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Detect Plant Stress by Measuring Chlorophyll Fluorescence Gain from Lamp PWM Signal

Master's thesis in Systems, Control and Mechatronics

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Abstract

An increasing world population and the spread of prosperity around the world are two major factors causing a shift within the global food production as we know it today. One major requisite for handling this shift lies within the efficiency of the cultivation and its related techniques - where controlled indoor environments for crop growth are likely to play a significant role. Heliospectra AB is a company that develops Light Emitting Diodes (LED) growing light for commercial use. LED lights have been receiving attention due to low power consumption for plant cultivation, and other applications. Plants exposed to illumination emit a light called chlorophyll fluorescence which has been shown to provide useful information about the state of the plant. The intensity of the chlorophyll fluorescence is low in relation to other light sources which makes it hard to isolate it when measured in a sunlight environment.

This thesis aims to develop a method that uses the lamp's inherent Pulse-Width Modulated (PWM) configuration to distinguish a chlorophyll fluorescence response in a dynamic light environment. Further, the study aims to investigate the ability to use this response to draw useful conclusions about the health of the subjected plant.

It was found that the method is able to distinguish the chlorophyll fluorescence from dynamic ambient light. Further, it was shown that an increased excitation frequency yields a more robust measurement in a more dynamic background light environment. The obtained chlorophyll fluorescence response indicates that it can be used for drawing useful conclusions about the plant's health. However, measurements have shown that the method puts a high demand on the sensor used for chlorophyll fluorescence measurements. Since the approach has shown good potential, an improvement of the sensor is suggested for future work.

Keywords: Chlorophyll fluorescence, plant stress, plant health, PWM.

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Nomenclature

F_v/F_m	Maximum quantum yield of PSII photochemistry
F'_m	Maximum value for chlorophyll fluorescence in quenched condition
F_0	Minimum value for chlorophyll fluorescence
F_m	Maximum value for chlorophyll fluorescence in un-quenched condition
F_v	Variable chlorophyll fluorescence, difference between F_m and F_0 or F'_m and F_0
$Y(II)$	Maximum quantum efficiency of PSII photochemistry in quenched condition
ETR	Electron Transport Rate
FOV	Field-of-view
HPS	High Pressure Sodium
LED	Light Emitting Diode
NPQ	Non-Photochemical Quenching
PAM	Pulse-Amplitude Modulation
PAR	Photosynthetic active radiation
PSII	PhotoSystem II
PWM	Pulse-Width Modulation

1

Introduction

Climate changes, an increasing world population and the spread of prosperity across the world are all major causes for a shift within the global food production as we know it today. According to the UN - The United Nations, the world population is expected to reach 9.8 billion by 2050, 32 percent higher than today [1].

One major requisite for handling these shifts of conditions and an increased demand lies within the efficiency of the cultivation and its related techniques - where controlled indoor environments for crop growth are likely to play a significant role. Up until recently, the conventional greenhouse lighting mainly comes from so-called High-Pressure Sodium (HPS) lamps [2]. Heliospectra AB is a company that develops Light Emitting Diodes (LED) growing light for commercial use. Within the transition from traditional HPS lamps to LED light, a wide range of advantages is expected to follow. LED lights have been receiving attention due to low power consumption for plant cultivation, and other applications [3] [4]. Additionally, LED lights possess a high responsivity along with its diodes being able to customise a spectrum's magnitude for given wavelengths which makes them a suitable option for scientific research and applications.

Studies have shown that remote sensing of chlorophyll fluorescence is a promising method for plant phenotyping and precision farming. The chlorophyll fluorescence exposes important growth factors such as plant health including stress level, which have the potential to pave the way for more sophisticated control and customisation of growth lights [5] [6] [7]. Light that hits a plant is either reflected off the leaves, absorbed or transmitted through the leaves. Chlorophyll fluorescence is one out of three processes that occurs during transmission along with heat dissipation of excess energy and photosynthesis, where the most energy is used driving the photosynthesis [2].

The relation between the amount of absorbed light, the amount of fluorescent light and the photosynthetic rate has been studied on both leaf level and canopy level and is dependent on plant health [8] [9]. For example, the fraction of chlorophyll fluorescence and photosynthesis is negatively correlated at low light intensity while it is positively correlated at a high light intensity and stress [10] [11]. However, the absolute quantities of both chlorophyll fluorescence and photosynthesis are expected to increase with an increased incident light intensity, though the photosynthetic rate will eventually saturate.

1.1 Background

Light energy absorbed by plants is used for photosynthesis, dissipated as heat or re-emitted as chlorophyll fluorescence. The spectral distribution of light has shown to affect the biomass of the plants [12], and chlorophyll fluorescence measurements contain information about the plant's physiology, which can be used to determine plant health [13]. Measurements of chlorophyll fluorescence in plant physiology research are often performed under defined conditions w.r.t. light, temperature, pre-darkening, radiation intensities and illumination times [14] [15] [16]. Some of these conditions are impractical when moving these experiments to a non-closed lab environment, where the chlorophyll fluorescence is challenged by fluctuating environmental conditions [17] [18] [19] [20]. A difficulty when measuring chlorophyll fluorescence is that the magnitude of the signal in relation to different sources of disturbance is rather weak and therefore hard to separate. The biggest influential factor is mainly sunlight which by itself can be overwhelmingly larger than the intensity of the chlorophyll fluorescence signal.

In order to be able to measure chlorophyll fluorescence remotely on canopy level and consequently open for a more scalable method than in a closed lab environment, Heliospectra AB believes that using Pulse-Width Modulation (PWM) for incident light has advantages when measuring chlorophyll fluorescence. Using signal processing (synchronous demodulation) enables a higher sensitivity over a broader dynamic range, which is beneficial for a greenhouse application. Such a method could simplify measuring chlorophyll fluorescence in a greenhouse environment, implicating varying sunlight compared to the methods that Heliospectra currently uses for measuring plant health parameters.

This study was conducted at Heliospectra AB with additional supervision from Chalmers University of Technology.

1.2 Aim

The aim of this study is to investigate and develop a method that analyses chlorophyll fluorescence response generated by PWM signals as incident light. The goal is to be able to measure and distinguish the chlorophyll fluorescence response in sunlight, independent of sunlight intensity. Further, the study aims to draw conclusions about plant health by studying the chlorophyll fluorescence response.

1.3 Limitations

This study was conducted in a closed indoor environment, e.g. no real sunlight was used for excitation of the plants during the experiments. The experiments were performed on a set of basil (*Ocimum basilicum*) Aroma 2 plants.

1.4 Objectives

1. **Is it possible to measure chlorophyll fluorescence gain in a simulated sunlight environment using a PWM based method?**
   According to [6], modern greenhouses having lighting systems are large consumers of electricity. Obviously, there is great potential in using the LED-light more efficiently. One way to do so is to regulate the light intensity based on current sunlight, e.g. lower the lamp intensity when the ambient sunlight is high enough - and the other way around. An identified problem when measuring chlorophyll fluorescence in an environment subjected to sunlight is that the wavelength of the sunlight overlaps with the chlorophyll fluorescence. This implies that the chlorophyll fluorescence signal becomes weak compared to the sunlight, and consequently difficult to distinguish. Further, an increased sunlight intensity also results in a lower Signal-to-Noise Ratio (SNR) due to the saturation of the fluorometer. Therefore, a method to investigate this problem may be to simulate sunlight using different light sources (LED lamps) in a laboratory. The reasoning above could be summarised as:

   - Is it possible to measure the chlorophyll fluorescence parameter in simulated sunlight using fast pulse excitation (e.g. using the lamp's PWM mode) in order to reduce the effect of noise caused by ambient light? This to study the chlorophyll fluorescence gain which is described as different level of chlorophyll fluorescence response in relation to different intensities of incident light.

2. **Is it possible to recognise any trends regarding chlorophyll fluorescence gain in relation to plant health?**
   As mentioned in the introduction in Chapter 1, a correlation between photosynthetic rate and chlorophyll fluorescence gain could be found [6]. To understand properties regarding chlorophyll fluorescence and plant health, additional research has to be taken into account. The following question will be studied:

   - Is it possible to detect plant stress by analysing the measured chlorophyll fluorescence gain?

2

Theory and related work

This chapter presents a set of concepts, both technical and biological. In order to give the reader an understanding sufficient for further reading, this chapter will present and explain the underlying theories of this thesis work. Further, related research areas will be summarised in order to give an overview of the background of this thesis.

2.1 Photosynthesis and chlorophyll fluorescence

Using water, carbon dioxide and light in the range 400-700 nm - bacteria, protistants and plants can create oxygen and chemically bounded energy in the form of glucose through a process called photosynthesis. Photosynthesis is one out of three processes resulting from the plant being exposed to light. This process is used to drive the plant's activity, thus an increasing photosynthesis is always desirable since this means that more chemical energy can be stored in the plants [2] [21].

Except driving the photosynthesis, the absorbed light is also dissipated as heat and re-emitted as chlorophyll fluorescence. The efficiency for converting light to chemical energy through the photosynthesis is 3-6%, and 1-2% of the total absorbed light is re-emitted as chlorophyll fluorescence [22] [23]. The chlorophyll fluorescence is within the range 670-800 nm with two peaks, located at 685 nm and 735-755 nm. Since the levels of photosynthesis, heat and chlorophyll fluorescence are correlated, information about the latter implies that information about the rate of efficiency of the photosynthesis can be obtained. Even though the amount of chlorophyll fluorescence is just a few percent and with a limited spectrum, the information about it is quite easy to access through measurements. This is due to the fact that the wavelength at the peak of the emitted chlorophyll fluorescence is higher than that of absorption.

2.2 Spectrometer and fluorometer data conversion

In order to measure light intensity, both a fluorometer and a spectrometer can be used. When using the fluorometer and the spectrometers described in Section 3.2 respectively Section 3.1, the measurements are obtained in the unit *counts*, which is described as a set of photons that are sorted with respect to wavelength, converted

to an electric charge which is read as a digital intensity number. Each count is specified between an interval of wavelengths, also called a pixel.

However, when using the spectrometer, the intensity of the light is generally presented as photon flux [$\mu mols^{-1}$]. By dividing the photon flux with m^2, it is converted to photon Flux Density (PFD) [$\mu molm^{-2}s^{-1}$] and at the same time made independent of the size of the collection area. To obtain a conversion from counts over pixels to irradiance over wavelengths several parameters and steps are needed along with specific calibration data for the corresponding spectrometer. The first step is to obtain the total energy in each pixel. The wavelength for each pixel has to be found, which is given by the spectrometer's wavelength calibration. Further, the total energy in each pixel is calculated by converting counts to energy. The calibration file is composed by measuring a calibration lamp of known intensity.

$$E = counts \cdot calibration[joules/counts] \tag{2.1}$$

The energy for a single photon, E_i, can be derived by using Planck's constant.

$$E_i = \frac{h \cdot c}{\lambda}, \tag{2.2}$$

where h is Planck's constant, λ the wavelength for the particular photon and c is the speed of light. The second step is to obtain the number of photons, $n_{photons}$, which is described by the total energy in each pixel, E, over the energy in each photon, E_i. The number of photons can be derived using the calibration file for absolute irradiance

$$n_{photons} = \frac{E}{E_i}, \tag{2.3}$$

which is specific for each spectrometer. By using Avogrado's constant, N_A, the number of photons can be converted into mol photons

$$mol\ photons = \frac{n_{photons}}{N_A}. \tag{2.4}$$

To convert mol photons to irradiance, an area, A, which describes the collection area [m^2] is required. Here,

$$A = \pi r_c^2, \tag{2.5}$$

at measurement level where r_c is the radius [m] for the attached cosine corrector. Lastly, by using the integration time, IT [s], the photon irradiance [$mol\ photons/m^2s$] can be calculated as

$$irr = \frac{mol\ photons}{A \cdot IT}. \tag{2.6}$$

2.3 Pulse-Width Modulation

Another application that has been discussed considering measuring chlorophyll fluorescence is to use modulated incident light, which implies measuring pulses of chlorophyll fluorescence response at high frequencies. By tuning the equipment to be able to detect chlorophyll fluorescence response at such frequencies enables determination of the chlorophyll fluorescence yield in the presence of background light, or more importantly, in presence of sunlight [23]. Heliospectra believes that using pulse-width modulation (PWM) as incident light could yield a similar result as using the PAM Fluorometry, but without requiring the precision work on leaf level. On the contrary, this method could be used remotely thus covering a larger area of plants when conducting experiments.

2.4 PAM fluorometry and reference measuring

One method to study chlorophyll fluorescence is to use Pulse-Amplitude Modulation (PAM) as incident light to the subjected plant. By using a series of short duration pulses, the PAM fluorometry can be used to separate chlorophyll fluorescence induced by these pulses from chlorophyll fluorescence induced by ambient light sources. Parameters such as heat dissipation can be neglected during an excitation since the average magnitude of the excitation is considered to be small [24]. Measurements using PAM fluorometry have to be conducted on single leaf level when studying chlorophyll fluorescence, which requires high precision work and sensors being placed close to the plant. This implies that the method using PAM fluorometry is mainly suitable for a low quantity of plants when performing measurements.

When performing experiments designed to detect plant stress, an obvious requirement is that the plant used for the experiment actually is stressed. To determine the stress level of the subjected plant, different parameters related to chlorophyll fluorescence must be determined both before and during the experiment. Here, reference measurements were conducted using PAM fluorometry in conjunction with the saturating pulse method.

F_0 denotes the minimum value of the chlorophyll fluorescence. This parameter is obtained by applying an excitation pulse on a dark-adapted leaf. This light is too low to induce any electron transport through PSII, but of an intensity high enough to gain a minimum level of chlorophyll fluorescence. When the maximum possible value for chlorophyll fluorescence is collected in un-quenched condition it is denoted as F_m, obtained by applying a saturating pulse to a dark-adapted leaf. When collected in quenched condition it is referred to as F'_m. F_v and F'_v are the variable chlorophyll fluorescence, which are the differences between F_m and F_0 or F'_m and F_0, i.e.

$$\begin{aligned} F_v &= F_m - F_0 \\ F'_v &= F'_m - F_0 \end{aligned} \tag{2.7}$$

F_v/F_m is a widely used parameter describing the maximum quantum efficiency of

PSII photochemistry. The dark-adapted value of F_v/F_m has, according to [25], been shown both empirically and theoretically to be a robust indicator of the maximum quantum yield of PSII chemistry. For unstressed dark-adapted leaves, the parameter is expected to be around 0.83, and highly consistent. When calculating this parameter in quenched condition it is referred to as Y(II), i.e.

$$Y(II) = F_v/F'_m. \tag{2.8}$$

NPQ, *non-photochemical quenching*, is a parameter used for describing the rate constant for heat dissipation of chlorophyll excitation energy, calculated according to

$$NPQ = \frac{F_m - F'_m}{F'_m}. \tag{2.9}$$

NPQ works as a safety mechanism by removing excess energy and preventing the formation of damaging free radicals within chlorophyll-containing complexes [25]. Upon illumination, there is a rapid increase in this parameter. If applying a saturating pulse to a dark-adapted leaf, a healthy non-stressed plant should give no NPQ since the saturating pulse should induce maximum fluorescence. Furthermore, when illuminating a stressed light-adapted plant, NPQ is expected to be higher compared to NPQ for a non-stressed plant exposed by the same light. Thus, this parameter is useful in order to determine whether the plant has been properly dark-adapted and whether it can be expected to have an increased stress level, where the latter is, in this study, considered to be of more importance.

3
Materials

OTHER than plants, several types of equipment have been used to aquire data for evaluating the objectives presented in Section 1.4. This chapter describes the complete list of equipment, including an overview of the experimental unit used for this study.

3.1 Spectrometers

The ambient light spectrum was measured in order to draw useful conclusions from the chlorophyll fluorescence measurements. This was done by using one spectrometer measuring the incident light and one spectrometer measuring the reflected light. The incident light spectrum was also used to map a specific chlorophyll fluorescence level to a known light spectrum and its intensity. When measuring different light intensities, the range of interest for this project was ~400-800 nm, where the chlorophyll fluorescence response was measured within ~730-750 nm. Both spectrometers should be considered as high-quality spectrometers with a sensitivity sufficient for the experiments carried out in this study.

Measuring reflected light was partially done by a Maya2000 Pro spectrometer from Ocean Optics [26]. This spectrometer covers a wavelength range of ~165-1100 nm, full angle field-of-view (FOV) of 28° determined by a Gershun tube. The goal of using the Maya2000 Pro is to measure the chlorophyll fluorescence response and thus covering the range of interest: ~730-750 nm. The number of pixels, which is the same as detector elements on the CCD (Charged-Coupled-Device) array, is 2068 for this specific spectrometer

The JAZ spectrometer from Ocean Optics covers a range of 340-1024 nm, FOV is 180° determined by the cosine corrector and was used to measure incident light from the lamps. The number of pixels is 2048 for this spectrometer.

3.2 Chlorophyll fluorescence measurement

The fluorometer used in this study has been developed by Heliospectra and measures chlorophyll fluorescence response from the plants on remote distance. The device consists of a gershun tube with a limited FOV of 14° and uses a photodiode along with an optical bandpass filter with Center Wavelength (CWL) of 740 nm and Full

Width-Half Max FWHM of 10 nm. The fluorometer contains a low-pass filter, non-causal filter and has a sampling frequency of 3.5 kHz. This device was used to characterise chlorophyll fluorescence responses.

3.3 Lamps

The lamps were provided by Heliospectra AB and used for both background illumination and excitation pulses. Two LX60G and one LX60G split plate were used for background illumination. One LX60G was used for light excitation. The lamps are controlled by specifying an input ranging from 0-1000 for each of the lamp's channels; 450 nm, 660 nm and 5700K for LX60G, respectively 450 nm, 451 nm, 660 nm, 661 nm, 5700K and 5701K for the LX60G split plate. The specific settings for each lamp are denoted as BR($X_{450} : X_{660} : X_{5700K}$) respectively BR($X_{450} : X_{451} : X_{660} : X_{661} : X_{5700K} : X_{5701K}$). Control and monitoring are managed either through Heliospectra's own web interface or software. The reason for using three LED lamps as background light was to enable high intensities during the experiments.

3.4 Plants

For all the experiments, groups of Basil (*Ocimum basilicum*) Aroma 2 were used. This variety of Sweet Basil is one of the most common herbals in greenhouse production. The specific plants were never replaced. Hence, used throughout the entire study.

3.5 Reference measurement

In line with the objective to examine chlorophyll fluorescence in relation to plant health, a reference plant unit was included in one of the conducted experiments. This required collection and evaluation of a separate set of parameters generally used for determining plant health. The hardware used to collect these parameters will be described later in this section.

3.5.1 Chlorophyll fluorescence

A JUNIOR-PAM chlorophyll fluorometer developed by Walz measures chlorophyll fluorescence response on leaf level, detected by using modulated measuring light, actinic light and saturating pulses. The JUNIOR-PAM chlorophyll fluorometer was used in order to collect reference measurements during the experiments specifically designed to determine whether plant stress could be detected by the PWM method or not.

3.5.2 Canopy temperature

The IR Radiometer from APOGEE is used for measuring infrared radiation on remote level [27], used to determine surface temperature on different objects with a half-angle FOV of 14°. Plant canopy temperature and housing temperature were also measured in this study to investigate the difference in heat dissipation when conducting experiments on salt exposed plants.

3.6 Experiment setup

The experiments were conducted in a closed lab environment and the experimental unit consists of a Styrofoam box (Figure 3.1) with the dimensions of approximately 70 x 70 x 90 (w x d x h) cm. The Styrofoam box had an open top to make room for the four lamps, one fluorometer, one IR radiometer and one spectrometer Maya2000 Pro. Two Junior-PAM fluorometers and one spectrometer JAZ were placed on canopy level along with the plants. The distance between the canopy and the lamps ranged between 91 and 101 centimetres.

Figure 3.1: Front view of the experimental unit. 1) Background lamp. 2) Excitation lamp. 3) Fluorometer. 4) IR Radiometer. 5) Maya2000 Pro. 6) JUNIOR-PAM. 7) JAZ.

The experimental unit was also used to grow plants for the experiments. For this purpose, a specific light schedule was designed and applied on one of the lamps when experiments were not conducted.

4
Methods

In order to retrieve sufficient knowledge and to validate the importance of chlorophyll fluorescence research in commercial farming, a literature study was first conducted. A set of experiments was later formulated in order to characterise the chlorophyll fluorescence gain which is related to the main research questions mentioned in Section 1.4. The decided experiments were then performed, and data were stored for validation. Stored data laid out the foundation determining whether using PWM signals as incident light is sufficient in order to achieve the desired chlorophyll fluorescence response in different environments.

4.1 Lamp configuration

This section presents the two configurations used for the excitation lamp and the configurations for the background lamps. These configurations were used in order to achieve the different conditions required for the experiments performed.

4.1.1 Excitation lamp settings

The induced light from the excitation lamp was pulsed with BR(100:0:0) at a frequency of approximately 1 Hz and a duty cycle of 50%. A frequency of exactly 1 Hz could not be achieved (Figure 4.1) due to limitations in the communication with the lamp. Communication with the lamp is done by manually write to its web user interface, which results in a small, but inconsistent, delay. This manual operation also generates a rise time for each excitation. This could have been avoided by configuring the lamp to have a PWM frequency of 1 Hz, but such a configuration was not possible to generate due to firmware limitations.

A faster excitation was obtained by continuously inducing light from the excitation lamp with settings BR(50:0:0). The lamp was configured to have an output signal consisting of pulses with a frequency of 208 Hz, and a duty cycle of 50%. Light intensities for each excitation lamp configuration can be found in Table 4.1.

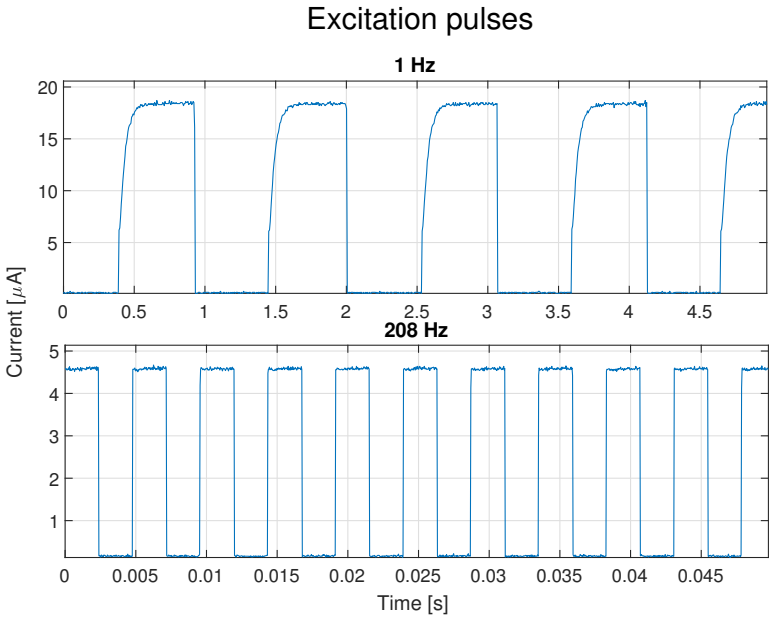

Figure 4.1: The two different excitation frequencies used in this study.

Table 4.1: Light intensity amplitude at the two different excitation frequencies.

Frequency [Hz]	**PFD** $[\mu molm^{-2}s^{-1}]$
1	13.5
208	5.4

Initially, the excitation frequency of 1 Hz was used to investigate whether the chlorophyll fluorescence response could be achieved using a PWM signal. A frequency of 1 Hz was considered to be high enough to capture the dynamics of the chlorophyll fluorescence response in a dynamic environment. However, an excitation frequency of 1 Hz could be expected to be disturbing in a working environment and also highly deviates from the lamp's default PWM frequency. This motivated the introduction of a higher excitation frequency that is clearly beyond the perception of the human eye. The upper bound of this frequency was restricted by the sample rate of the fluorometer which was known to be approximately 3.5 kHz. In consultation with Heliospectra AB, using a lamp configuration with an excitation frequency of ~208 Hz was decided.

4.1.2 Background lamp settings

The two different excitation configurations were applied according to Subsection 4.1.1 in parallel with sequences of different background lamp settings generating varying ambient light environments, see Table 4.2. The length of each background step was initially set to 7 minutes, considered to be a sufficient length based on findings presented in [28]. However, early pre-experiments for this thesis showed

this to not to be sufficient in order to reach steady-state chlorophyll fluorescence at each level - probably as a consequence of using higher incident light intensities than in [28]. As a consequence, each background step was extended to 12 minutes for Sequences 1-3, see below.

Table 4.2: Lamp settings (blue:red:white) used to create the various incident background light environments applied in this study.

Step	**Sequence 1**	**Sequence 2**	**Sequence 3**	Step	**Sequence 4**
1	100:100:0	100:100:0 - One lamp	100:100:100 - One lamp	1	*
2	200:200:0	100:100:0 - Two lamps	100:100:100 - Two lamps	.	S
3	300:300:0	100:100:0 - Three lamps	100:100:100 - Three lamps	.	U
4	400:400:0	150:150:0	200:200:200	.	N
5	500:500:0	200:200:0	300:300:300	.	L
6	600:600:0	250:250:0	400:400:400	.	I
7	700:700:0	300:300:0	500:500:500	.	G
8	800:800:0	500:500:0	600:600:600	.	H
9	900:900:0	700:700:0	800:800:800	.	T
10	1000:1000:0	1000:1000:0	1000:1000:1000	N	*

- **Sequence 1** was implemented in order to measure the emitted light from the plants at the full range of the lamp settings (0-1000). The white channel was excluded from this experiment in order to avoid interference with the fluorometer since white light partially consists of wavelengths in the chlorophyll fluorescence range.
- **Sequence 2** was implemented due to findings from running experiments using Sequence 1. It was found that a local maximum for chlorophyll fluorescence gain was located in the first half of the range of background PAR intensities, and a higher resolution than the initial background steps was consequently implemented for these intensities.
- **Sequence 3** was implemented to investigate if higher incident background light intensities could cause light saturation of the plants. Higher intensities were obtained by including the white channel in the sequence.
- **Sequence 4** used the background lamps to simulate a true sunlight environment. The step time between each lamp setting is different from the other sequences. In order to mimic true sunlight as good as possible the step time was set to 0.2937 seconds which is related to the original sampling rate, see Subsection 4.2.1.

4.2 Sunlight simulation

In order to investigate whether it is possible to measure chlorophyll fluorescence gain in a sunlight environment, a method for simulating sunlight using an arbitrary number of background lamps was developed.

Two models, one for each channel, were derived. The models characterise the background lamp's relation between lamp input and output light intensity used to yield

the same dynamic behaviour as in Figure 4.3. The model was obtained by collecting irradiance measurements from each separate channel using the JAZ spectrometer. The white channel in the lamp was excluded from the measurements since it contains wavelengths which cover the wavelengths of chlorophyll fluorescence. The measurements contain an intensity for a given lamp input (Table 4.3). Two measurements, one for each channel, were performed and ten samples for each channel were collected. The lamp input was increased each second and sampling was performed with a frequency of 2 Hz.

Table 4.3: Lamp input for characterising blue and red channel in relation to light intensity.

Blue channel			**Red channel**		
Blue	**Red**	**White**	**Blue**	**Red**	**White**
100	0	0	0	100	0
200	0	0	0	200	0
300	0	0	0	300	0
400	0	0	0	400	0
500	0	0	0	500	0
600	0	0	0	600	0
700	0	0	0	700	0
800	0	0	0	800	0
900	0	0	0	900	0
1000	0	0	0	1000	0

The dynamics of the LX60G lamps that were used in this study are partially linear due to the different dynamics in different regions of lamp input. The region [100-1000] is by design linear with minor abnormalities. Linear regression is used to derive a linear model that characterises output light in relation to lamp input:

$$\begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix} = \begin{bmatrix} p_1 \\ p_2 \\ \vdots \\ p_n \end{bmatrix} \begin{bmatrix} x_1 & x_2 & \cdots & x_m \\ \vdots & \ddots & & \\ x_1 & & & \end{bmatrix} + \begin{bmatrix} \beta_1 \\ \vdots \\ \beta_n \end{bmatrix} \quad \text{where} \quad i = 1, 2, ..., n, \tag{4.1}$$

where n is the number of lamp inputs, and y_i describes output intensity for a given lamp input, x_i. The variable p_i describes the coefficient for $y_i(x_i)$ and β_i describes the intersection with the y-axis. The estimated model, y, for channels blue and red can be seen in Figure 4.2.

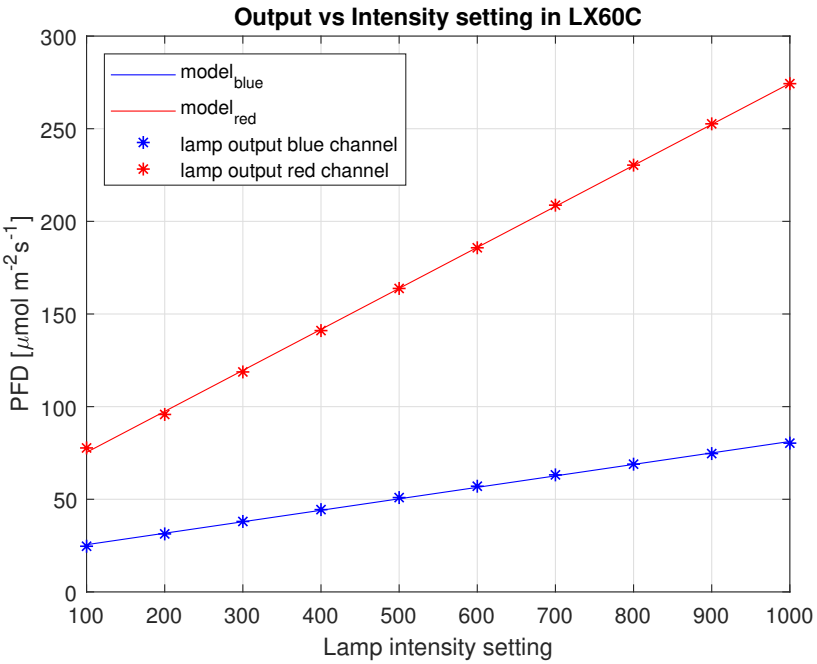

Figure 4.2: The derived model of the LX60G lamps using linear regression. The blue line describes the relation between output light and lamp input for the blue channel. The red line describes the relation between output light and lamp input for the red channel. The marks describe the samples that were collected in each measurement.

These two models were used to determine the output intensity for each lamp input ranging from [100-1000] which enabled mapping a specific lamp input to a corresponding output intensity. A data set, y_{model}, was derived containing the mapped values used to determine the lamp input necessary to obtain a simulation similar to the real sunlight data.

4.2.1 Sunlight intensity data

The sunlight data used for simulation, collected and provided by Heliospectra AB, are presented in Figure 4.3. As shown, a fraction of the complete data was segmented and used for simulating sunlight using the developed models. This specific segment was chosen due to its suitable variations and magnitude. The data set contains irradiance intensities presented as PFD $[\mu molm^{-2}s^{-1}]$ which were converted to lamp input for two of the lamps three different channels, blue and red.

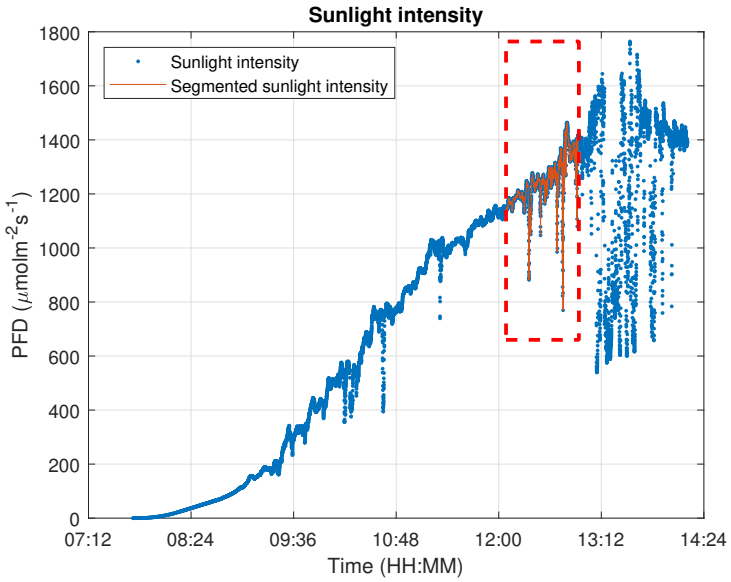

Figure 4.3: Collected sunlight intensities. Data inside checked rectangle was segmented and used for simulating the model.

When running a simulation, the specific lamp input was found by comparing the models to the sunlight data for each time-step. Due to limitations of the output intensity of the lamp, the magnitude of the sunlight data had to be down-scaled. This was done by dividing the maximum value of the sunlight with the maximum possible intensity generated from the lamp according to the model, i.e.

$$ratio = \frac{max(sunlight)}{max(y_{model})} \tag{4.2}$$

This ratio was applied to the complete sunlight data in order to fit the data within the output range of the lamp. Further, restrictions on the resolution of the lamp, meaning that a lamp setting can only be represented as an integer, resulted in that not all intensities in the sunlight data could be simulated using the lamp. Therefore, for each data point, the integer x_i generating the closest intensity for both the blue and the red channel was calculated by

$$x_i = \min_y |y_{model} - y_{sunlight,i}| \quad \text{for i} = 1 \ ... \ \text{N} \tag{4.3}$$

Applying this on three lamps generated simulated sunlight according to Figure 4.4. By using additional lamps, a higher intensity close to the real sunlight could also be achieved.

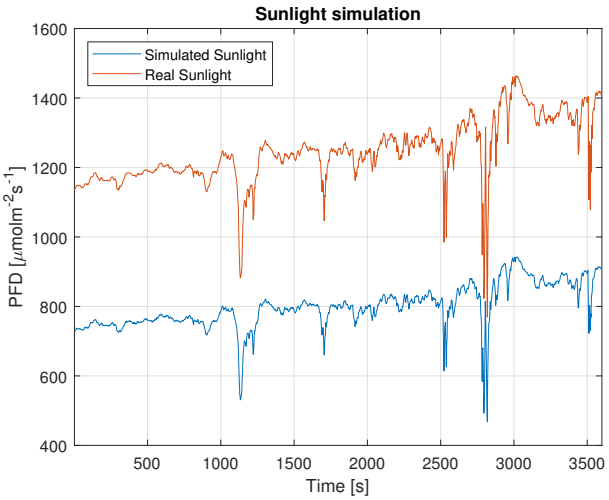

Figure 4.4: Real sunlight data in PAR along with simulated sunlight.

A higher resolution than the collected raw sunlight data was desirable in order to achieve a more true sunlight behaviour. The sunlight data was originally sampled approximately every 2.7 seconds, which was considered too seldom since sunlight could possibly change during this time interval. Linear interpolation was performed in order to increase the resolution of the sunlight simulation. A measurement of the sunlight in Gothenburg was conducted with a frequency of 10 kHz to examine whether a linear interpolation could be performed without the interference of too much noise. The weather conditions were blue sky and no clouds. It could be seen that the dynamics of the sunlight were small enough to validate the use of linear interpolation. In other words, the sunlight intensity was relatively constant on such a short time interval. The linear interpolation performed resulted in a sample every 0.2937 seconds.

4.3 Calculating chlorophyll fluorescence gain

Three main steps were used to calculate chlorophyll fluorescence gain from measured chlorophyll fluorescence response. This procedure was conducted on fluorescence data measured using both the fluorometer and the spectrometer Maya2000 Pro.

1. **Outlier reduction** - A median filter was used in order to reduce the impact of outliers as a pre-processing step before calculating the emitted chlorophyll fluorescence gain. This procedure is visualised in Figure 4.5. The implemented median filter was a first-order filter which ran through the collected measurements entry by entry and created segments including a number of preceding and subsequent data points. These segments were used to calculate a median value replacing the raw collected measurements.

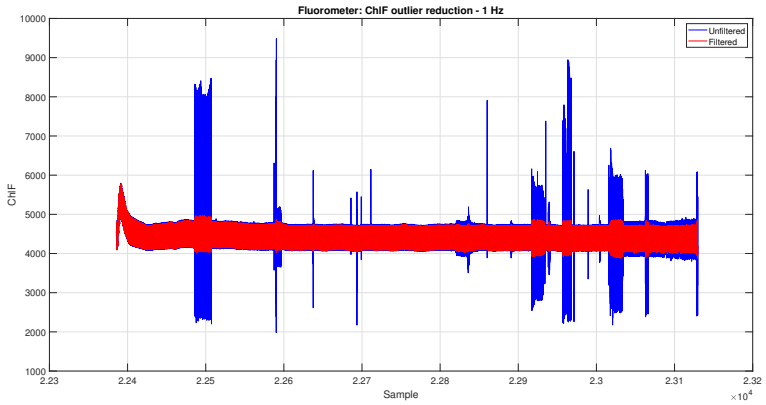

Figure 4.5: The blue line represents raw chlorophyll fluorescence data and the red line represents the chlorophyll fluorescence data after reduction of outliers. All data were collected with the fluorometer.

2. **Average filter** - An average filter was implemented in order to reduce the noise level. The filter was equivalent to three passes of a sliding average filter, which used a pre-determined window width to derive the mean of each excitation. For the experiments using an excitation frequency of 1 Hz, the window width was 50 samples, and for the experiments using an excitation frequency of 208 Hz, the width was 4 samples.

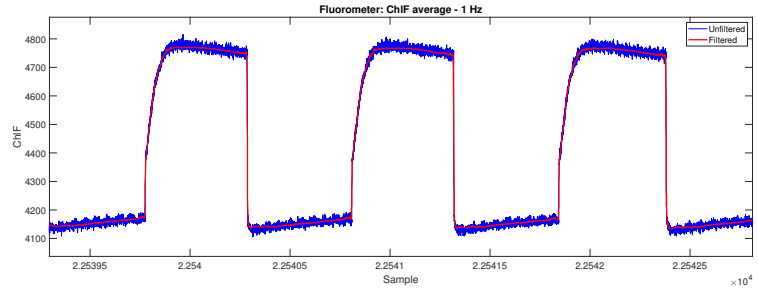

Figure 4.6: The blue line represents raw chlorophyll fluorescence data and the red line represents the chlorophyll fluorescence data after averaging the top and bottom of each excitation. All data were collected with the fluorometer.

3. **Local extreme points** - Local maximum and local minimum points were found to determine the gain at each excitation. Each local maximum was found by defining an interval corresponding to the length of one excitation and locating the maximum value within that interval. The data was later inverted and the same procedure was conducted in order to find the local minimum for that specific period. A chlorophyll fluorescence gain could be derived from subtracting each local minimum from the corresponding local maximum.

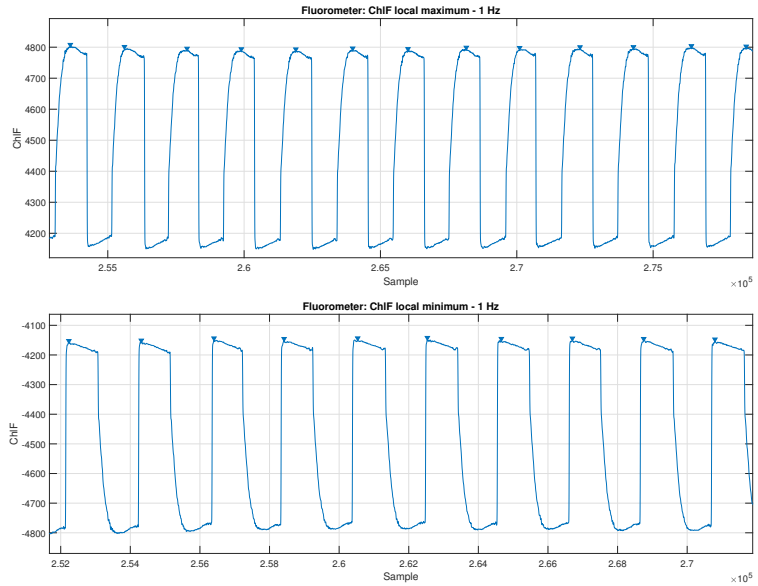

Figure 4.7: The upper plot presents the local maximum of each excitation. The lower plot presents the inverted data in order to find the minimum of each excitation.

Since the input of the excitation light was constant for each experiment, the intensity of the excitation was expected to be constant. To ensure this to be valid, a comparison between excitation light and background light, denoted as ΔI, was conducted (Figure A.1 in Appendix A). To determine the effect of these deviations on the chlorophyll fluorescence gain parameter, another comparison was conducted where the impact of ΔI was taken into consideration. Results from this analysis showed that the impact of ΔI is negligible (Figure A.2 in Appendix A). Thus, chlorophyll fluorescence gain was throughout this study calculated without taking ΔI into consideration.

4.4 Analysing chlorophyll fluorescence

In order to draw conclusions regarding the chlorophyll fluorescence response, the signal was analysed in three different ways;

- Chlorophyll fluorescence response with respect to time throughout the entire experiment.
- Chlorophyll fluorescence response with respect to time for a separate background light level.
- Chlorophyll fluorescence pulse response with respect to both time and incident light intensity.

Chlorophyll fluorescence gain was analysed in two ways. It is shown in [28] that the chlorophyll fluorescence response exhibits changes in its dynamics over time when the plant is exposed to excessive light. Hence, this study aims to analyse also changes in chlorophyll fluorescence gain with respect to time and at different

light environments, referred to as ambient light dynamics and intensities. Thus, calculation of chlorophyll fluorescence gain was also done with respect to incident light.

An average steady-state chlorophyll fluorescence gain was derived from the last 3 minutes of each background step level (Table 4.2). The steady-state chlorophyll fluorescence gain was investigated in order to characterise its behaviour relative to different incident background intensities.

4.5 Plant stress validation

When validating obtained chlorophyll fluorescence gain to detect plant stress, measurements were compared to the parameters described in Section 2.4. To obtain sufficient parameters, a JUNIOR-PAM fluorometer was used before, during and after each experiment. The measurements were collected on leaf level on one or two plants which were randomly selected from the current batch. The leaf was placed in a metallic clip situated a few millimetres from the optical fibre. The leaves used for measurements were chosen among the most recent fully developed pair of leaves.

Firstly, F_v/F_m was collected on a dark-adapted leaf before initiating each of the experiments. The same parameter was collected throughout the entire experiments with a sample rate of 1 sample per minute. After finishing the experiments, F_v/F_m was continued to be collected with the same sample rate in order to record a recovery period when the lamps were turned off.

The parameters were then used to derive NPQ and Y(II). These composed the foundation for analysis whether there was a correlation between calculated chlorophyll fluorescence gain and plant health.

The chlorophyll fluorescence measurements obtained for plant stress validation were validated using two different approaches. These approaches will be denoted as *average chlorophyll fluorescence gain level* and *excitation level* throughout the report. The average chlorophyll fluorescence gain was obtained by calculating the mean of the chlorophyll fluorescence gain from the three last minutes on each background light level. This generated information about the chlorophyll fluorescence gain after the plant had been light adapted to respectively background light intensity. The chlorophyll fluorescence on so-called excitation level generates potential information about the dynamics of each excitation. This response was expected to vary depending on plant health.

4.6 Data processing

The measurements obtained from using the spectrometers contains a bias over the whole spectrum which has to be compensated for. This bias is dependent on integration time in the spectrometers which is dynamically set as input to the specific spectrometer in order to yield an optimal SNR. Integration time determines the

amount of time the spectrometer absorbs light at each sample. A higher integration time yields a greater signal intensity in the unit counts but needs to be regulated to avoid saturation. The integration time is determined based on operating point and light intensity, which is based on the pixels with the highest count that corresponds to ≈ 80-90 % of the level of saturation at 64 000 counts [29]. Therefore, the integration time is required to be adjusted depending on the experiment. In this study, the aim is to collect samples only when an excitation is either on or off which results in an integration time smaller or equal to the length of an excitation. Thus, potentially resulting in the pixel with the highest count not reaching ≈ 80-90 % of the level of saturation. As a result, a lower SNR is obtained as a consequence of the excitation times in this study.

4.6.1 Dark zero correction

Measuring the bias, also called spectrum correction, is performed by collecting spectrometer data when the unit has no exposure to light. This is done by covering the spectrometer with a cap to delimit the light intake. The measurement is repeated for different integration times resulting in a table of dark zero spectra for each integration time. The dark zero correction is done by subtracting the dark zero spectrum, $y_{b,i}$, from the output signal, $y_{raw,i}$, of the spectrometer for the corresponding integration time, i, that was used in the experiment, i.e.

$$y_i = y_{raw,i} - y_{b,i}. \tag{4.4}$$

This is usually performed in parallel with the data collection, but for a fixed integration time. In this study, it is performed as a post-processing feature due to the use of dynamic settings of the integration time as a function of light intensity and excitation time.

4.6.2 Electrical dark zero correction

Another factor affecting the dark spectrum is caused by the dark current, which is dependent on the sensor's temperature [30]. This part of the dark spectrum is identified by comparing the dark pixels in the spectrometer from the dark spectrum to the raw data of the spectrometer. These pixels are subtracted from the raw spectrum. The dark pixels can be identified by analysing the beginning and end points of the dark spectrum.

5
Experiments

This chapter describes the experiments performed for this thesis. The experiments were designed by combining the different background light sequences with the two different excitation lamp configurations described in Section 4.1. This resulted in five different experiments, with the common goal of calculating chlorophyll fluorescence gain under different conditions. The combinations are presented in Table 5.1 and described in detail by the flowcharts in Figure 5.1 and Figure 5.2

Table 5.1: Experiments conducted by combining incident background light with a specific excitation frequency.

	Excitation 1 Hz	**Excitation 208 Hz**
Experiment 1 - 100:100:1000	X	X
Experiment 2 - Low intensities	X	X
Experiment 3 - Sunlight	X	X
Experiment 4 - Salt stress	X	

5.1 General information

All the experiments conducted in this study has some common features. Firstly, the species used in all experiments was basil (*Ocimum basilicum*) Aroma 2.

Before the experiments started, the plants were dark-adapted for 20 minutes. Typically, a minimum dark-adaption time of 20-30 minutes has to be carried out to ensure that all the PSII reaction centres in the plants are in an open state [31]. However, in this study, the dark-adaption was considered of less importance since the behaviour of the emitted chlorophyll fluorescence is studied rather than its magnitude. Further, the conducted dark-adaption should not be considered as a proper dark-adaption due to exposure of low ambient light.

Each experiment started with collecting Fv/Fm data using the JUNIOR-PAM, followed by applying the first background light level for the used sequence (Table 4.2) for 10 minutes without collecting any sensor data except from the JUNIOR-PAM data. This was done for all experiments except Experiment 3, described in Section 4.2. The reason for applying this initial phase was to ensure that the plants reached chlorophyll fluorescence steady-state before any measurements were collected. When the initial phase was finished, the same background light level was retained and kept

for 12 minutes. Simultaneously, the excitation lamp was initiated and measurements using fluorometer, spectrometers and IR radiometer were collected. The entire procedure was repeated for each step in the sequences, see Figure 5.1 and Figure 5.2 for detailed work-flow. Y(II) was measured using the JUNIOR-PAM throughout the whole experiment.

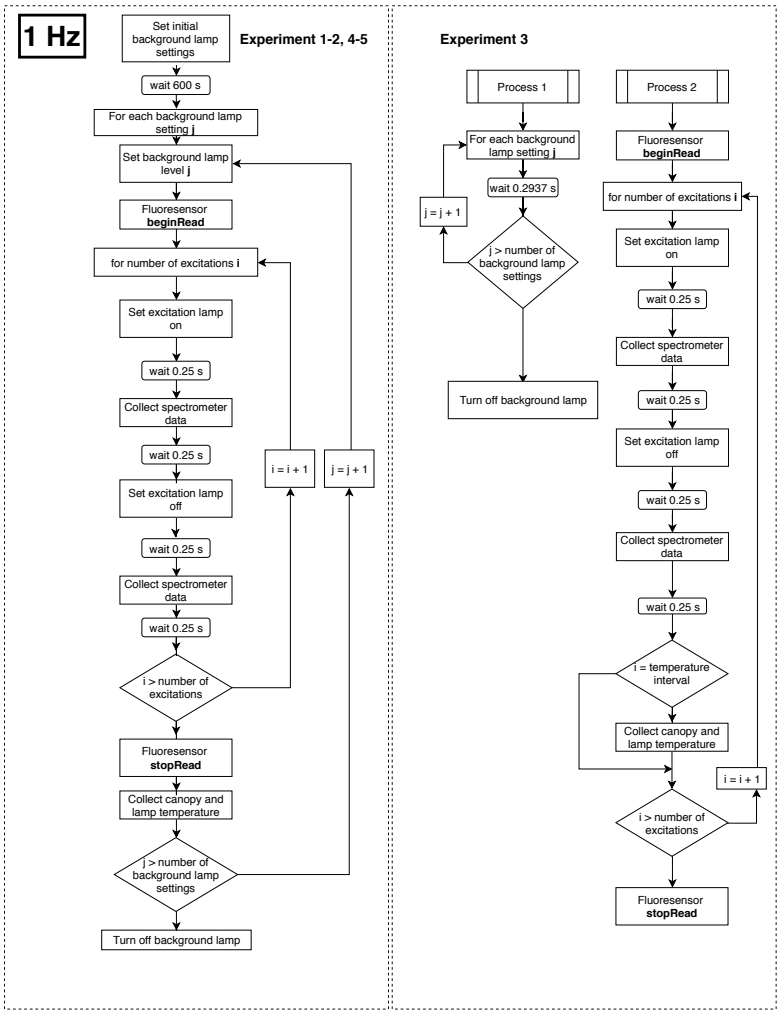

Figure 5.1: Work scheme when conducting experiments with an excitation frequency of 1 Hz.

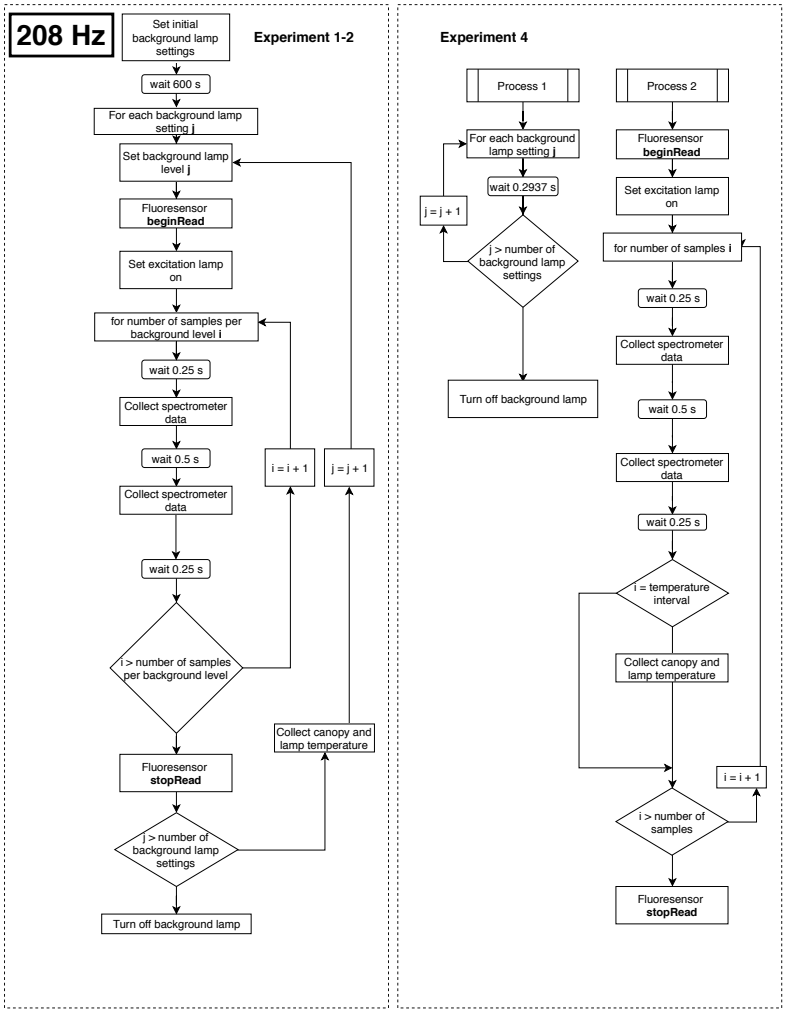

Figure 5.2: Work scheme when conducting experiments with an excitation frequency of 208 Hz. Note that the same sampling frequency of the spectrometer was used also in this setup, but the collected data was disregarded due to the high excitation frequency. Thus, the chlorophyll fluorescence response was measured by using only the fluorometer.

Since plant stress limits the growth, a set of experiments were also conducted on stressed plants to investigate how the chlorophyll fluorescence gain is related to the current stress level. A JUNIOR-PAM chlorophyll fluorometer was used during the experiments to obtain reference measurements used in order to ensure that plants exposed to stress actually became stressed.

5.2 Experiment 1 - Standard

This experiment used a background lamp sequence according to Sequence 1 in Table 4.2. Two repetitions of this experiment were conducted for each of the two different excitation frequencies (Table 4.1). Figure 5.1 presents the procedure that was performed during the experiment. Two trays of basil were used, which contained 12

and 13 plants respectively. This specific experiment was developed to investigate whether a similar pattern of the chlorophyll fluorescence response could be achieved using different excitation frequencies. A chlorophyll fluorescence with the same pulsing behaviour would make it possible to distinguish chlorophyll fluorescence caused by an excitation from chlorophyll fluorescence caused by ambient light.

5.3 Experiment 2 - Higher resolution at low intensities

More information about chlorophyll fluorescence gain at lower light intensities was desirable. Consequently, another background light sequence was developed. This sequence was used in Experiment 2 for two separate runs in combination with an excitation lamp frequency of both 1 Hz and 208 Hz. As in Experiment 1, two trays of basil containing 12 and 13 plants were used during each run.

5.4 Experiment 3 - Sunlight

Since one of the objectives for this study was to distinguish chlorophyll fluorescence gain in sunlight environment, background lamp Sequence 4 described in Section 4.2 was used. Both the excitation lamp frequencies 1 Hz and 208 Hz were used in separate runs. Repeatedly, two trays of basil with 12 and 13 plants were used during each run.

5.5 Experiment 4 - Light stress

The second objective of this study was to investigate chlorophyll fluorescence gain in relation to plant health. Growth and development of plants are highly affected by light intensities [32]. In order to study the effect of excess light, background light intensities according to Sequence 3 in Table 4.2 were used. In this sequence, the white channel was included which results in saturated chlorophyll fluorescence measurements at high intensities using the fluorometer. Thus, only the spectrometer Maya2000 Pro was used in this experiment for chlorophyll fluorescence measurements. Due to limitations in sampling frequency of the spectrometer Maya2000 Pro, this experiment was performed only with an excitation frequency of 1 Hz. Two trays of basil with 12 and 13 plants were used for the experiment.

5.6 Experiment 5 - Salt stress

To study a more diverse set of stress parameters, the subjected plants were repeatedly exposed to salt water with a concentration of 500 mM/l. Each experiment was repeated three times. The irrigation protocol used is given in Table 5.2. Background light Sequence 2 described in Table 4.2 along with an excitation frequency of 1 Hz was used for all repetitions. The reason why an excitation frequency of 208

Hz was not used for this experiment was due to excessive noise of the fluorometer measurements caused by hardware malfunctions. The lamp configurations used in this experiment was also applied to a reference unit of plants that had been irrigated with solely nutrient solution. This was done in order to ensure that the unit which had been exposed to salt water actually showed a deviating behaviour.

Table 5.2: Irrigation scheme for salt treated unit and reference unit. The concentration of the salt solution was 500 mM/l.

	Salt treated			**Reference**	
	Tray 1	Tray 2		Tray 1	Tray 2
December 2 12:00 PM	500 ml	500 ml	December 2 12:00 PM	500 ml	500 ml
December 3 10:50 AM	**Run 1**	**Run 1**	December 3 2:20 PM	**Run 1**	**Run 1**
December 4 9:00 AM	1000 ml	1000 ml	December 3 9:00 AM	1000 ml	1000 ml
December 4 12:04 PM	**Run 2**	**Run 2**	December 4 3:14 PM	**Run 2**	**Run 2**
December 5 8:57 AM	**Run 3**	**Run 3**	December 12:32 PM	**Run 3**	**Run 3**

Two trays of basil containing 12 plants each were used both for the salt treated unit and the reference unit.

6
Results

This chapter presents the results obtained from the experiments carried out in this study. The results mainly consist of chlorophyll fluorescence data produced and examined in different ways. Chlorophyll fluorescence measured in simulated sunlight is presented and examined in order to gain useful information about the potential of using a PWM signal to distinguish chlorophyll fluorescence in a dynamic environment. A detailed review of the relation between reference measurements and measurements from plants treated with salt is presented to identify possible trends on the chlorophyll fluorescence response for plants exposed to stress.

6.1 Chlorophyll fluorescence response

Measurements presented in Figure 6.1 and Figure 6.2 were collected during the same experiment but clearly have different characteristics on excitation level due to the sampling frequency of the two sensors. The trends on background step level show similar behaviour. Note that the y-axis of the background step differs between the two figures. The measurements presented in Figure 6.1 are in $\mu molm^{-2}s^{-1}$, whereas measurements in Figure 6.2 are expressed as counts.

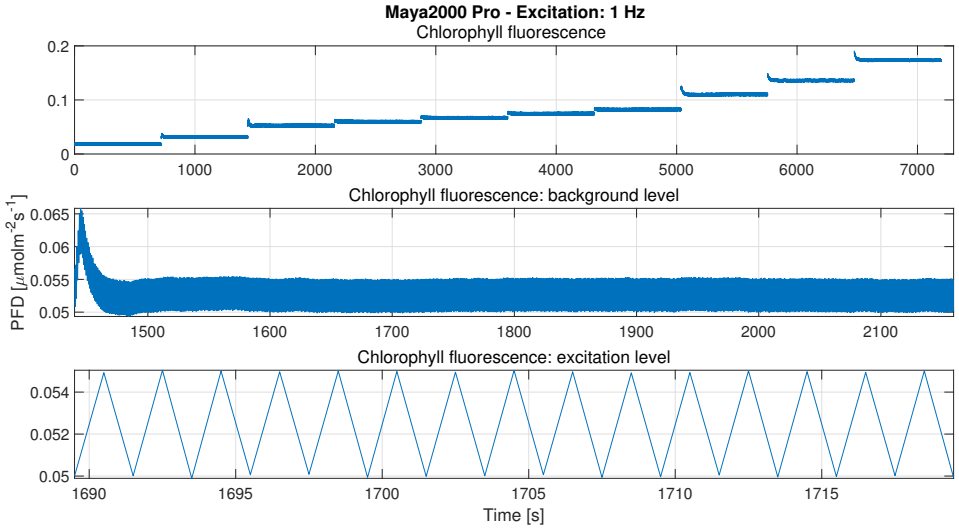

Figure 6.1: Chlorophyll fluorescence response for complete experiment, on background step level and on excitation step level. Measurements were collected using spectrometer Maya2000 Pro. The presented background level is from time ~1500-2250 seconds which had an average incident background light of 264.8 $\mu molm^{-2}s^{-1}$.

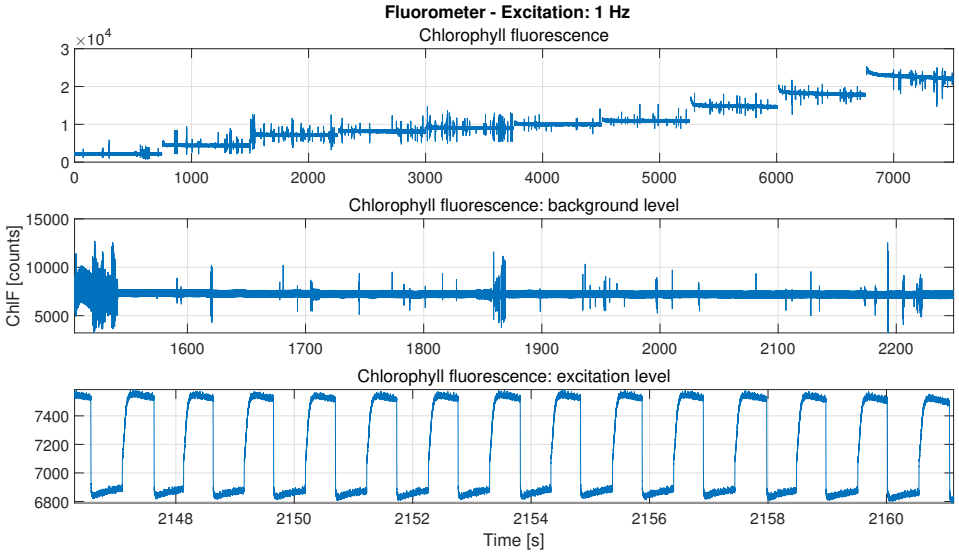

Figure 6.2: Chlorophyll fluorescence response for the complete experiment at 1 Hz, on background step level and on excitation step level. Measurements were collected using the fluorometer. The presented background level is from time ~1500-2250 seconds which had an average incident background light of 264.8 $\mu molm^{-2}s^{-1}$.

As can be seen in Figure 6.3 the chlorophyll fluorescence response is clearly distinguishable also when running the excitation lamp on 208 Hz. The measurements show a tendency of double maximum and minimum for each excitation and have a

different shape compared to the pulse responses from using an excitation frequency of 1 Hz. Note the various peaks on background step level which are probably caused by internal hardware noise in the fluorometer.

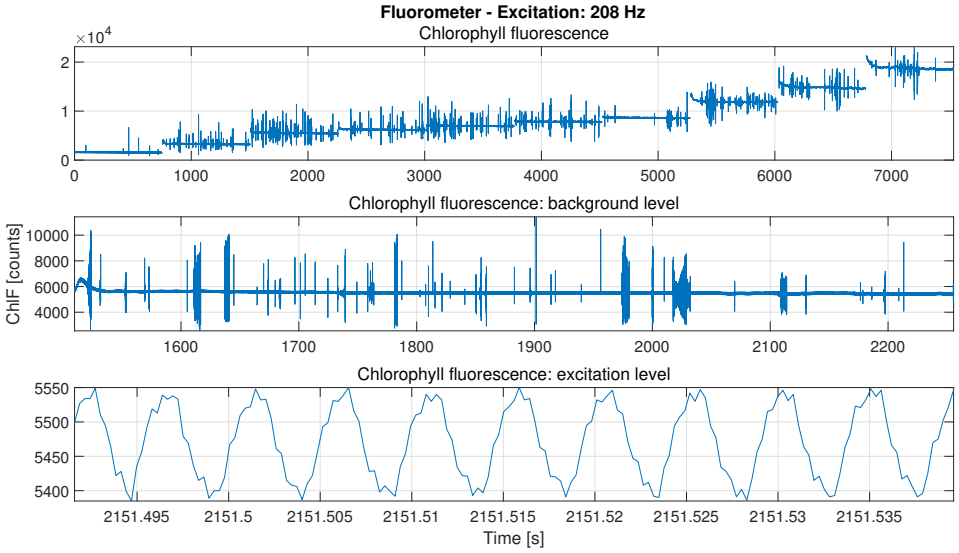

Figure 6.3: Chlorophyll fluorescence response for the complete experiment at 208 Hz, on background step level and on excitation step level. Measurements were collected using the fluorometer. The presented background level is from time ~1500-2250 seconds which had an average incident background light of 269.8 $\mu molm^{-2}s^{-1}$.

6.2 Chlorophyll fluorescence gain

To distinguish chlorophyll fluorescence in a dynamic environment it is crucial to identify the chlorophyll fluorescence gain. This section presents the chlorophyll fluorescence gain in relation to time and light intensity which gives information about the characteristics of the dynamic behaviour under different light conditions.

6.2.1 Experiment 1 - Standard

It can be seen from the measurements in Figure 6.4 that the two sensors yield different behaviour at an excitation frequency of 1 Hz. The spectrometer Maya2000 Pro shows a more consistent as well as a distinct decline in chlorophyll fluorescence gain for increasing background light intensities.

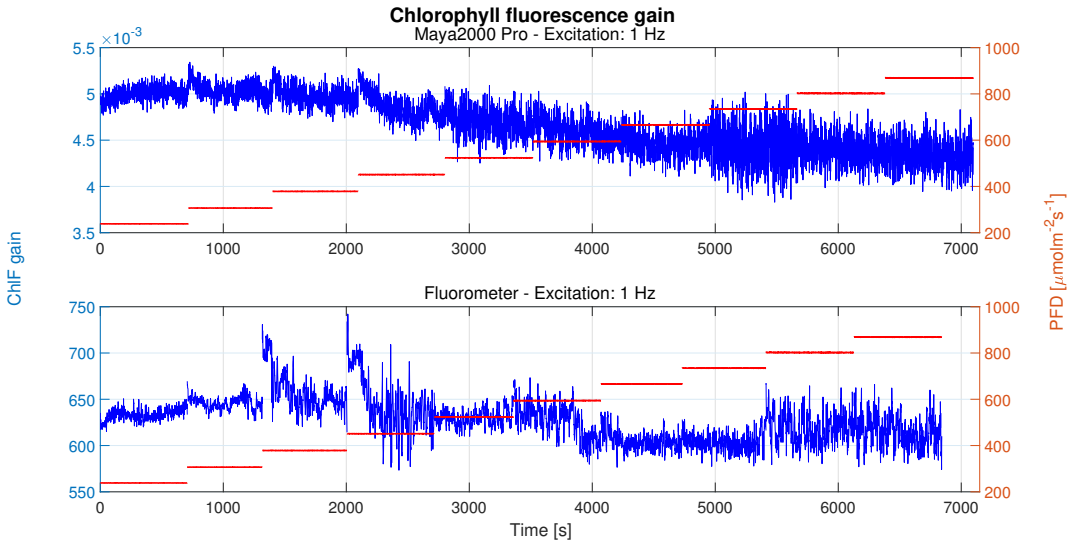

Figure 6.4: The upper plot presents chlorophyll fluorescence gain using the spectrometer Maya2000 Pro along with incident background light. The lower plot shows chlorophyll fluorescence gain using the fluorometer along with incident background light. Both plots present the chlorophyll fluorescence gain derived from the same experiment using an excitation frequency of 1 Hz.

Once again, according to Figure 6.5, the fluorometer shows a varying behaviour for each background light intensity at an excitation frequency of 208 Hz. A lower amplitude in chlorophyll fluorescence gain was obtained compared to using the fluorometer for an excitation frequency of 1 Hz in Figure 6.4, partly due to a smaller step size. A decline for increasing background light intensities can also be noted when using an excitation frequency of 208 Hz.

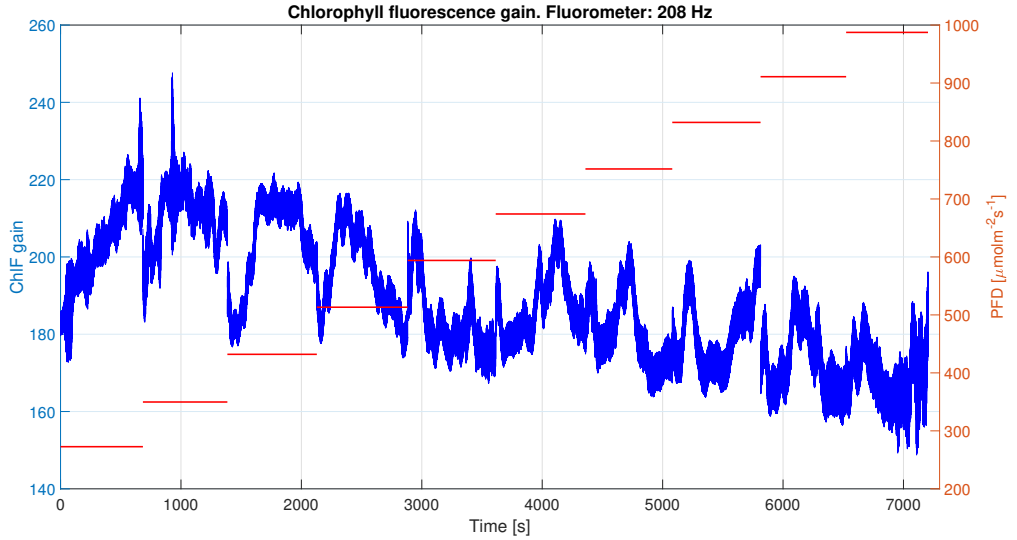

Figure 6.5: Chlorophyll fluorescence gain collected using the fluorometer, along with incident background light and an excitation frequency of 208 Hz.

6.2.2 Experiment 2 - Low intensities

Figure 6.6 showing the results for 1 Hz. This shows a local maximum for intensities ranging from approximately 200-300 $\mu molm^{-2}s^{-1}$. This trend is the most apparent when using the spectrometer Maya2000 Pro but can also be seen by the fluorometer.

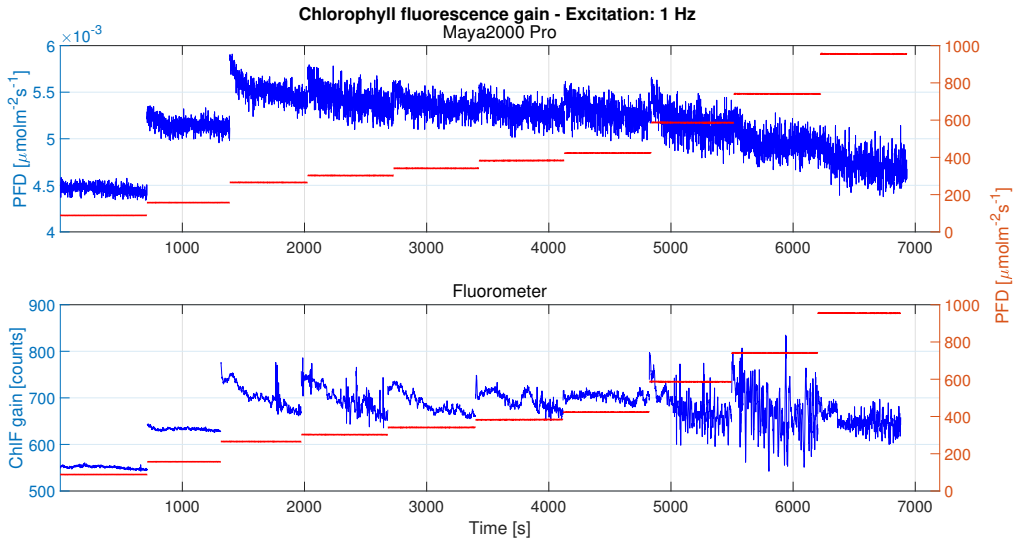

Figure 6.6: The upper plot presents chlorophyll fluorescence gain collected using the spectrometer Maya2000 Pro along with incident background light. The lower plot shows chlorophyll fluorescence gain collected using the fluorometer along with incident background light. Both plots present the chlorophyll fluorescence gain derived from the same experiment using an excitation frequency of 1 Hz.

For data at 208 Hz, large variations for the chlorophyll fluorescence gain can be seen, see Figure 6.7. The results show a lack of both consistency and distinct trends.

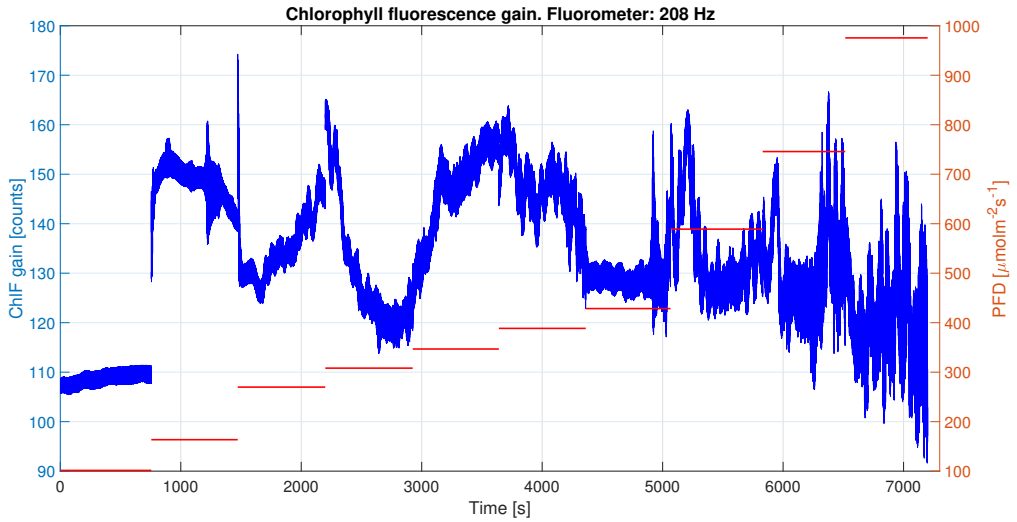

Figure 6.7: Chlorophyll fluorescence gain collected using the fluorometer along with incident background light and an excitation frequency of 208 Hz.

6.2.3 Experiment 3 - Sunlight

The measurements presented in this subsection shows the chlorophyll fluorescence response, incident background light intensities and chlorophyll fluorescence gain based on the sunlight simulation derived in Section 4.2 as background light environment. This experiment investigated the effects on a fast-changing light environment and its effects on calculating the chlorophyll fluorescence gain.
Results from using the Maya2000 Pro (Figure 6.8) and fluorometer (Figure 6.9) show that the chlorophyll fluorescence gain fluctuates at ~1100, 1600, 2500, 2750 and 3400 seconds. These fluctuations coincide with larger changes in chlorophyll fluorescence response, which is a result of changes in incident background light.

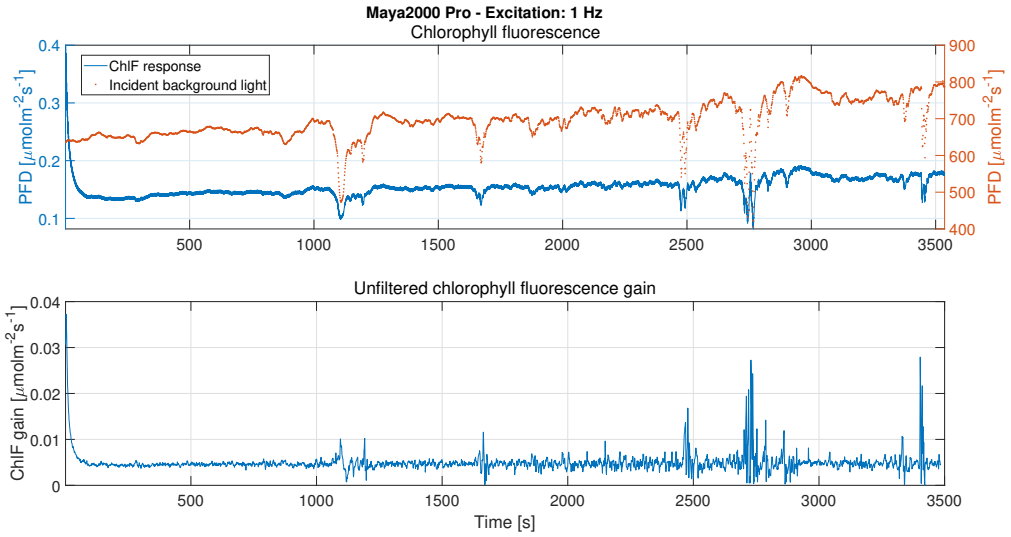

Figure 6.8: The upper plot presents chlorophyll fluorescence collected using the spectrometer Maya2000 Pro along with incident background light at excitation frequency 1 Hz. The lower plot shows chlorophyll fluorescence gain for each excitation.

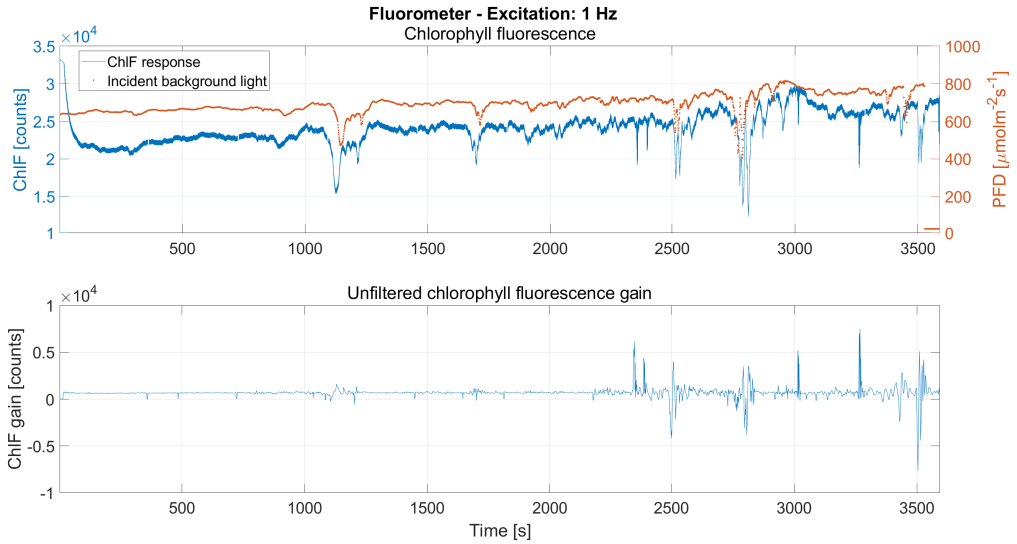

Figure 6.9: The upper plot presents chlorophyll fluorescence collected using the fluorometer along with incident background light at an excitation frequency 1 Hz. The lower plot shows chlorophyll fluorescence gain for each excitation. Note the small misalignment between chlorophyll fluorescence and incident background light caused by a removal of outliers in the chlorophyll fluorescence data.

Filtering was used on the calculated chlorophyll fluorescence gain due to large variations caused by rapid changes in incident background light (Figure 6.9). By inspecting the data, it is visible that the incident background light interferes with the step response (Figure 6.10) causing less distinct pulse responses from the excitations.

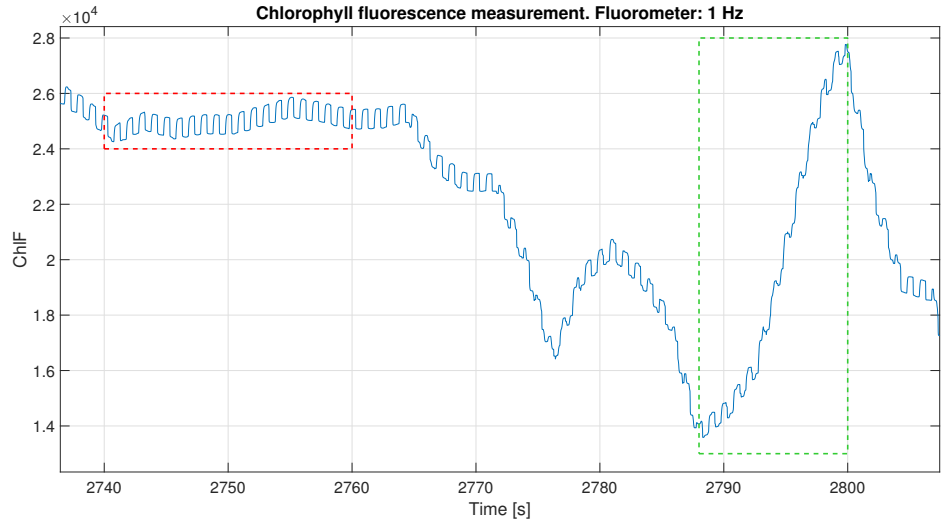

Figure 6.10: Chlorophyll fluorescence measured during a short segment of varying incident background light dynamics (Figure 6.9). The pulses inside the red box coincide with a segment where no rapid changes in incident background light occurred, thus the measured pulse responses are easy to detect. The pulses inside the green box coincide with a segment where rapid changes in incident background light occurred, which generated deviated pulse responses difficult to process.

Filtering segments of the pulse responses generated a less fluctuating chlorophyll fluorescence gain (Figure 6.11). Note that the chlorophyll fluorescence is still affected by rapid changes in incident background light.

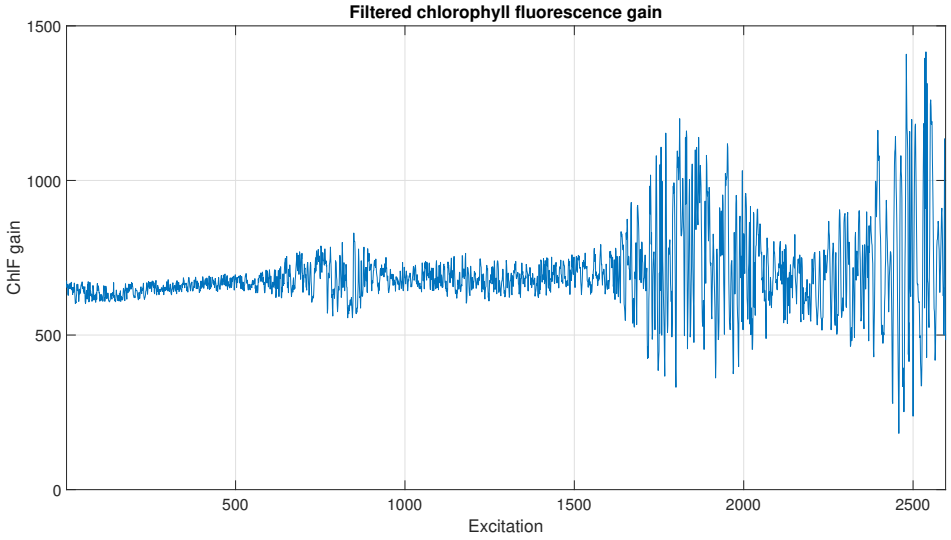

Figure 6.11: Filtered chlorophyll fluorescence gain derived from results presented in 6.9. Chlorophyll fluorescence gain is presented over number of excitations since outliers were discarded when filtering the data in Figure 6.9.

The results in Figure 6.12 show that using an excitation frequency of 208 Hz generates a less varying chlorophyll fluorescence gain. Some disturbances are still present, most distinct at the beginning and the last segments of the data. To handle these disturbances the data was filtered, resulting in data presented in Figure 6.13.

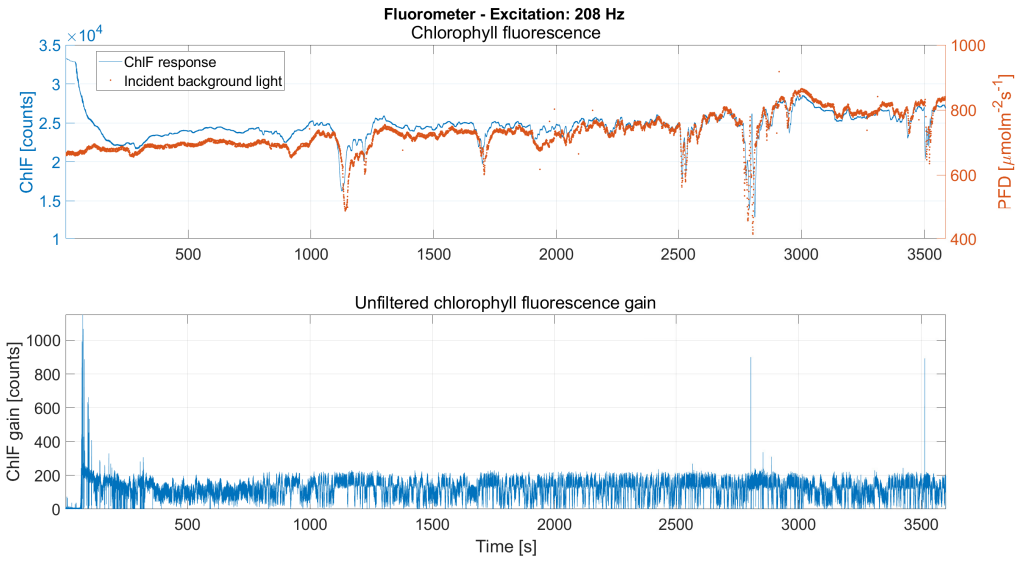

Figure 6.12: The upper plot presents filtered chlorophyll fluorescence gain along with incident background light at excitation frequency 208 Hz using the fluorometer. The lower plot shows chlorophyll fluorescence gain for each excitation.

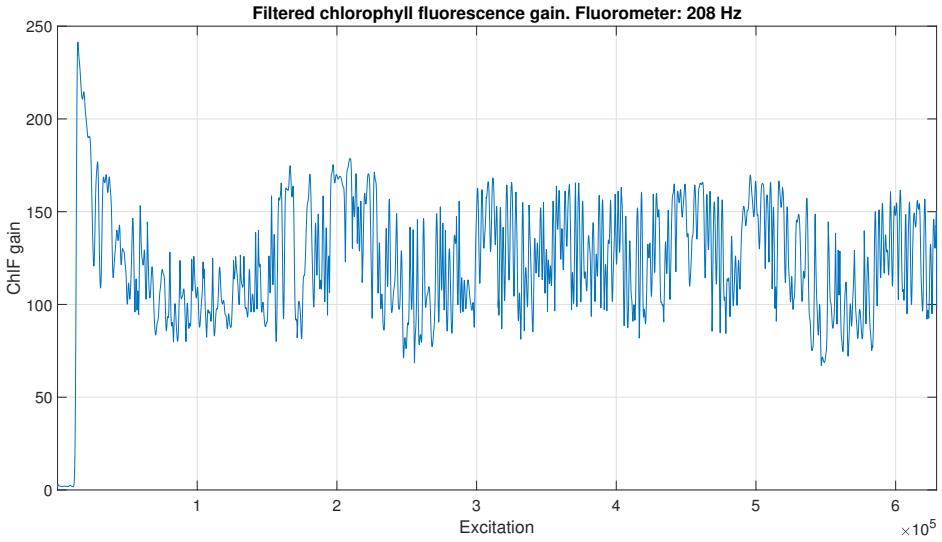

Figure 6.13: The plot presents filtered chlorophyll fluorescence gain derived from results presented in Figure 6.12.

6.3 Plant health analysis

An experiment was conducted on a reference unit along with a comparable unit with salt treated plants. The experiment was repeated three times per unit in order to investigate the effects on chlorophyll fluorescence gain for plants subjected to stress. This section presents and compares chlorophyll fluorescence of the two units. These measurements were examined with respect to parameters generally related to plant health.

6.3.1 Chlorophyll fluorescence dynamics

The results show a distinct variation within the characteristics of the chlorophyll fluorescence response on excitation level between plants exposed for stress and the reference plants. The results indicate that the dynamics of the chlorophyll fluorescence on excitation level are dependent on a possible stress exposure, both from salt treatment and higher incident background light intensities, see Figure 6.14, Figure 6.15 and Figure 6.16. Dynamics on a reference unit exposed to light of low intensities has consistently a negative gradient starting from the top of each excitation. Similarly, the same has a positive gradient starting at the bottom of each excitation. This shape is less distinct on plants subjected to moderate stress treatment (Figure 6.15), or even reversed for plants subjected to high salt concentrations and high light intensities (Figure 6.16). The difference in amplitude of the output between salt treated plants and the reference unit could be explained by the varying canopy height of the two units.

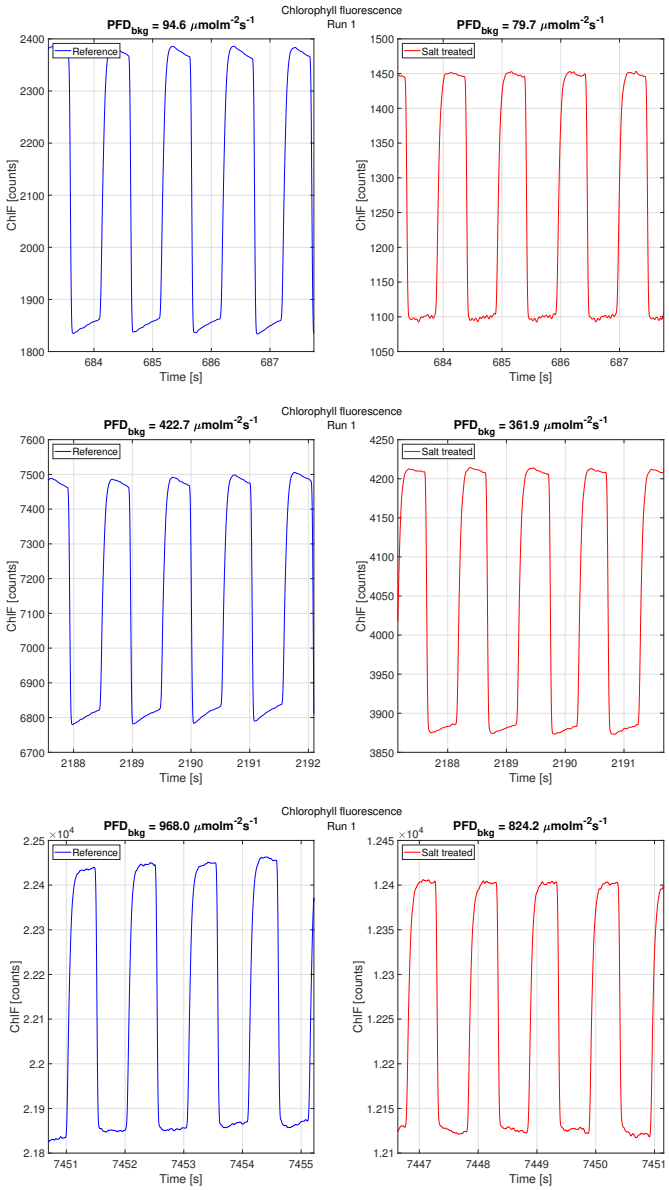

Figure 6.14: Chlorophyll fluorescence response at three different background light intensities from Experiment 5 run 1. The left figures present results from measurements on the reference plants and the right figures present the results from plants that were salt treated.

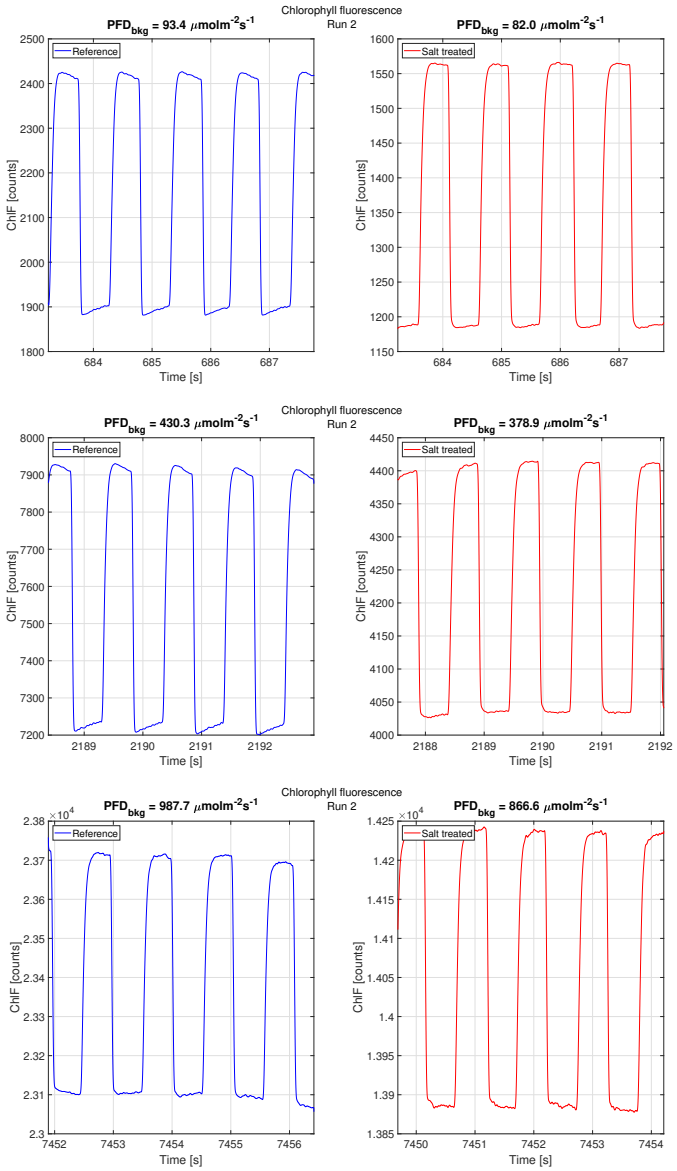

Figure 6.15: Chlorophyll fluorescence response at three different background light intensities from Experiment 5 run 2. The left figures present results from measurements on the reference plants and the right figures present the results from plants that were salt treated.

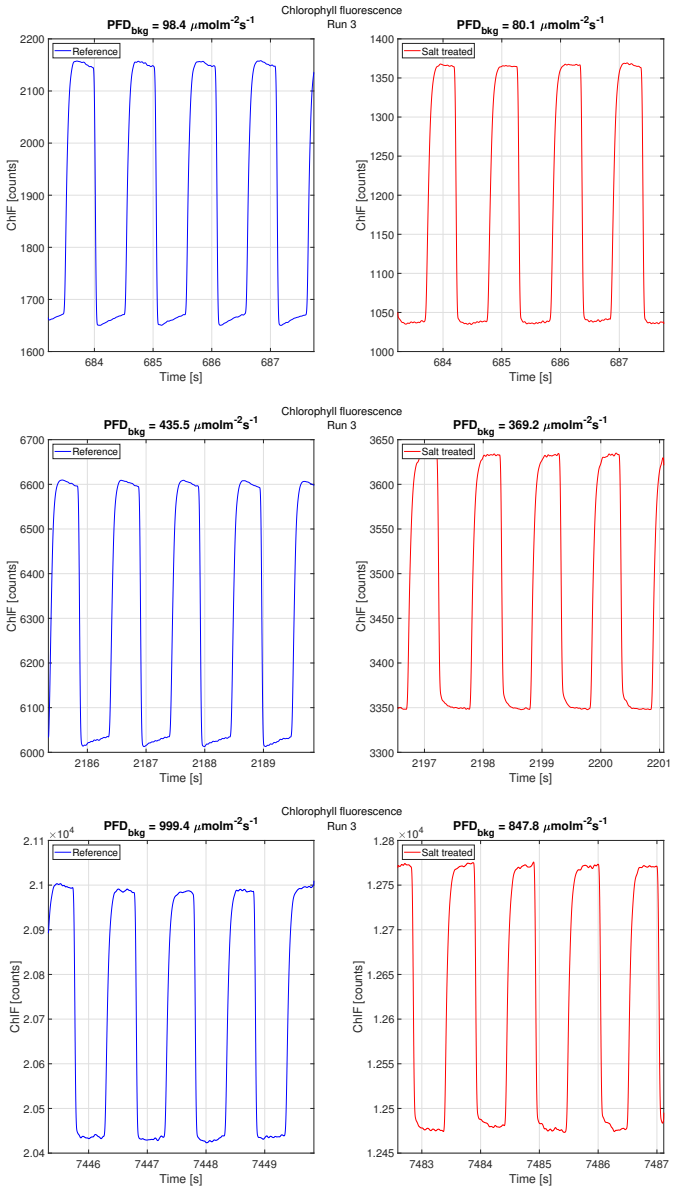

Figure 6.16: Chlorophyll fluorescence response at three different background light intensities from Experiment 5 run 3. The left figures presents results from measurements on the reference plants and the right figures presents the results from plants that were salt treated.

6.3.2 Chlorophyll fluorescence gain

The results from the reference unit show a similar trend where a local maximum in chlorophyll fluorescence gain can be located for incident background intensities of ~280-450 $\mu molm^{-2}s^{-1}$ (Figure 6.17). This trend can be found even in the early stages of salt treatment, however the decline in chlorophyll fluorescence gain appears at lower incident background light intensities. The last experiment on the salt treated unit (Run 3) indicates an immediate decline in chlorophyll fluorescence gain when exposed to incident background light.

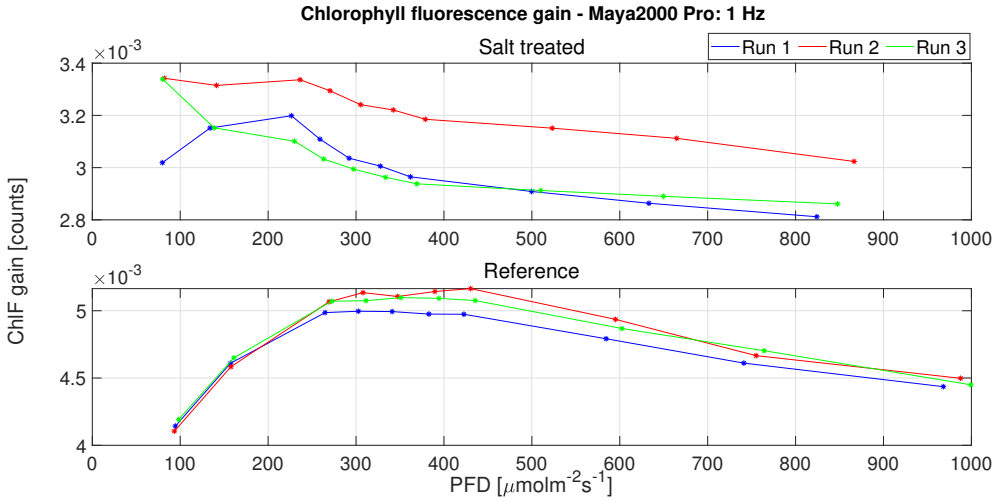

Figure 6.17: Average chlorophyll fluorescence gain for reference and salt treated plants measured in experiment 5.

6.3.3 Reference measurements

The reference unit for all the runs shows that an increase in NPQ rate appears around ~280-450 µ$molm^{-2}s^{-1}$ (Figure 6.18). When studying NPQ from the salt treated unit it is shown that an increase in NPQ rate occurs for lower incident background light intensities compared to the reference unit. Additionally, the magnitude of NPQ becomes greater for later stages of salt treatment.

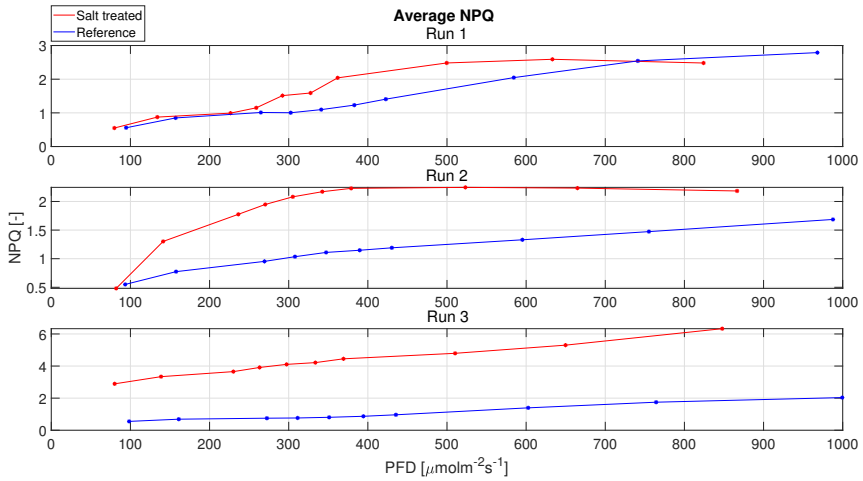

Figure 6.18: Average NPQ for salt treated plants and reference plants measured in experiment 5.

Comparing canopy temperature measurements between the reference unit and salt treated unit presents a coherent greater temperature in all repetitions for the salt treated unit (Figure 6.19). Housing temperature measurements were also collected during all repetitions which clearly indicated a similar trend (Appendix A.3). This

result can be used to confirm that all the experiments were conducted under similar conditions.

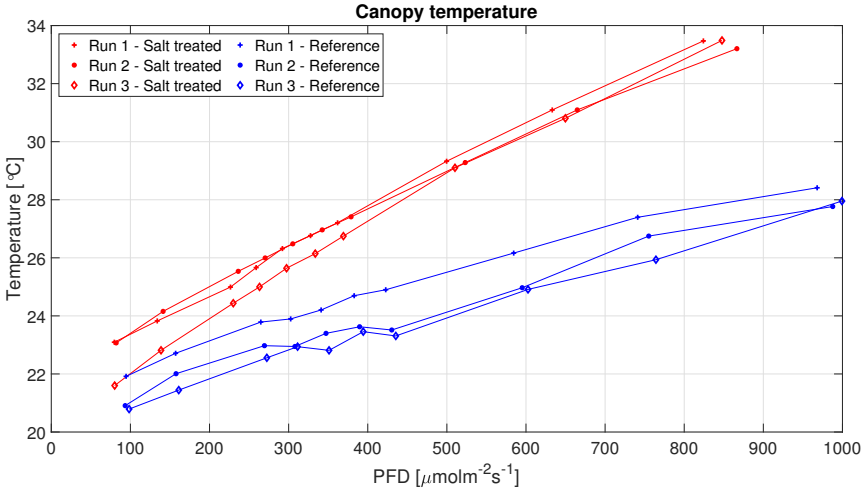

Figure 6.19: Canopy temperature collected every 12 minutes for reference and salt treated plants in Experiment 5.

7
Discussion

This chapter discusses the potential of detecting chlorophyll fluorescence in diverse light environments, and an evaluation is done whether using the developed PWM method is sufficient to determine plant health. Further, suggestions about future work are discussed.

7.1 Detecting chlorophyll fluorescence

Results clearly indicate that measuring chlorophyll fluorescence is possible using the PWM method. However, the properties of the response have shown to be highly dependent on a combination of the excitation frequency and sensors being used. Common for the two excitation frequencies is that the chlorophyll response could be distinguished in both a static and in a dynamic light environment with moderate variations. However, large fluctuations in incident light intensities have shown to aggravate the discrimination of the chlorophyll response to the excitation pulse from the chlorophyll response to the ambient light. The effect of large fluctuations in incident light can be reduced by using a higher excitation frequency, where the sufficient excitation frequency depends on the magnitude and the rate of the fluctuations with respect to time.

In the experiment carried out in this study, an excitation frequency of 1 Hz implied that the dynamics of the chlorophyll fluorescence pulse response, due to too large variations in incident light intensities, become less uniform. As a result of this, the pulse response appears without any clear extreme value. Since the method for calculating chlorophyll fluorescence gain in this study is based on comparing local extreme values for each pulse response, this behaviour makes the developed method inapplicable in a light environment with large variations. Even if a pulse could be distinguished, i.e. even if an extremum could be detected, a too low frequency will cause interference between the chlorophyll fluorescence caused by the excitation, and the chlorophyll fluorescence from variations in ambient light. Hence, the difference could not be derived.

An increased excitation frequency has from experiments shown that these issues can be resolved for the light environment examined in this study. A high frequency makes it possible to identify a pulse response within a change in incident light intensity, that compared to the excitation is small enough to be negligible.

Another factor that has shown great significance concerning the interpretation of the chlorophyll fluorescence response is the choice of the sampling frequency of the spectrometer and the fluorometer. The majority of the experiments were conducted with both a slow and a fast sampling frequency - 2 Hz respectively 3.5 kHz.

By using a slow sampling frequency, a consistent measurement of the chlorophyll fluorescence is obtained when using an excitation frequency of 1 Hz. However, due to the low sampling frequency the resolution of the measurements is not sufficient to obtain detailed information about the pulse response. In this case, the absence of information could result in a faulty interpretation of the local extreme values for each pulse response, thus generating an inaccurate chlorophyll fluorescence gain. For the experiments using an excitation frequency of 208 Hz, the slow sampling frequency of 2 Hz was discarded since it is not able to gain information of each pulse response.

By using a fast sampling frequency in the experiments conducted with an excitation frequency of 1 Hz it could be seen that sufficient information about the pulse response was obtained. A similar dynamic behaviour could not be seen when applying a fast sampling frequency for an excitation frequency of 208 Hz. The difference in dynamics could indicate that a sampling frequency of 3.5 kHz is too low to obtain useful information about the dynamics in each pulse response.

An important difference between the two sampling frequencies is the effect on locating local extreme values to determine chlorophyll fluorescence gain. A sampling frequency of 2 Hz generates a single value when the pulse response is high and a single value when it is low. This sample is not necessarily the highest or lowest value, but the samples are consistently collected at the same time instances of each pulse response. This ensures that the sample is collected during the same phase of the response. However, a sampling frequency of 3.5 kHz measures the response with a higher resolution, thus the obtained measurements reflect the true response more accurately and generate more accurate representations of the local extreme values. This could explain the differences in calculated chlorophyll fluorescence gain in Figure 6.6.

The design of the digital filter in the fluorometer used for fast sampling has shown to have a substantial impact on the measurements of chlorophyll fluorescence response. A pulse response with a tendency of a sine wave is obtained when running a simulation of the filter using high excitation frequencies (Figure 7.1). The same figure shows that this characteristic declines for lower excitation frequencies, which also can be concluded when comparing Figure 6.2 and 6.3. Thus, the consequence of the firmware filter implies that although the chlorophyll fluorescence can be measured, it is not necessarily the representation of the true chlorophyll fluorescence response. However, this does not affect the potential of using the PWM method for measuring chlorophyll fluorescence, but rather emphasises the need of sufficient firmware.

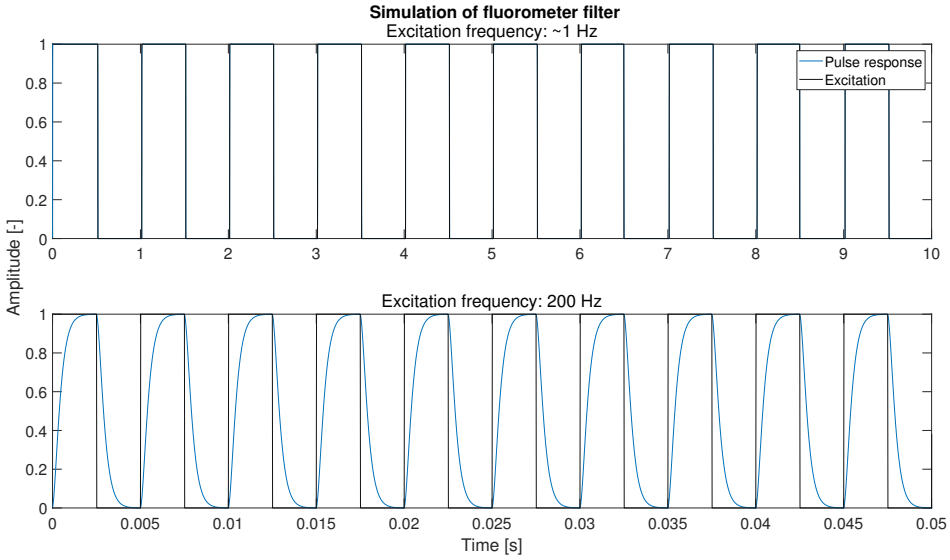

Figure 7.1: Simulation of the firmware filter designed for the fluorometer. The grey line presents the input and the blue line presents the pulse response. The upper plot has an input signal with an excitation frequency of 1 Hz. The lower plot has an input signal with an excitation frequency of 200 Hz. The filter has a sampling frequency of 3.5 kHz.

7.2 Determining plant health

Considering chlorophyll fluorescence response for each background level, such as in Figure 6.1, the results are consistently hard to interpret. The trend of chlorophyll fluorescence response on each background level is consistent, which makes it difficult to recognise any deviating behaviour when considering plant health. At the same time, the chlorophyll fluorescence gain for each background level is shown to be substantially inconsistent (Subsection 6.2.1 and Subsection 6.2.2), which also on this level makes it hard to gain useful information. For this reason, when evaluating the potential of using the PWM method, and consequently using chlorophyll fluorescence response to analyse a plant's health, two different approaches are taken into consideration.

7.2.1 Average chlorophyll fluorescence gain level

A coherent trend was identified when calculating an average of the chlorophyll fluorescence gain for plants from the reference unit. The average gain was derived from the last three minutes of each background light level. This trend is visualised in Figure 6.17 and indicates that there is a specific light intensity where the chlorophyll fluorescence gain has a local maximum. Since an increase in chlorophyll fluorescence gain is desirable, this local maximum could imply that there is a specific light intensity where a healthy plant is in its optimum state. Findings from [33] also shows a local maximum in chlorophyll fluorescence gain where a peak for a specific light intensity occurs where the plants become light-saturated. This supports the results

obtained regarding average chlorophyll fluorescence gain.

Interestingly, the local maximum in chlorophyll fluorescence gain seems to drift towards lower intensities, or even disappear when the plant becomes more stressed. The first repetition on a salt treated unit stills shows a similar trend where a local maximum is present, yet it appears at lower intensities compared to the first repetition for the reference unit. At the second repetition this trend is not as distinct for the salt treated unit. However, a local maximum still appears around the same light intensities as for repetition one. This trend is not present for the third repetition where the chlorophyll fluorescence gain immediately drops already from the first background light level.

These observations are supported by the relation to NPQ. Similarly, there is coherence between NPQ of the reference unit and salt treated unit in repetition one. However, an increase in NPQ rate for the salt treated unit appears at lower intensities, as a result of the chlorophyll fluorescence gain for the same unit decreasing at lower intensities compared to the reference unit. At the beginning of the second repetition, the NPQ rate appears to have a greater increase for the salt treated unit compared to the reference unit. This can be a result of the chlorophyll fluorescence gain being virtually constant for the corresponding background light levels. Results from the third repetition show that the NPQ rate for the salt treated unit is fairly constant, yet substantially higher compared to the other repetitions.

The JUNIOR-PAM used to collect measurements used for calculating NPQ is known to not yield high precision measurements, and is therefore generally not used in comprehensive scientific studies. The absolute values obtained should not be considered to be accurate for a scientific purpose. However, the results obtained in this study has shown consistency regarding trends, which in this case motivates the usage of this instrument in the conducted experiments. Further, to validate the presence of stress the results obtained from the IR radiometer (Figure 6.19) show a coherent higher temperature of the unit exposed to salt, compared to the reference unit. Since a higher temperature is expected to appear as a consequence of an increased NPQ, this result further supports the results obtained by the JUNIOR-PAM. This reasoning is supported by [33], which states that there is a relation between chlorophyll fluorescence gain and NPQ, where chlorophyll fluorescence gain normally is close to constant or declining as a result of an increased NPQ rate.

7.2.2 Chlorophyll fluorescence response on excitation level

The ability to detect plant stress by the usage of the PWM method was also evaluated by studying the chlorophyll fluorescence response on excitation level. This approach showed useful information when comparing the salt treated unit to the reference unit. The dynamics that were observed in Subsection 6.3.1 clearly indicate that there are parameters that differ between a plant subjected to stress factors compared to a plant not exposed to stress. The negative gradient starting from the top of each pulse can be an indicator of the plant being in a healthy state, com-

pared to a non-negative gradient which occurred on plants exposed to stress. The negative gradient became less steep, or even flat, at higher light intensities also for the plants in the reference unit. This behaviour is not necessarily a stress indicator, but rather an indication of the plant being light saturated. This indicates that a plant exposed to stress becomes light saturated at lower light intensities compared to a non-stressed plant. This reasoning is supported by the findings when studying the dynamics of average chlorophyll fluorescence gain where the gain decreased at lower light intensities for a plant exposed to stress (Subsection 7.2.1).

7.3 Future work

An experiment that was not conducted in this thesis was one using the sunlight simulation when analysing plant health on excitation level. Such an experiment would investigate an approach more applicable in commercial farming. Considering the findings from Subsection 6.3.1, it would be of interest to study the dynamics on excitation level also in a dynamic sunlight environment. If similar results could be achieved, a continuation of this thesis could be to characterise the dynamics of the chlorophyll fluorescence response to different types of stress factors. The gradient around the local maximum/minimum for each response has potential to be different depending on the stress factor for which the plant has been exposed to. The trends found on excitation level shows a potential to use system identification to determine a mathematical model applicable to describe the chlorophyll fluorescence in different states of the plant, or even to describe the specific stress factor for which the plant has been exposed to.

When using the fluorometer to calculate the chlorophyll fluorescence gain, an improvement of the algorithm to determine its gain is sufficient. The algorithm must be able to consistently identify the same part of the chlorophyll fluorescence response since the local extreme values are varying in phase for each response, depending on the state of the plant.

Finally, an improvement of the fluorometer is required to more precisely measure the chlorophyll fluorescence response. The fluorometer has consistently generated measurements containing a large amount of noise potentially caused by surrounding electrical disturbances. The hardware significantly amplifies the measured signal due to the low amplitude of the chlorophyll fluorescence. Consequently, undesirable signals are also amplified. In order to exclude signals not being part of the chlorophyll fluorescence, a reconstruction of the fluorometer must be considered. Further, a higher sampling frequency is also desirable since this could enable the use of even higher excitation frequencies which increases the accuracy of the chlorophyll fluorescence gain in dynamic background light environments. By deriving a more accurate gain it would be possible to determine the chlorophyll fluorescence at two different operating points. This could be used as an indicator for whether the ambient light should be increased or decreased in order to achieve an increased chlorophyll fluorescence gain.

8

Conclusion

In this thesis, it has been studied whether using the LED lamp's inherent PWM configuration can be used to distinguish chlorophyll fluorescence response. Results presented in this report have clearly shown that the approach is a promising method for this purpose.

The experiments conducted with an excitation frequency of 1 Hz resulted in distinct discrimination of chlorophyll fluorescence, which paved the way for using the method in a more applicable configuration. Thus, the same approach was conducted with an excitation frequency of 208 Hz which again resulted in a distinguishable chlorophyll fluorescence response, even in a dynamic background light environment. However, this configuration induced factors that have to be taken into consideration.

Using the higher excitation frequency puts a great demand on the sensor used for measuring the chlorophyll fluorescence response. The fluorometer used in this study consistently caused partially noisy measurements which could, if not taken into consideration, yield a faulty interpretation of the obtained chlorophyll fluorescence. Fortunately, such disturbances could be handled by filtering. Further, for higher excitation frequencies the fluorometer generated an output with characteristics similar to a sine wave which deviates from an expected square wave. It should be investigated whether these dynamics are caused by plant dynamics and/or firmware malfunctions. In the case where it is showed to be a consequence of firmware properties, sensor improvements are expected to resolve the issue.

The information obtained from the chlorophyll fluorescence response has shown to have good potential for being used as an indicator of plant health. However, due to sensor malfunctions, these conclusions are drawn only from experiments using an excitation frequency of 1 Hz. Interpretations of the chlorophyll fluorescence response are established both by analysing the dynamics on excitation level and by calculating an average chlorophyll fluorescence gain. Both cases show a distinction between a healthy plant and a plant exposed to stress.

The first case generates a more immediate response dependent on only a few, or even a single, excitation. The second case depends on measurements collected during a longer period which makes it more sensitive to noise. More importantly, a closed lab environment with a low dynamic background light is required to draw any useful conclusions from the chlorophyll fluorescence gain which makes it less suitable in a commercial application. However, the use of an excitation frequency along with a

sufficient sampling frequency is expected to be of great importance. By being able to calculate the chlorophyll fluorescence gain at two different operating points it would be possible to determine how the incident light should be regulated in order to yield an increase in chlorophyll fluorescence gain.

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Appendix 1

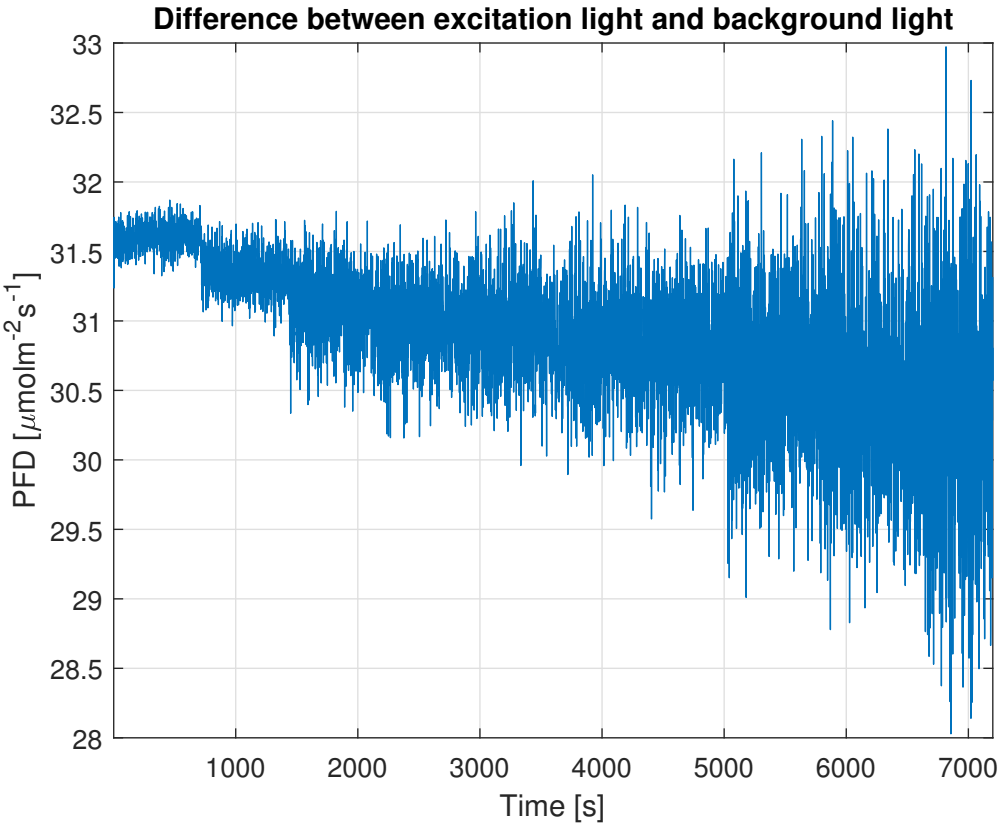

Figure A.1

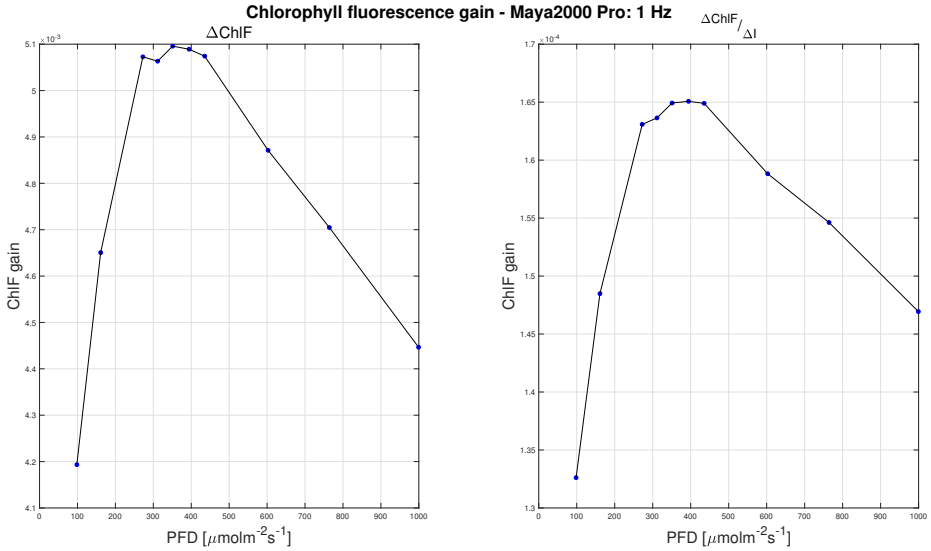

Figure A.2

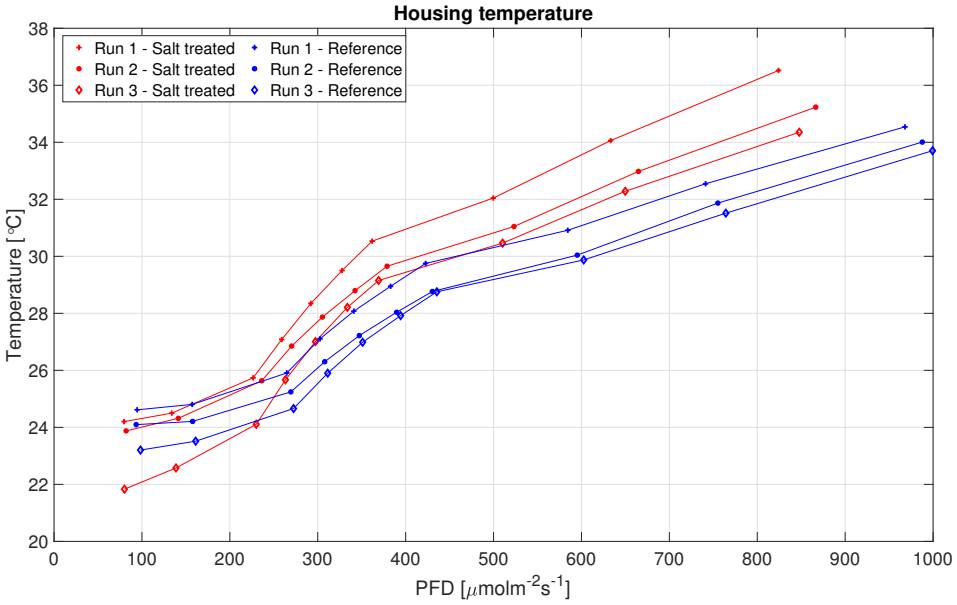

Figure A.3