	1,0730	0,2745	1,3475	102,685	76,20408
53	Yes	Low	1	0,2787	0,0816	0,1420	0,2216	0,0000	0,5023	0,2216	0,7239	74,711	103,2062
54	Yes	Low	2	0,5171	0,0557	0,2755	0,4042	0,1760	1,0243	0,4042	1,4285	86,166	60,31922
55	Yes	Low	2	0,4257	0,0567	0,2675	0,2548	0,1605	0,9104	0,2548	1,1652	91,072	78,15997
56	Yes	Low	2	0,4069	0,0583	0,2109	0,2178	0,2245	0,9006	0,2178	1,1184	97,677	87,33637

Appendix 3 MATLAB code boxplot

```
figure (1)
set(gca,'FontSize',14)
%LLYH First
x1 =[62.75548,99.61147,97.71439,95.20823,80.60157,89.40311];
x1=x1';
%LLNH First
y1 =[72.69912,51.9938,72.37758,74.14142];
y1=y1';
%HLYH First
z1 =[70.55117,80.52642,57.51797,76.27804,99.8237,53.77916];
z1=z1';
%HLNH First
a1 =[42.14384,77.36042,102.75698,71.09545,61.16887];
a1=a1';
group1 = [repmat({'LLYH'}, 6, 1); repmat({'LLNH'}, 4, 1); repmat({'HLYH'},
6, 1); repmat({'HLNH'}, 5, 1)];
M1=[x1;y1;z1;a1];
boxplot([M1], group1)
% legend ({'Low Light Yes HBED','Low Light No HBED','High Light Yes
HBED','High Light No HBED'})
title ('A')
ylabel('Jmax (µmol m^{-2} s ^{-1})')
ylim([40 110])

%autosave
saveas(gcf,([pwd '/Figures/Jmax in Soybean First destruction']))
saveas(gcf,([pwd '/Figures/Jmax in Soybean First destruction.jpg']))
% ------------------------------------------------------------------------
-
figure (2)
set(gca,'FontSize',14)
%LLYH Second
x2 =[95.75834,65.81402,79.5003,75.4966,42.5389,82.20041];
x2=x2';
%LLNH Second
y2 =[104.55982,92.25692,94.08412,85.11062,96.84701,91.38146];
y2=y2';
%HLYH Second
z2 =[51.89676,58.4723,66.98955,82.29418,71.12307,52.91675];
z2=z2';
%HLNH Second
a2 =[56.31963,63.10498,99.90255,94.91501,53.89896,70.57106];
a2=a2';
group2 = [repmat({'LLYH'}, 6, 1); repmat({'LLNH'}, 6, 1); repmat({'HLYH'},
6, 1); repmat({'HLNH'}, 6, 1)];
M2=[x2;y2;z2;a2];
boxplot([M2], group2)
% legend ({'Low Light Yes HBED','Low Light No HBED','High Light Yes
HBED','High Light No HBED'})
title ('B')
ylabel('Jmax (µmol m^{-2} s^{-1})')
ylim([40 110])

%autosave
saveas(gcf,([pwd '/Figures/Jmax in Soybean Second destruction']))
saveas(gcf,([pwd '/Figures/Jmax in Soybean Second destruction.jpg']))
```

Appendix 4 MATLAB code two-way ANOVA

```
%% Jmax of a two way anova

close all
clear all
%LLYH First
x1 =[56.31889,107.47291,66.25932,45.34916,78.08221,69.73973];
%LLNH First
Y1 =[107.40043,40.00555,48.42535,49.20088];
%HLYH First
z1 =[32.97174,117.88629,39.80821,59.32614,125.51325,39.12022];
%HLNH First
a1 =[24.2086,109.57421,79.19384,56.79238,58.56822];

%LLYH Second
x2 =[65.87312,33.78979,61.17875,51.30593,23.59371,51.18955];
%LLNH Second
Y2 =[69.21882,46.24068,38.59342,54.86337,53.51151,59.72385];
%HLYH Second
z2 =[28.46271,46.0808,42.47129,54.47173,45.84762,25.89337];
%HLNH Second
a2 =[23.52208,100.16525,53.61414,56.3622,34.17005,30.62362];

%% First fit the data in a matrix (dat1a)
Data1 = NaN(100,4);
l = length(x1);
Data1(1:l,1) = x1;
l = length(Y1);
Data1(1:l,2) = Y1;
l = length(z1);
Data1(1:l,3) = z1;
l = length(a1);
Data1(1:l,4) = a1;

%%Second fit for the data in a matrix (data2)
Data2 = NaN(100,4);
l = length(x2);
Data2(1:l,1) = x2;
l = length(Y2);
Data2(1:l,2) = Y2;
l = length(z2);
Data2(1:l,3) = z2;
l = length(a2);
Data2(1:l,4) = a2;
%% Plot the data in a boxplot first fase
figure(1)
labels = {'LLYH','LLNH','HLYH','HLYH'};
boxplot(Data1)
title('A');
xticklabels(labels)

%% Plot the data in a boxplot secpnd fase
figure(2)
labels = {'LLYH','LLNH','HLYH','HLYH'};
boxplot(Data2)
title ('B');
xticklabels(labels)
%% Use anovan function for unbalanced design (for anova2 you need equal
sample sizes in all groups)
% First make a matrix with indentifiers for each data point
```

```
%First fase Anovan test
% One identifier for treatment 1
Treatment1 = NaN(100,4);
l = length(x1);
Treatment1(1:l,1) = 1;
l = length(Y1);
Treatment1(1:l,2) = 1;
l = length(z1);
Treatment1(1:l,3) = 2;
l = length(a1);
Treatment1(1:l,4) = 2;

% One identifier for treatment 2
Treatment2 = NaN(100,4);
l = length(x1);
Treatment2(1:l,1) = 1;
l = length(Y1);
Treatment2(1:l,2) = 2;
l = length(z1);
Treatment2(1:l,3) = 1;
l = length(a1);
Treatment2(1:l,4) = 2;

y1 = reshape(Data1,[],1);
g1 = reshape(Treatment1,[],1);
g2 = reshape(Treatment2,[],1);

% Second fase Anovan Test
 % One identifier for treatment 1
Treatment3 = NaN(100,4);
l = length(x2);
Treatment3(1:l,1) = 1;
l = length(Y2);
Treatment3(1:l,2) = 1;
l = length(z2);
Treatment3(1:l,3) = 2;
l = length(a2);
Treatment3(1:l,4) = 2;

% One identifier for treatment 2
Treatment4 = NaN(100,4);
l = length(x2);
Treatment4(1:l,1) = 1;
l = length(Y2);
Treatment4(1:l,2) = 2;
l = length(z2);
Treatment4(1:l,3) = 1;
l = length(a2);
Treatment4(1:l,4) = 2;

y2 = reshape(Data2,[],1);
g3 = reshape(Treatment3,[],1);
g4 = reshape(Treatment4,[],1);
 %% Now do the two-way anova first fase
p1 = anovan(y1,{g1,g2},'model','interaction','varnames',{'Treatment
1','Treatment 2'})
%% Now do the two-way anova second fasep2 =
anovan(y2,{g3,g4},'model','interaction','varnames',{'Treatment
3','Treatment 4'})
```

Appendix 5 Plant parameter data table

Plant sample	FeHBED	Light condition	Harvest	Above ground (g)	Below ground (g)	Plant weight (g)	Total leaf area (cm^2)	LAR (g/cm^2)	V_{cmax} ($\mu mol\ m^{-2}\ s^{-1}$)	J_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	Jmax:Vcmax ratio	cit
1	No	High	1	0,5703	0,2030	0,7733	85,221	110,2043	24,2086	42,14384	0,574427959	165,7667
2	No	High	2	1,3474	0,4146	1,7620	128,787	73,09137	23,52208	56,31963	0,417653312	166,6299
3	No	High	2	0,9817	0,3232	1,3049	86,658	66,40969	100,16525	63,10498	1,587279641	341,7372
4	No	High	2	1,1510	0,3620	1,5130	96,408	63,71976	53,61414	99,90255	0,536664379	428,0596
5	No	High	2	1,5549	0,3682	1,9231	143,98	74,8687	56,3622	94,91501	0,593817564	295,579
6	No	High	1	0,4692	0,2830	0,7522	79,369	105,5158	109,57421	77,36042	1,416411777	38,16489
7	No	High	2	0,6369	0,2017	0,8386	69,169	82,48152				
8	No	High	1	0,3715	0,1701	0,5416	53,472	98,72969	79,19384	102,75698	0,770690614	217,2035
9	No	High	2	0,4987	0,1396	0,6383	48,11	75,37208				
10	No	High	1	0,6473	0,2067	0,8540	71,525	83,75293				
11	No	High	2	1,1840	0,3497	1,5337	112,157	73,12838	34,17005	53,89896	0,633964923	307,1106
12	No	High	2	0,9539	0,3722	1,3261	91,19	68,76555	30,62362	70,57106	0,433940202	251,7824
13	No	High	1	0,3925	0,1351	0,5276	55,877	105,9079	56,79238	71,09545	0,798818771	108,8462
14	No	High	1	0,6352	0,2414	0,8766	86,96	99,20146	58,56822	61,16887	0,957484093	122,6139
15	Yes	High	2	0,9436	0,3217	1,2653	91,364	72,20738	28,46271	51,89676	0,548448689	440,8219
16	Yes	High	1	0,6743	0,3089	0,9832	97,347	99,01037	32,97174	70,55117	0,467345049	211,5894
17	Yes	High	2	1,4178	0,5967	2,0145	114,991	57,08166	46,0808	58,4723	0,788079142	212,9176
18	Yes	High	2	1,6339	0,4558	2,0897	128,552	61,51696				
19	Yes	High	1	0,7615	0,2885	1,0500	102,414	97,53714	117,88629	80,52642	1,463945498	232,4811
20	Yes	High	2	1,3435	0,4048	1,7483	100,282	57,35972	42,47129	66,98955	0,633998736	330,8755
21	Yes	High	2	1,3193	0,3899	1,7092	108,042	63,21203	54,47173	82,29418	0,661914731	277,7987
22	Yes	High	1	0,6738	0,2370	0,9108	89,935	98,74286	39,80821	57,51797	0,692100399	168,055
23	Yes	High	2	1,5017	0,4455	1,9472	112,968	58,01561				
24	Yes	High	1	0,6437	0,3153	0,9590	90,241	94,09906	59,32614	76,27804	0,777761725	177,946
25	Yes	High	2	1,6566	0,5263	2,1829	130,457	59,76316	45,84762	71,12307	0,644623749	306,4138
26	Yes	High	1	0,5870	0,2517	0,8387	78,073	93,08811	125,51325	99,8237	1,257349207	123,0272
27	Yes	High	1	0,5645	0,2160	0,7805	80,482	103,116	39,12022	53,77916	0,727423411	155,135
28	Yes	High	2	1,3359	0,4351	1,7710	96,335	54,39582	25,89337	52,91675	0,489322757	319,0034
29	No	Low	1	0,3119	0,1176	0,4295	50,118	116,6892				
30	No	Low	1	0,3673	0,2003	0,5676	78,957	139,1068	107,40043	72,69912	1,477327786	136,3229
31	No	Low	2	0,8900	0,2220	1,1120	103,122	92,73561				
32	No	Low	2	1,0992	0,2898	1,3890	115,519	83,16703	69,21882	104,55982	0,662002096	291,5722
33	No	Low	2	1,0427	0,2280	1,2707	103,779	81,67073	46,24068	92,25692	0,501216386	390,8941
34	No	Low	1	0,4709	0,1702	0,6411	83,271	129,8877	40,00555	51,9938	0,76942924	230,3954
35	No	Low	2	0,9169	0,2301	1,1470	106,578	92,91892	38,59342	94,08412	0,410201211	439,537
36	No	Low	2	0,8179	0,2676	1,0855	101,355	93,37172	54,86337	85,11062	0,644612505	351,0104
37	No	Low	2	0,7712	0,2072	0,9784	90,117	92,1065	53,51151	96,84701	0,552536521	396,9596
38	No	Low	1	0,3655	0,1177	0,4832	75,427	156,0989				
39	No	Low	2	1,0970	0,2639	1,3609	121,713	89,43567				
40	No	Low	1	0,5086	0,2370	0,7456	96,548	129,4903	48,42535	72,37758	0,669065614	297,3451
41	No	Low	1	0,5176	0,1858	0,7034	84,377	119,9559	49,20088	74,14142	0,663608547	274,8073
42	No	Low	2	1,0615	0,2318	1,2933	101,706	78,64069	59,72385	91,38146	0,65356638	199,3845
43	Yes	Low	2	1,2702	0,5138	1,7840		0	65,87312	95,75834	0,687910003	213,4628
44	Yes	Low	1	0,6622	0,2382	0,9004	95,459	106,0184	56,31889	62,75548	0,897433818	183,8728
45	Yes	Low	1	0,5365	0,1831	0,7196	83,715	116,3355	107,47291	99,61147	1,078921032	154,3546
46	Yes	Low	1	0,6647	0,2700	0,9347	98,643	105,5344	66,25932	97,71439	0,678091732	218,5899
47	Yes	Low	2	0,8996	0,2460	1,1456	78,56	68,57542	33,78979	65,81402	0,513413251	255,7375
48	Yes	Low	2	1,0091	0,2424	1,2515	93,115	74,40272	61,17875	79,5003	0,769541121	133,6723
49	Yes	Low	2	1,0882	0,2376	1,3258	102,336	77,18811				
50	Yes	Low	1	0,5568	0,2051	0,7619	83,277	109,3017	45,34916	95,20823	0,476315545	472,1291
51	Yes	Low	1	0,8613	0,2791	1,1404	114,669	100,5516	78,08221	80,60157	0,968743041	163,1172
52	Yes	Low	2	1,0730	0,2745	1,3475	102,685	76,20408				
53	Yes	Low	1	0,5023	0,2216	0,7239	74,711	103,2062	69,73973	89,40311	0,780059329	199,9162
54	Yes	Low	2	1,0243	0,4042	1,4285	86,166	60,31922	51,30593	75,4966	0,679579345	212,2463
55	Yes	Low	2	0,9104	0,2548	1,1652	91,072	78,15997	23,59371	42,5389	0,55463846	443,1027
56	Yes	Low	2	0,9006	0,2178	1,1184	97,677	87,33637	51,18955	82,20041	0,622740811	374,1618

Appendix 6 Raw DTPA data

Disclaimer

Waarden onder de detectielimiet worden weergegeven in het grijs. Deze waarden zijn zeer onbetrouwbaar en kunnen niet meegenomen worden in berekeningen.
Values less then the detection limits are given in gray. This values are unreliable and can't be used for calculations.

Waarden boven 2x de line-range worden weergegeven het rood. Deze waarden kunnen een meetfout hebben van > 10% en moeten als indicatievewaarden beschouwd worden.
Values above 2 times the line range are given in red. This value can have an error of analysis > 10% and should be seen as an aproximate valuee.

Waarden weergegeven in deze kleur liggen onder de BEC(*). Deze waarden kunnen een meetfout hebben van > 10% en moeten als indicatieve waarden beschouwd worden.
Values below the BEC(*) are given in blue. This value can have an error of analysis > 10% and should be seen as an aproximate value

(*) BEC = Background Equivalent Concentration
The BEC value is the concentration of an element which would produce the same emission intensity as the plasma background measured at the analyte wavelength. The BEC checks torch alignment, plasma viewing height (only meaningful in radial view), nebulizer gas flow rate, and incident RF power.

Date of analysis	31-Jan
Task	water2012
unit	mg/kg

Data in measured solution		Al 394.401	As 189.042	B 249.677	Ba 455.404	Be 313.107	Ca 315.887	Cd 228.802	Cl 134.724	Co 230.786	Cr 205.618	Cu 324.754	Fe 239.562	K 766.491	Li 670.780	Mg 280.270
BEC		1.01	0.118	0.0642	0.0125	0.00546	0.245	0.0213	19.5	0.0581	0.0208	0.0575	0.0429	2.09	0.0718	0.00254
Line range		29.3	5.85	5.85	5.85	5.85	176	5.85	309	5.85	5.85	5.85	12.5	58.5	5.85	5
detection limit		0.0117	0.0043	0.000545	0.00019	0.0000651	0.00465	0.000227	2.75	0.000736	0.000403	0.000778	0.000522	0.131	0.00064	0.000265
practical detection limit		0.0351	0.0129	0.001635	0.00057	0.0001953	0.01395	0.000681	8.25	0.002208	0.001209	0.002334	0.001566	0.393	0.00192	0.000795
Recovery QC's		**98**	**99**	**99**	**102**	**101**	**97**	**101**	**104**	**99**	**100**	**100**	**100**	**97**	**100**	**100**
	Ter info:	Al 394.401	As 189.042	B 249.677	Ba 455.404	Be 313.107	Ca 315.887	Cd 228.802	Cl 134.724	Co 230.786	Cr 205.618	Cu 324.754	Fe 239.562	K 766.491	Li 670.780	Mg 280.270
Sample Name	Toegepaste **verdunr**	**Al 394.40**	**As 189.0**	**B 249.67**	**Ba 455.4**	**Be 313.1**	**Ca 315.8**	**Cd 228.8**	**Cl 134.72**	**Co 230.7**	**Cr 205.61**	**Cu 324.7**	**Fe 239.5**	**K 766.49**	**Li 670.78**	**Mg 280.2**
Walter 2.1	1	-0.183	0.008	0.118	0.002	0.000	401.345	0.000	719.705	-0.001	0.004	0.004	0.019	16.894	0.309	0.037
Walter 2.2	1	-0.189	0.009	0.115	0.002	0.000	395.506	0.000	713.864	-0.001	0.003	0.004	0.016	16.736	0.307	0.051
Walter 2.3	1	0.100	0.008	0.115	0.127	0.000	402.344	0.000	708.292	-0.001	0.002	0.039	0.041	17.189	0.305	1.032
Walter 2.4	1	0.101	0.006	0.115	0.123	0.000	400.281	0.000	703.876	-0.001	0.002	0.034	0.051	16.999	0.299	1.056
Walter 2.5	1	-0.169	0.008	0.158	0.512	0.000	568.495	0.001	715.955	0.004	0.002	0.159	0.496	14.971	0.275	38.017
Walter 2.6	1	-0.186	0.006	0.161	0.499	0.000	587.739	0.001	722.737	0.004	0.002	0.177	0.507	14.830	0.269	41.004
Walter 2.7	1	-0.212	0.007	0.169	0.483	0.000	660.368	0.002	718.505	0.005	0.001	0.268	0.607	14.323	0.255	61.831
Walter 2.8	1	-0.214	0.008	0.171	0.502	0.000	659.853	0.001	735.857	0.005	0.001	0.274	0.608	14.364	0.256	61.660
spoel	1	0.013	0.000	0.001	0.004	0.000	1.767	0.000	5.397	-0.001	0.000	0.000	0.002	0.043	-0.001	0.003

Data in measured solution		Mn 257.611	Mo 202.095	Na 589.592	Ni 231.604	P 177.495	Pb 168.215	S 182.034	Sb 187.115	Sb 217.581	Sc 424.683	Si 251.612	Sr 407.771	Ti 334.187	V 292.402	Y 324.228	Zn 202.613	Zr 343.823
BEC		0.00738	0.0343	0.502	0.0722	0.084	0.166	0.4	0.461	0.28	0.00414	0.106	0.00405	0.0804	0.0541	0.0228	0.0116	0.0773
Line range		5.85	5.85	58.5	5.85	29.5	5.85	59.5	5.85	5.85	0.919	59.1	5.85	5.85	5.85	5.86	5.85	5.87
detection limit		0.0000801	0.0016	0.0452	0.00108	0.00554	0.00742	0.146	0.0123	0.00693	0.0000118	0.00457	0.0000469	0.00287	0.0000892	0.000179	0.000427	0.00127
practical detection limit		0.0002403	0.0048	0.1356	0.00324	0.01662	0.02226	0.438	0.0369	0.02079	0.0000354	0.01371	0.0001407	0.00861	0.0002676	0.000537	0.001281	0.00381
Recovery QC's		**100**	**98**	**100**	**100**	**100**	**95**	**96**	**100**	**101**	**100**	**105**	**98**	**100**	**99**	**100**	**101**	**101**
	Ter info:	Mn 257.611	Mo 202.095	Na 589.592	Ni 231.604	P 177.495	Pb 168.215	S 182.034	Sb 187.115	Sb 217.581	Sc 424.683	Si 251.612	Sr 407.771	Ti 334.187	V 292.402	Y 324.228	Zn 202.613	Zr 343.823
Sample Name	Toegepaste **verdunr**	**Mn 257.6**	**Mo 202.0**	**Na 589.5**	Ni 231.604	P 177.495	Pb 168.215	S 182.034	Sb 187.115	Sb 217.581	Sc 424.683	Si 251.612	Sr 407.771	Ti 334.187	V 292.402	Y 324.228	Zn 202.613	Zr 343.823
Walter 2.1	1	0.001	0.000	9.865	0.004	0.017	0.022	0.204	-0.014	0.001	0.000	0.048	7.140	0.000	0.000	0.000	0.005	0.000
Walter 2.2	1	0.000	-0.001	11.411	0.002	0.015	0.028	0.204	-0.016	0.001	0.000	0.036	7.037	0.000	0.000	0.000	0.007	0.000
Walter 2.3	1	0.005	0.000	10.406	0.001	0.041	0.064	1.143	-0.020	0.000	0.000	0.257	7.039	0.003	0.001	0.001	0.018	0.000
Walter 2.4	1	0.006	0.000	10.521	0.001	0.038	0.056	1.154	-0.020	-0.001	0.000	0.259	7.069	0.003	0.001	0.001	0.016	0.000
Walter 2.5	1	0.861	0.002	21.197	0.041	0.059	0.134	8.017	-0.013	0.003	0.000	3.127	10.145	0.000	0.023	0.022	0.071	0.003
Walter 2.6	1	0.952	0.006	22.205	0.045	0.058	0.147	8.491	-0.017	-0.001	0.000	3.240	10.515	0.000	0.024	0.023	0.053	0.003
Walter 2.7	1	1.512	0.002	32.427	0.077	0.078	0.199	14.033	-0.012	0.002	0.000	3.558	12.239	0.000	0.023	0.038	0.075	0.003
Walter 2.8	1	1.509	0.003	32.503	0.076	0.077	0.202	13.660	-0.020	0.002	0.000	3.580	12.312	0.000	0.023	0.037	0.082	0.003
spoel	1	0.000	0.000	0.951	0.000	0.007	-0.005	-0.035	-0.008	-0.002	0.000	0.127	0.004	-0.001	0.000	0.000	0.002	0.000

Appendix 7 Raw SPAD data

			18-3-2019			20-3-2019			22-3-2019					25-3-2019						27-3-2019					
Samples	FeHBED	Light	New		Gem.	New		Gem.	Old		Gem.	New		Gem.	Old		Gem.	New		Gem.	Old		Gem.	New		Gem.
1	No	High	24,2	26,2	25,2	25,2	24,9	25,05	29,5	29,7	29,6	27,7	26	26,85	31,1	29,2	30,15	28,1	27,6	27,85	31,4	32,1	31,75	28,6	28	28,3
2	No	High	25,2	30	27,6	24,7	28,1	26,4	31,9	29,4	30,65	28,3	23	25,65	29,3	31,7	30,5	31,2	32,4	31,8	33,4	32,6	33	31,6	34,5	33,05
3	No	High	28,5	29,2	28,85	31,6	32,5	32,05	44,4	44,2	44,3	32,8	33,2	33	34,1	34,8	34,45	28,1	28,8	28,45	35,4	33,6	34,5	31,6	27,4	29,5
4	No	High	33,9	34	33,95	34,9	33,6	34,25	48	44,9	46,45	35,1	35,6	35,35	34,4	37,7	36,05	29,5	28,8	29,15	38,4	37,5	37,95	32	30,1	31,05
5	No	High	25,8	21,3	23,55	26,5	23,9	25,2	31,9	29,2	30,55	26,2	24,8	25,5	31,9	33	32,45	29,3	29,4	29,35	34,4	29,1	31,75	27,9	27,7	27,8
6	No	High	27	26	26,5	27,8	28,7	28,25	41,1	41,2	41,15	29,7	26,9	28,3	26,9	31,7	29,3	27,7	23,1	25,4	32,3	33,4	32,85	24,4	28,5	26,45
7	No	High	29,2	25,8	27,5	27,9	25,1	26,5	42,7	39,1	40,9	36,9	28,2	32,55	40,1	36,7	38,4	28,7	30,1	29,4	31,2	30,1	30,65	27,7	28,5	28,1
8	No	High	35,6	31,3	33,45	30,5	34,5	32,5	42,6	38,5	40,55	35,6	30,5	33,05	45	38,8	41,9	37,3	34,8	36,05	37,5	36,3	36,9	33,4	32,5	32,95
9	No	High	28,2	26	27,1	33,8	28,2	31	36,5	39,7	38,1	27,2	21,6	24,4	35,6	35	35,3	31,7	26,7	29,2	33,5	27	30,25	25,9	24,9	25,4
10	No	High	14,4	22,5	18,45	27,7	20,1	23,9	33,8	36,6	35,2	30,1	22,3	26,2	35,5	38,9	37,2	18,7	28,5	23,6	31,4	21,7	26,55	25,9	29,3	27,6
11	No	High	26,8	28,4	27,6	27,8	27,3	27,55	28,4	37	32,7	29,3	27	28,15	28,9	29,5	29,2	30,3	28,6	29,45	30,5	31,3	30,9	30,6	32,8	31,7
12	No	High	30,9	27,1	29	30,5	29,3	29,9	45,9	42,2	44,05	32,6	32,6	32,6	29,2	34,9	32,05	25,2	26,6	25,9	35,1	34,6	34,85	29	29,4	29,2
13	No	High	30,8	31,3	31,05	30	28,7	29,35	37,7	40,4	39,05	31,3	30,2	30,75	39,3	41,3	40,3	29,4	29,8	29,6	29,6	31,7	30,65	23	25,3	24,15
14	No	High	29,2	29,9	29,55	32,9	32,6	32,75	32,3	33,5	32,9	29,6	31,1	30,35	34,3	34,8	34,55	29,6	29,4	29,5	34,9	34,7	34,8	32,1	32,4	32,25
15	Yes	High	41,6	41,8	41,7	41,9	41,3	41,6	49,2	47,7	48,45	38,6	41,6	40,1	43,6	41,6	42,6	35,1	34,9	35	41,8	41	41,4	36,4	36,2	36,3
16	Yes	High	44	42,2	43,1	44,2	47	45,6	43,1	46,5	44,8	45,3	42,3	43,8	48,8	46,8	47,8	41,7	45,3	43,5	48,7	46,5	47,6	40,2	42,3	41,25
17	Yes	High	43,2	45,3	44,25	44,8	46	45,4	46,2	46,2	46,2	40,2	41,8	41	50,8	44,7	47,75	40,7	40,6	40,65	52,4	50,1	51,25	41,8	42,5	42,15
18	Yes	High	42,7	40,3	41,5	39,7	41,2	40,45	47,9	47,5	47,7	40	41,3	40,65	45,7	47,2	46,45	42,5	40,6	41,55	49,8	47,3	48,55	42	42,6	42,3
19	Yes	High	43,5	44	43,75	45,4	43	44,2	45,4	46,7	46,05	41,2	40,9	41,05	49,2	49,1	49,15	41,7	40,7	41,2	51,9	51,2	51,55	41,3	39,1	40,2
20	Yes	High	46,2	44,2	45,2	46,5	45,1	45,8	47,6	46,4	47	38,5	41	39,75	47,5	50	48,75	40,6	41,6	41,1	47,6	48,8	48,2	42,8	40,4	41,6
21	Yes	High	42,2	46,4	44,3	46,5	46,9	46,7	44,8	47,2	46	37,9	39,7	38,8	48,4	51,3	49,85	40,8	40,1	40,45	49	50,2	49,6	43,8	40,5	42,15
22	Yes	High	44,9	44,3	44,6	46,2	45,5	45,85	46	48,3	47,15	39,1	39,2	39,15	49,8	46,9	48,35	41,3	39,2	40,25	48	50,7	49,35	40,6	42,7	41,65
23	Yes	High	47,7	44,1	45,9	47,7	46,2	46,95	50,3	43,3	46,8	42,2	41,8	42	49,2	46	47,6	42,2	42,3	42,25	51,3	49,6	50,45	45,5	42,5	44
24	Yes	High	42	41,9	41,95	44,8	47	45,9	45,3	48,4	46,85	40,4	40	40,2	44,9	47,5	46,2	40	40,8	40,4	45,1	49,8	47,45	40,8	37,6	39,2
25	Yes	High	45,5	46,7	46,1	41,3	41	41,15	49,7	45,8	47,75	41,7	41,2	41,45	51,3	51,2	51,25	42,9	40,7	41,8	48,7	48,5	48,6	42,7	43,9	43,3
26	Yes	High	42,4	45,2	43,8	41,9	46,6	44,25	43,4	46,6	45	41,3	39,6	40,45	43,1	47,8	45,45	37,9	39,1	38,5	54	46,5	50,25	40,1	40,1	40,1
27	Yes	High	44,5	42,5	43,5	44,2	44,1	44,15	46,5	45,3	45,9	38,2	40,1	39,15	43,4	45,7	44,55	39,4	38,9	39,15	48,3	43,2	45,75	38	40,9	39,45
28	Yes	High	44,5	40,9	42,7	44,6	46	45,3	50,9	50,7	50,8	46	46,7	46,35	46,8	46,1	46,45	40,4	39,5	39,95	46,8	47,7	47,25	41,7	39,8	40,75
29	No	Low	33,5	33,8	33,65	32,9	31,9	32,4	45,7	43,4	44,55	33,9	32,4	33,15	45,5	46,6	46,05	35,3	33,2	34,25	46,8	47,5	47,15	35,9	35,9	35,5
30	No	Low	28,1	29	28,55	25,7	25,3	25,5	35,7	34,4	35,05	23,6	25,4	24,5	35,7	39	37,35	27	25,6	26,3	26,5	28,8	27,65	29,2	31,2	30,2
31	No	Low	22,5	24,5	23,5	24,9	22,1	23,5	40,1	37,8	38,95	25,8	23,6	24,7	41,6	36	38,8	28,5	27,1	27,8	30,1	30	30,05	31	30,4	30,7
32	No	Low	27,3	27,4	27,35	23,5	23,3	23,4	42,4	42,5	42,45	26,5	26	26,25	30,7	30	30,35	32,8	29,7	31,25	32,5	31,9	32,2	33,3	32,5	32,9
33	No	Low	21,2	20,9	21,05	23,5	21	22,25	23	25,4	24,2	23,2	22,9	23,05	25,4	27,9	26,65	27,2	25,6	26,4	27,5	29,8	28,65	29,3	27,9	28,6
34	No	Low	16,1	15,8	15,95	15,8	14,7	15,25	14,5	13,8	14,15	8,5	14,3	11,4	26,1	27,2	26,65	26,8	27,3	27,05	17,4	15,7	16,55	15,3	13,2	14,25
35	No	Low	15,2	13,1	14,15	11,6	12,4	12	12,6	12	12,3	13,8	16,6	15,2	11,8	10,7	11,25	18,2	22,6	20,4	15,9	19	17,45	23,1	23,8	23,45
36	No	Low	23,4	24,4	23,9	23,9	23,4	23,65	40,3	41,1	40,7	25,9	22,8	24,35	37,9	43,8	40,85	26	26,1	26,05	28,6	28,1	28,35	29,1	26,9	28
37	No	Low	21,5	22,4	21,95	20,9	18,5	19,7	29,6	40,2	34,9	18,6	20,8	19,7	21,6	18,5	20,05	16	18	17	21,1	22,3	21,7	23,4	22,2	22,8
38	No	Low	22,6	23,2	22,9	19,3	19,6	19,45	17,5	16,6	17,05	8,2	11,9	10,05	20,1	17,9	19	17,1	23,4	20,25	19,8	23,5	21,65	25,2	21,5	23,35
39	No	Low	28,7	30,4	29,55	27,1	28,4	27,75	33,6	32	32,8	28,7	29,4	29,05	33,8	33,7	33,75	31,8	33,1	32,45	37,4	38	37,7	33,8	35,9	34,85
40	No	Low	25,4	25,9	25,65	28,3	25	26,65	27,3	28	27,65	19,2	23	21,1	28,9	30,5	29,7	28,2	23,5	25,85	29,6	30,8	30,2	26,7	29,8	28,25
41	No	Low	18,9	18,5	18,7	21,1	18,8	19,95	23,1	21,6	22,35	21,3	22	21,65	27,8	23,9	25,85	25,8	27,2	26,5	31,3	33,5	32,4	30	25,8	27,5
42	No	Low	27,8	25,7	26,75	25,6	22,3	23,95	25,8	23,1	24,45	14,9	15,2	15,05	29,8	26,2	28	13,4	17	15,2	29,7	25,6	27,65	19,6	22,1	20,85
43	Yes	Low	37,3	37,5	37,4	38,4	39,4	38,9	42,6	42,9	42,75	33	34,1	33,55	43,5	44,8	44,15	36,3	38,6	37,45	48,8	46,6	47,7	38,4	40	39,2
44	Yes	Low	43,3	44,1	43,7	42,5	46,9	44,7	45,1	45,7	45,4	40,4	39,9	40,15	46,4	50,4	48,4	43,1	42,3	42,7	46,3	41	43,65	45,5	40,6	43,05
45	Yes	Low	40,3	42,1	41,2	45,1	45,7	45,4	48,7	47,8	48,25	40,4	40,5	40,45	51,1	51	51,05	38,9	37,6	38,25	47,9	53,9	50,9	40,9	40,7	40,8
46	Yes	Low	45,5	44,6	45,05	44,6	46,1	45,35	49,4	48,2	48,8	43,7	42,5	43,1	51,3	51,6	51,45	43,1	46,2	44,65	50,5	50,2	50,35	47,3	45,4	46,35
47	Yes	Low	37,2	37,9	37,55	38,2	38,8	38,5	44,5	47,3	45,9	37,6	40,8	39,2	41	43,7	42,35	39	36,3	37,65	46	46,8	46,4	39,6	40,7	40,15
48	Yes	Low	40,1	42,6	41,35	44	44,6	44,3	44,7	45,3	45	42,5	40,9	41,7	47,3	45,6	46,45	39,1	40,8	39,95	47,8	50,3	49,05	38,7	42,6	40,65
49	Yes	Low	41,2	40,7	40,95	45,3	44,8	45,05	47,6	49,4	48,5	44,9	45,5	45,2	46,2	49,8	48	39,1	38,1	38,6	49,5	50,9	50,2	41	42,5	41,75
50	Yes	Low	41,7	40,4	41,05	40,5	44,2	42,35	43,3	44,7	44	43,1	41,6	42,35	48,4	44,1	46,25	39,3	36	37,65	47,9	47,3	47,6	39,3	39,6	39,45
51	Yes	Low	41,8	44,3	43,05	42,5	42,1	42,3	44,6	40,9	42,75	35,2	37,2	36,2	44,2	42,6	43,4	40,3	38,9	39,6	49,4	46	47,7	40,7	40,9	40,8
52	Yes	Low	42,4	42,2	42,3	44,7	41	42,85	42,6	44	43,3	42,3	43,1	42,7	47,5	46,3	46,9	37,4	38,8	38,1	44,9	44,6	44,75	38,5	42	40,25
53	Yes	Low	41,5	40,6	41,05	43,5	44,2	43,85	51,7	52,1	51,9	44,8	43,7	44,25	49,2	47,3	48,25	40,1	40	40,05	40,1	39,8	39,95	50,7	48,7	49,7
54	Yes	Low	40,9	40,4	40,65	41,7	41,5	41,6	44,4	46,3	45,35	41	42,7	41,85	28,2	47,7	37,95	40	41	40,5	49,2	49	49,1	41,4	41,7	41,55
55	Yes	Low	40,9	40,4	40,65	38,6	40,7	39,65	43,8	40	41,9	41	38,1	39,55	44,4	43,6	44	34,4	35,7	35,05	46	41,8	43,9	36,1	36,4	36,25
56	Yes	Low	38,8	37,1	37,95	39,2	38,3	38,75	40,3	43,2	41,75	33,9	33,5	33,7	45,4	46,7	46,05	37,7	36,7	37,2	47,2	49	48,1	40,5	39,9	40,2

29-3-2019						1-4-2019						3-4-2019						5-4-2019					
Old		Gem.	New		Gem.	Old		Gem.	New		Gem.	Old		Gem.	New		Gem.	Old		Gem.	New		Gem.
32,9	34,5	**33,7**	33,5	35,2	**34,35**	35,7	37	**36,35**	38,3	38	**38,15**	36,3	35,8	**36,05**	35,4	37,5	**36,45**	36,7	38,3	**37,5**	37,1	38,5	**37,8**
35,8	35,6	**35,7**	28,6	29	**28,8**	36,2	35,4	**35,8**	31,3	30,9	**31,1**	37,6	36,7	**37,15**	34,4	31,6	**33**	37,9	37,8	**37,85**	35,7	34,2	**34,95**
38,6	37,3	**37,95**	32,7	32,1	**32,4**	37,3	38,4	**37,85**	34,7	35	**34,85**	38,7	38,8	**38,75**	36	32,5	**34,25**	39,9	40,2	**40,05**	35,8	38	**36,9**
34,8	30,1	**32,45**	30,6	28,4	**29,5**	34,8	32,8	**33,8**	34,8		**34,8**	33,8	34,2	**34**	30,1	31,6	**30,85**	34,9	33,7	**34,3**	30,1	32,2	**31,15**
31,9	30,7	**31,3**	29,4	29,3	**29,35**	31,5	30,7	**31,1**	28,4	31,5	**29,95**	31,3	30,6	**30,95**	31,2	30,3	**30,75**	31	31,3	**31,15**	31,2	32,8	**32**
31,7	24,9	**28,3**	29	28,4	**28,7**	35,4	31,3	**33,35**	30,7	31,4	**31,05**	37,5	28,4	**32,95**	30,5	35	**32,75**	37	31,1	**34,05**	34,4	32,1	**33,25**
31,9	31	**31,45**	30,7	32,2	**31,45**	32,4	33,6	**33**	36,7	32,4	**34,55**	33,4	32,3	**32,85**	34,7	35,5	**35,1**	35,5	34,1	**34,8**	34,8	30,2	**32,5**
35,7	32,1	**33,9**	29,9	28,6	**29,25**	37	33,7	**35,35**	32,5	32,6	**32,55**	36,7	34,3	**35,5**	33,1	31,7	**32,4**	36,3	34	**35,15**	33,5	33,3	**33,4**
41,1	42,8	**41,95**	35,3	35	**35,15**	41,6	42,7	**42,15**	37,6	31,5	**34,55**	44,5	41,3	**42,9**	35,9	37,4	**36,65**	41,7	40	**40,85**	37,4	35,3	**36,35**
49,7	48,3	**49**	40,5	43,5	**42**	49,1	51,5	**50,3**	43,6	36,9	**40,25**	44,1	47,5	**45,8**	38,1	36,5	**37,3**	50,1	49	**49,55**	38	37,5	**37,75**
47,8	47,1	**47,45**	42,9	40,7	**41,8**	44	44,7	**44,35**	40,6	47,2	**43,9**	44	49,1	**46,55**	40,4	37,2	**38,8**	44,5	44,8	**44,65**	41,2	39,5	**40,35**
47,9	50,8	**49,35**	43,1	43,5	**43,3**	51,1	51,3	**51,2**	43,8	38,5	**41,15**	46,5	44,2	**45,35**	36,7	37,3	**37**	45,2	45,5	**45,35**	37,3	39,4	**38,35**
50,3	52,2	**51,25**	43,5	41,3	**42,4**	41,5	45,5	**43,5**	37	44,5	**40,75**	45,5	46,7	**46,1**	34,5	36,2	**35,35**	46,8	47	**46,9**	36,7	37,9	**37,3**
51,4	48,3	**49,85**	44,3	44,5	**44,4**	45,5	45,9	**45,7**	41,1	35,5	**38,3**	44,5	46,2	**45,35**	40,8	39	**39,9**	47,8	45,8	**46,8**	40,4	39,4	**39,9**
51	49,2	**50,1**	43,4	46,1	**44,75**	51,5	51,1	**51,3**	44,9	38,9	**41,9**	47,6	45,7	**46,65**	37,2	40,5	**38,85**	48,1	47,8	**47,95**	38,9	41,4	**40,15**
48,1	48	**48,05**	41,4	41,6	**41,5**	49,1	50,5	**49,8**	42,7	46,6	**44,65**	49,6	51,5	**50,55**	41,6	42,9	**42,25**	50,5	50,3	**50,4**	43,9	44,1	**44**
30,3	31,6	**30,95**	33,1	33,3	**33,2**	30,9	33,2	**32,05**	37,5	42,6	**40,05**	33,1	35,5	**34,3**	38,6	37,9	**38,25**	34,9	35,4	**35,15**	40,9	40,1	**40,5**
33,5	33,3	**33,4**	33,7	33,8	**33,75**	35,5	34,7	**35,1**	37,2	36,6	**36,9**	36,9	35,7	**36,3**	37,5	36,2	**36,85**	38	37,5	**37,75**	41,1	38,6	**39,85**
26,1	29,9	**28**	31,9	32,1	**32**	29,7	31,6	**30,65**	35,7	36,4	**36,05**	30,2	33,1	**31,65**	38,4	36,6	**37,5**	36,7	37	**36,85**	37,4	37	**37,2**
18,1	15,6	**16,85**	23,9	27,5	**25,7**	19,7	17,7	**18,7**	30	34,3	**32,15**	32,9	33,5	**33,2**	35,3	34,4	**34,85**	34,5	36,1	**35,3**	31,6	35,2	**33,4**
29	26,2	**27,6**	29,5	29,1	**29,3**	31,2	30,4	**30,8**	32,3	30,5	**31,4**	31,6	33,1	**32,35**	32,9	33,9	**33,4**	32,4	32,2	**32,3**	34,9	36,5	**35,7**
23,2	22,2	**22,7**	26,6	25,8	**26,2**	22,8	25,3	**24,05**	32,4	33,2	**32,8**	22,8	27,2	**25**	35,3	35,4	**35,35**	36,8	37,5	**37,15**	31,7	35,7	**33,7**
32,2	37,2	**34,7**	29,5	29,3	**29,4**	36,2	37,7	**36,95**	32	31,6	**31,8**	36,5	39,3	**37,9**	31,9	30,3	**31,1**	40,3	36,2	**38,25**	33,9	33,8	**33,85**
30,2	27,2	**28,7**	23,9	25,2	**24,55**	29,3	30,8	**30,05**	37,2	31,3	**34,25**	32,4	33,3	**32,85**	35,2	34,8	**35**	33,7	34,8	**34,25**	37,5	34,2	**35,85**
46	48,2	**47,1**	41,9	41,6	**41,75**	49,5	49,9	**49,7**	46,1	33,2	**39,65**	46,9	47,1	**47**	38,6	36,4	**37,5**	49,6	45,2	**47,4**	38,9	39,7	**39,3**
49,5	48,9	**49,2**	42,9	42,1	**42,5**	52,3	49,1	**50,7**	42,1	47,9	**45**	54,8	53,2	**54**	45	48,3	**46,65**	52,5	53	**52,75**	50,6	50	**50,3**
52,8	51,2	**52**	39,3	43,2	**41,25**	49,7	50,9	**50,3**	41,3	47,3	**44,3**	50,8	50,9	**50,85**	44,2	49,5	**46,85**	52,3	52,8	**52,55**	47,4	45,7	**46,55**
51,8	54,5	**53,15**	41,7	42,6	**42,15**	54,5	47,8	**51,15**	43,7	43,2	**43,45**	56	55,5	**55,75**	45,5	43,1	**44,3**	56	55,2	**55,6**	44,6	47,8	**46,2**
48,1	52,9	**50,5**	41,4	41,8	**41,6**	51	49	**50**	45,5	45,7	**45,6**	49	52		43,7	46,3		44,5	43,6		37,3	41,6	
										42,6													
50,5	47,7	**49,1**	43,4	42,4	**42,9**	51,1	52,3	**51,7**	44,1	45,1	**44,6**	54	53,1	**53,55**	45,7	45,2	**45,45**	51,5	52,5	**52**	47,6	46,9	**47,25**
45,7	45,1	**45,4**	37,9	37,3	**37,6**	47,7	47,2	**47,45**	38,3	39,7	**39**	48,6	47,6	**48,1**	39,1	39,2	**39,15**	49,7	46,6	**48,15**	39,6	42,1	**40,85**
49,2	48,4	**48,8**	42,2	42,4	**42,3**	48,4	52,8	**50,6**	46,1	45,6	**45,85**	48,8	48,4	**48,6**	36,8	38,4	**37,6**	48,1	47,9	**48**	40,1	37,8	**38,95**

8-4-2019						10-4-2019						12-4-2019						15-4-2019						17-4-2019					
Old		Gem.	New		Gem.	Old		Gem.	New		Gem.	Old		Gem.	New		Gem.	Old		Gem.	New		Gem.	Old		Gem.	New		Gem.
38	39,2	**38,6**	40,3	35,6	**37,95**	40,7	39	**39,85**	36,5	40,4	**38,45**	37,1	38,6	**37,85**	37	39,3	**38,15**	41,6	40,4	**41**	38,3	40,3	**39,3**	39,8	38,5	**39,15**	42,7	42	**42,35**
38,3	36,9	**37,6**	34,7	38,3	**36,5**	38,9	37	**37,95**	38	37,7	**37,85**	38,6	39,1	**38,85**	39,1	37,5	**38,3**	39,3	38,3	**38,8**	39,6	38,7	**39,15**	40,1	38,2	**39,15**	38,8	40,3	**39,55**
39,7	40,1	**39,9**	37,6	37,8	**37,7**	40,5	40,1	**40,3**	39,2	37,6	**38,4**	38	38,7	**38,35**	37,1	37,4	**37,25**	38,6	37,6	**38,1**	36,7	41,9	**39,3**	37,2	39,3	**38,25**	37,6	41,3	**39,45**
37,4	33,5	**35,45**	32,2	33,2	**32,7**	36,9	37	**36,95**	32,4	31,3	**31,85**	35,3	35,8	**35,55**	33,3	34,7	**34**	38,8	38,2	**38,5**	34,2	34,6	**34,4**	39,7	37,9	**38,8**	36,6	33,9	**35,25**
32,2	28,5	**30,35**	31,4	32,3	**31,85**	30,7	30,3	**30,5**	33,7	36,1	**34,9**	32,2	30,8	**31,5**	34,6	34,5	**34,55**	30,6	32,8	**31,7**	35,6	36,1	**35,85**	35,1	34,5	**34,8**	32,5	35,4	**33,95**
36,7	32,2	**34,45**	37,4	36,2	**36,8**	38,5	33,2	**35,85**	36,3	38,3	**37,3**	38,5	32,5	**35,5**	37	39,3	**38,15**	39,3	32,5	**35,9**	40	39,7	**39,85**	39,7	40,3	**40**	32,7	31	**31,85**
38	33,3	**35,65**	33,4	36,4	**34,9**	37,9	34,7	**36,3**	31,9	32,8	**32,35**	38,6	45,3	**41,95**	33,3	31,3	**32,3**	39,7	36,8	**38,25**	35	34,3	**34,65**	41,3	38,7	**40**	34,2	32,4	**33,3**
33,9	33,2	**33,55**	33,6	35,8	**34,7**	30,2	34	**32,1**	36,2	35,9	**36,05**	32,5	27,6	**30,05**	35,1	34,7	**34,9**	35,4	28,7	**32,05**	35,4	33,1	**34,25**	33,4	32,8	**33,1**	35,8	35,5	**35,65**
34,7	42,9	**38,8**	39,4	37,8	**38,6**	40,5	42,9	**41,7**	38,8	40,3	**39,55**	40,8	41,6	**41,2**	39,5	38,6	**39,05**	44,2	42	**43,1**	40,4	40,6	**40,5**	42,1	40,5	**41,3**	38,7	40,5	**39,6**
48,1	47,5	**47,8**	38,6	40,4	**39,5**	47,3	50,5	**48,9**	40,6	42,9	**41,75**	47,6	48,4	**48**	42,6	38,1	**40,35**	51,7	49,5	**50,6**	43,2	40,8	**42**	49,9	49,7	**49,8**	40,5	42,8	**41,65**
46,6	46,1	**46,35**	42,6	39,9	**41,25**	44,6	44,4	**44,5**	44,7	41,8	**43,25**	46,5	50,2	**48,35**	43,5	42,1	**42,8**	45,9	49,7	**47,8**	45,7	41	**43,35**	47,3	48,7	**48**	43,9	42,7	**43,3**
47,6	46,9	**47,25**	39,3	41,2	**40,25**	48,6	46	**47,3**	41,8	41,9	**41,85**	48,9	48,5	**48,7**	42,2	40,1	**41,15**	48,2	48,6	**48,4**	41,6	44,5	**43,05**	48,1	47,2	**47,65**	43,2	42,5	**42,85**
47,5	48,1	**47,8**	40,3	38,1	**39,2**	48,6	48,8	**48,7**	41,6	39,5	**40,55**	46,5	47,4	**46,95**	42,8	45,9	**44,35**	48,2	49,2	**48,7**	43,9	42,4	**43,15**	45,6	50,5	**48,05**	43,4	43,5	**43,45**
46,8	48	**47,4**	41	43,8	**42,4**	49,6	49,7	**49,65**	41,8	43,2	**42,5**	48,3	47,5	**47,9**	43,6	43,1	**43,35**	47,3	45,6	**46,45**	44,8	44,1	**44,45**	49,7	48	**48,85**	43,5	44,2	**43,85**
49,9	47,5	**48,7**	40	43,3	**41,65**	49,3	48,3	**48,8**	42,8	45,4	**44,1**	46,3	49,9	**48,1**	42,2	46,9	**44,55**	50,6	49,7	**50,15**	42,3	44,7	**43,5**	48,9	48,6	**48,75**	43,9	47,1	**45,5**
50,4	47,7	**49,05**	44,2	44,9	**44,55**	46,3	45,5	**45,9**	44,4	43,3	**43,85**	47,9	47,4	**47,65**	43,3	42,9	**43,1**	47,7	45,2	**46,45**	41,9	44,3	**43,1**	44,1	47,2	**45,65**	43,4	43,1	**43,25**
34,9	36	**35,45**	42,3	40,7	**41,5**	35,7	35,2	**35,45**	42,3	43,3	**42,8**	43,7	41	**42,35**	35,6	39,5	**37,55**	43,3	44,6	**43,95**	31,3	32,5	**31,9**	44,5	43,3	**43,9**	38,2	37,2	**37,7**
38	37	**37,5**	41,2	40,3	**40,75**	37,3	38,3	**37,8**	44,4	42,6	**43,5**	42,3	43,7	**43**	34,3	32,9	**33,6**	42,9	42,7	**42,8**	38,7	37,1	**37,9**	43,5	43,7	**43,6**	38,1	39,6	**38,85**
30,5	33,2	**31,85**	38	40,7	**39,35**	31,4	33,9	**32,65**	41	41,9	**41,45**	42,3	41,4	**41,85**	34,3	35,5	**34,9**	41,9	41,7	**41,8**	36,1	38,2	**37,15**	41	40,6	**40,8**	30,3	35,8	**33,05**
36,8	37	**36,9**	35,1	39,5	**37,3**	37,3	37	**37,15**	35,9	40,2	**38,05**	37,2	38,7	**37,95**	38,3	42,2	**40,25**	36,2	39	**37,6**	39	42,8	**40,9**	36,5	38,2	**37,35**	42,3	43,5	**42,9**
32,6	31,1	**31,85**	35,9	36,6	**36,25**	32	30,6	**31,3**	36	39,6	**37,8**	29,9	31,9	**30,9**	35,2	38,6	**36,9**	30,3	30,8	**30,55**	38	38,4	**38,2**	31,5	30,2	**30,85**	36,3	37,5	**36,9**
38,8	38,1	**38,45**	38,1	36,9	**37,5**	38,6	42,7	**40,65**	40,2	37,6	**38,9**	41,8	40,4	**41,1**	41,8	39,9	**40,85**	40,7	42	**41,35**	44,6	43,1	**43,85**	43,8	42,2	**43**	44,9	44,6	**44,75**
35,4	39,1	**37,25**	32,2	33,9	**33,05**	40,3	38	**39,15**	36,4	37,5	**36,95**	36	36,3	**36,15**	28,4	28,4	**28,4**	37,3	39,4	**38,35**	32,7	31	**31,85**	37	37,6	**37,3**	35	32,5	**33,75**
33,5	35,4	**34,45**	39,5	39,4	**39,45**	35,8	37,9	**36,85**	40,8	40,7	**40,75**	36,9	37,2	**37,05**	40,5	42,6	**41,55**	33,9	36,6	**35,25**	45,6	44,5	**45,05**	35,9	37,6	**36,75**	42,6	44	**43,3**
47,9	47,5	**47,7**	40,4	42,1	**41,25**	48,8	42,8	**45,8**	43	42,9	**42,95**	52,1	50,5	**51,3**	42,9	45,1	**44**	51,6	51,8	**51,7**	45,2	45	**45,1**	48,1	49,3	**48,7**	46,7	44,6	**45,65**
53,6	53,4	**53,5**	50,5	51,7	**51,1**	54,1	45,5	**49,8**	53,7	50,6	**52,15**	52	51,3	**51,65**	38,7	39,7	**39,2**	51	54,9	**52,95**	39,7	41,7	**40,7**	48,8	54,5	**51,65**	42,4	44,3	**43,35**
55,6	53,9	**54,75**	46,1	49,1	**47,6**	54,7	56,1	**55,4**	47,2	50,3	**48,75**	48,3	47,5	**47,9**	40,6	41,5	**41,05**	48,1	48,5	**48,3**	42,6	43,1	**42,85**	46,2	50,6	**48,4**	42,7	41,3	**42**
47,5	45,5	**46,5**	41	42,9	**41,95**	47,7	51,5	**49,6**	41,8	41,3	**41,55**	51,1	48,1	**49,6**	41,6	43,8	**42,7**	50,6	51,1	**50,85**	42,6	44,4	**43,5**	49,3	48,2	**48,75**	45,8	46,5	**46,15**
45,4	48,5		41,4	41,8		48	49,7		40,4	44,2		50,1	45,3		41,3	39,4		46,7	50,2		42,1	42,5		47,1	49,6		43,1	43,6	
49,2	49,4	**49,3**	38,2	41,6	**39,9**	50,3	50,4	**50,35**	43,4	40,9	**42,15**	51	50,5	**50,75**	40,6	39,8	**40,2**	49	48,3	**48,65**	38,7	40,6	**39,65**	53	53,8	**53,4**	41	40,3	**40,65**
41,6	40,4	**41**	35,4	36,2	**35,8**	43,9	42,8	**43,35**	36,6	32,1	**34,35**	44	43,7	**43,85**	37,1	35,2	**36,15**	42,7	44,2	**43,45**	35,5	35,3	**35,4**	41,7	42,2	**41,95**	37,4	36,5	**36,95**
49	48,1	**48,55**	42,1	39,6	**40,85**	50,1	50,1	**50,1**	41,9	43,8	**42,85**	51	49,8	**50,4**	41,8	44,6	**43,2**	51	49,2	**50,1**	43,6	43	**43,3**	50,6	52,3	**51,45**	44,1	43	**43,55**