THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

Fluorescence as a biological feedback signal for energy optimising spectra for greenhouse LED-illumination

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To my husband

Abstract

The introduction of LED-lighting for greenhouses has enabled new ways of controlling the light, both spectrum and intensity, to save energy and increase crop production. We have investigated a new method of automatically tuning the spectrum based on biological feedback. More specifically, we have performed experiments in order to evaluate if steady-state chlorophyll *a* fluorescence, measured at canopy level, can be a candidate signal to be used as feedback for spectrum optimisation. The experimental results show a strong correlation between fluorescence and photosynthetic rate, both in the case when one LED colour is used at a time, and under various background light. This indicates that fluorescence can indeed be used as an indirect measure of growth.

Furthermore, we have investigated different methods of evaluating how much the different diodes in the armature enhance the fluorescence and short term photosynthetic rate. At different background light, one LED colour at a time was changed, and the consequent change in fluorescence was measured. We refer to the fluorescence gain, for the specific LED colour, as the change in fluorescence divided by the change in photon flux, caused by the specific LED colour. When comparing the fluorescence gains for the different LED colours, the mutual relation was constant for a given plant species, independent of the background light spectra. Hence, if implementing the suggested controller for a predefined intensity, aiming to find the optimal spectra for maximised plant growth, the controller will maximise the intensity input to the most efficient LED colour and minimise the intensity to all other LEDs.

When evaluating the performance of the different LED colours, as a function of different intensity levels, changes were noticed in the mutual relations of the fluorescence gains. For cucumber, we found that when passing the light level where the photosynthesis saturate, no further changes in the mutual relation of the fluorescence gains were noticed. This opens up for using our method, i.e. comparing the fluorescence gains caused by excitation of light of different colours, to determine the light level where light saturation occurs.

Acknowledgements

I have been studying Chemical Engineering with Physics, which means that I have a slightly different background than the majority of the people at our division. That has not always been easy and I have felt like I'm talking another language. But I'm slowly learning a new language and it was, after all, the will of learning new things that took me to this position.

Towards the end of my master, after taking an advanced control course with Claes Breitholtz, I decided I wanted to slightly shift path. I wanted to somehow work within the automatic control field, preferably within the food industry. Via Jonas Sjöberg I found a master thesis project, at a company in Switzerland that produces equipment for the food industry. Towards the end of my master thesis project, I came to a point where the next step was to develop a control system to find the optimal settings for the model that I had built. I had no clue how to do that. My colleagues at the company did not have the possibility or knowledge enough, to teach me how to. That was when I decided to apply for a position as a PhD student, to get the possibility to learn more within the automated control field.

I would like to thank my supervisor Torsten Wik, first of all for that you encouraged me to apply for the position but foremost for all support and advice you have shared. Anna-Maria and Daniel, thanks for being my colleagues as well as friends. I greatly appreciate our collaborations and discussions about everything from plants, light and (even) biology, to kids, houses and wellbeing.

As engineers, we are all familiar with the fact that everything is relative. This has recently been clear to me as my scale of love and challenges have widely been stretched since our kids arrived. Having a toddler and twin babies makes you manage things you did not know was possible. Malte, Folke and Majken, I am happy that you broaden my mind and I love you all! Last but not least I would like to thank my husband, Marcus. Your support, patience and love are more than I could ever ask for. I expect the ratio of produced kids and produced papers to soon shift center of gravity and I look forward to work my last years as a PhD a bit more uninterrupted.

Linnéa Ahlman
Sävedalen, 2018

List of appended papers

Paper I. L. Ahlman, D. Bånkestad and T. Wik,
Using chlorophyll *a* fluorescence gains to optimize LED light spectrum for short term photosynthesis,
Computers and Electronics in Agriculture, vol. 142, pp. 224-234, 2017.

Paper II. L. Ahlman, D. Bånkestad and T. Wik,
LED spectrum optimisation using steady-state fluorescence gains,
Acta Horticulturae, vol. 1134, pp. 367-374, 2016.

Paper III. L. Ahlman, D. Bånkestad and T. Wik,
The correlation between changes in photosynthetic rate and changes in canopy level chlorophyll fluorescence in various background light and excitation of different LED colours,
To be submitted, 2018.

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Part I

Introductory chapters

Chapter 1

Introduction

During the spring and summer this year, 2018, we have experience an exceptionally warm and dry weather in a large part of the northern latitudes. Such extreme weather conditions have a big impact for many farmers and the food industry (Lesk et al., 2016). Due to global warming, experts foresee that we will experience more extreme weather conditions in the future (Stott, 2016). In this context, protected cultivation has the advantage of being less affected by unforeseen weather conditions. Protected cultivations have developed from simple row covers in open fields to greenhouses that most resembles high-tech plant factories (Ting et al., 2016) with climate control, stepwise movement of the plants from sowing area to the harvesting area and automated irrigation and fertilization (Shamshiri et al., 2018). Artificial light can also be used, either as a complement to sunlight; for increasing light intensities or daylight extension, or as the only light source in a closed environment. In the future, with an increasing world population, ongoing urbanisation and technical development and inventions within the food industry, it is likely to belief that the use of artificial light will increase, though preferably without increasing the energy consumption. Traditionally, high pressure sodium (HPS) lamps have been used in greenhouses. Due to the technical advances development, light emitting diode (LED) lamps are now a competitive alternative that enables an energy saving potential. Not only because of their high luminous efficacy but also because both spectrum and light intensity can be tuned and optimised for desired plant growth. The work within this thesis concerns spectrum optimisation of LED lamps for greenhouses. In particular, the possibility of automatic controlling the spectrum, based on biological feedback from the plants.

1.1 Greenhouse lighting

Artificial light can either be used as the only light source for plant growth, or as a complement to the sunlight. In the latter case either to increase the light intensity, or for daylight extension, which is of course particularly important in wintertime at northern latitudes. For these purposes there are a couple of common light types available, whereof

the most common are described further here.

1.1.1 High intensity discharge lights

In a high intensity discharge (HID) lamp, an electric current is flowing through a gas mixture (Durner, 2013). The gas becomes excited by the electrical energy and emits photons. The spectral distribution of the light is mainly determined by the gases involved and the pressure within the lamp (Björn, 2002). If high light levels are needed (500–1500 $\mu mol\, m^{-2}\, s^{-1}$), HID lamps are normally used. All high-pressure lamps should be handled carefully, due to the potential risk of explosion.

High pressure sodium (HPS) lamps and metal halide (MH) are two of the most common types of HIDs. Both types are used in growth chambers and growth rooms as well as in greenhouses. In addition to a high intensity level, they also have a long life-time, about 30,000 h for HPS and 15,000 h for MH (Durner, 2013). The HPS lamps have the highest photosynthetic photon flux, PPF (emitted photons having wavelengths 400–700 nm, per square meter and seconds), per unit electricity and are the most common lamp type used in greenhouses. However, when used as the only light source the spectrum from HPS may be deficient in blue for many species, especially if the total light level is below 700 $\mu mol\, m^{-2}\, s^{-1}$. The spectrum from MH lamps on the other hand, is satisfactory for most plant growth. Table 1.1 shows the spectral distribution of the lamps.

1.1.2 Fluorescent lights

Fluorescent lights are not as intense as HID lights and they have a shorter life-time, typically 5000–10,000 h. The lamp life-time is also affected by the numbers of starts, making them unsuitable for frequent turning on and off. However, for low light intensities fluorescent light is favorable, for examples for starting seedlings or for simulating spring conditions. Hence, they are extensively used in growth chambers and growth rooms (ibid.), i.e. boxes/rooms with temperature control and light fixtures to study plant growth and development under controlled simulated outdoor conditions. The standard choice is cool white fluorescent bulbs. They have an acceptable spectral distribution (see Table 1.1) and also the greatest photosynthetic photon flux, PPF, compared to other fluorescent bulbs.

1.1.3 Light emitting diodes

Light emitting diodes, LEDs, are solid-state light emitting devices. Thereby they can instantly be turned on or off, without requiring warm-up time (Morrow, 2008). Advanced LED lamps combine diodes which generate different wavelengths (from broad-band light to narrow-spectrum), into an array with light peaks at several wavelengths (Durner, 2013). As each diode can be dimmable individually, the spectrum can be varied. That makes LED lamps suited for control of spectrum as well as intensity.

The energy efficacy (efficiency where the numerator and denominator have different units, here µmol/J) varies among diode types and develops with time. Today, many LED lamps has an efficacy above 2 µmol/J, compared to the most efficient HPS lamp having an

Table 1.1: Spectral comparison of common horticultural light sources (adapted from Deitzer, 1994). The illumination is normalised to 100 µmol m^{-2} s^{-1} within PAR (400–700 nm).

Light	UV–B (250–350)	UV–A (350–400)	Blue (400–500)	Green (500–600)	Red (600–700)	Far–red (700–750)
Sunlight	2.9	6.2	29.2	35.2	35.6	17.0
HPS	0.2	0.5	6.5	56.6	36.9	4.0
MH	0.7	6.7	20.4	55.5	24.1	4.0
Cool White	0.03	1.1	24.8	52.6	22.6	1.4

efficacy of 0.9 or 1.7 µmol/J (for a 400 W or 1000 W HPS lamp). Independent researchers foresee a development were LEDs within ten years, have increase there maximum efficacy by 75% (Runkle and Bugbee, 2017). LEDs do not burn out like a bulb. The most commonly used "life-time" metric for LEDs is instead the time when the maximum intensity has been reduced by 30%, which occurs after about 50,000 h (Bourget, 2008).

Another advantages are that LEDs do not contain mercury, do not have a high pressure, have a low voltage requirements and do not generate excessive heat (Durner, 2013). LEDs do not radiate heat directly, but do produce heat that must be removed to ensure maximal performance and life-time (Bourget, 2008). This can be done by conduction through a liquid cooling device, or through natural or forced convection, the latter case by using a fan. LEDs can also be placed closer to the plants, without the risk of sunburned leaves, since they do not radiate heat directly. The fact that LEDs do not radiate heat directly, opens for the ability to place the lamps in close proximity to the plant tissue (Morrow, 2008). This enables an energy saving potential, as less light is lost to the surrounding (the light intensity is proportional to the inverse of the square root of the distance to the plant). This also opens up for intermediate lighting or dense vertical farming, with an increased production per growing area.

1.2 Photobiology

Light has multiple roles in plant functioning and growth, with photosynthesis being the most important. Besides its role in photosynthesis, light also constitute an important control signal through activation/deactivation of certain genes and enzymes (Jiao et al., 2007). In this way light controls different aspects of plant development, such as pigment composition, timing of flowering and plant morphology. However, our focus has been on the effect of light quality (and to some extent quantity) on short term photosynthetic rate.

1.2.1 Light absorption

It is mainly light in the wavelength span 400–700 nm that is absorbed by plants and used for photosynthesis (Lawlor, 2001). The photon flux within these wavelengths are referred

to as the photosynthetic active radiation, PAR. It is the chlorophyll (and to some extend carotenoid) pigments that absorbs the light for photosynthesis.

On atom level the energy absorption (and emission) is quantified, as electrons excites from the ground stat to a higher excited state. However, on molecular level and further in vivo where pigments are tightly packed into protein complex, the absorbtion occurs instead from a spectra of wavelengths. This is due to vibrational energy and more closely spaced molecular orbitals from which electronic transitions occurs (Porcar-Castell et al., 2014). For extracted, diluted pigments in a solution, the absorption spectra are well known, but in vivo the spectra will differ due to increased complexity.

1.2.2 Photosynthesis

The photosynthesis is the reaction where light energy is transformed into chemical energy, primarily stored as carbohydrates in the plant. The phenomena is described in many text books, for example Björn (2002), Durner (2013), and Lawlor (2001). Besides light energy, carbon dioxide and water are consumed to form carbohydrates and oxygen roughly according to

$$6\,CO_2 + 6\,H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6\,O_2.$$

The photosynthesis takes place in the plant cell, within an organelle (part of the cell) called chloroplast. The chloroplast is filled with a liquid, the stroma, and contains stacks of thylakoid membranes. In these membranes the chlorophyll and the carotenoid pigments are found. These are the pigments that absorbs the light energy in the first part of the photosynthesis, called the light reaction.

In the light reaction, light photons are absorbed by the chlorophyll and carotenoid molecules, which become excited. The energy absorbed by the carotenoid is transferred to the chlorophyll molecules. The carotenoid is also vital for protecting the reaction centers from photodamage (Vershinin, 1999). The energy of the excited chlorophyll molecules is passed to other molecules within the thylakoid, through the reaction centers called photosystem I (PSI) and II (PSII). They are connected in series and can be regarded as "electron pumps" powered by light photons (Björn, 2002). The energy is eventually used to synthesize ATP and NADPH (adenosine triphosphate and nicotinamide adenine dinucleotide phosphate), where the energy is temporary stored until it is used in the next step, the dark reaction. In the formation of ATP and NADPH, electrons are removed from PSII. These are replaced by hydrogen ions which are formed from the oxidation of water,

$$2\,H_2O \rightarrow O_2 + 4\,H^+ + 4\,e^-.$$

The oxygen diffuses out of the chloroplast and eventually exits the leaf to the air.

The dark reaction takes place in the liquid part of the chloroplast, the stroma. It occurs either in the dark or in the light, and the driving force for the reactions are the energy from ATP and NADPH. A series of reactions ends up in the Calvin cycle, where CO_2 is reduced to carbohydrate which is a biologically stable storage of the energy initially harvested from the sun.

1.2.3 Chlorophyll *a* fluorescence

Chlorophyll fluorescence is the emission of photons when excited molecules return to a lower energy level (Porcar-Castell et al., 2014). Most emission at normal temperatures derives from chlorophyll a, thereby the term "chlorophyll *a* fluorescence" (Lawlor, 2001). In solution, chlorophyll *a* emits almost one third of the absorbed light as fluorescence, while in vivo the amount is less than one or up to a few percentage. The fluorescence is emitted in the near infrared region, having peak wavelengths around 685 nm and 740 nm. Figure 1.1 shows an example of (a) an incident light spectrum using four different diode colours and (b) the corresponding reflection and the fluorescent light. In the experiments conducted within this thesis, the fluorescence signal is defined as the integral over the wavelength span 735–745 nm, illustrated by the coloured area in the figure.

Fluorescence is one of three possible pathways for absorbed light energy (see Figure 1.2). The probability of a photon being reemitted as fluorescence competes with the probability of photochemistry and heat re-emission. Hence, fluorescence emission indirectly contains information about the quantum efficiency of photochemistry and heat dissipation (Murchie and Lawson, 2013). This close relationship, between chlorophyll fluorescence and photosynthesis, has made it an indispensable tool in studying photosynthesis in a wide range of applications on-leaf as well as remotely from satellites (Murchie and Lawson, 2013; Porcar-Castell et al., 2014).

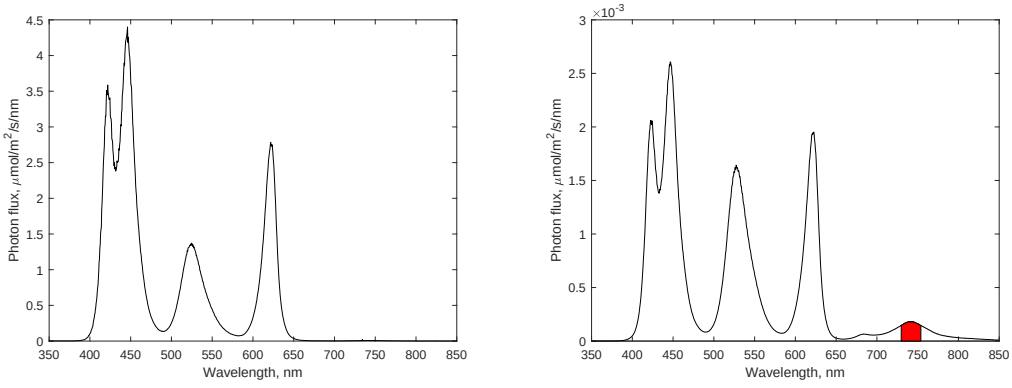

Figure 1.1: (a) Incident light spectrum using four diode colours. (b) Reflected and fluorescent light. The fluorescence signal, F740, we define as the integral over the wavelength span 735–745 nm, i.e. the coloured area under the curve.

The main tools used for measuring and analyzing chlorophyll fluorescence are the pulse amplitude modulation fluorometer (PAM) developed by Schreiber (2004) and the photosynthetic efficiency plant analyser (PEA) developed by Strasser et al. (2004). The PAM measures the fluorescence response to short duration (micro second) light pulses and discriminates chlorophyll fluorescence induced by the excitation pulse from chlorophyll fluorescence induced by ambient light through synchronous detection. The actual yields of photochemistry and heat dissipation, respectively, are sorted out from the fluorescence

signal by so called fluorescence quenching analysis (Schreiber, 2004). The PEA measures the fluorescence rise during the first second of saturating illumination, and analysis photosynthesis through inflection points on the resulting fluorescence curve (Strasser et al., 2004). These methods both require a precise measurement of the fluorescence from fully dark adapted plants, which is compared with the fluorescence at fully saturating light. These requirements limits the use of these methods for remote sensing and during day time, implying a restriction of their use to mainly on-leaf measurements. For semi-remote sensing applications, tracking of the development of the steady-state fluorescence yield has proven useful (Evain et al., 2004). However, at longer distances, i.e., measurements from satellites, passive methods are more suitable, such as, solar induced fluorescence and fluorescence detection within the Fraunhofer lines, the dark lines in the solar spectrum, caused by absorption by chemical elements in the earth atmosphere (Kirchhoff, 1860; Moya et al., 2004).

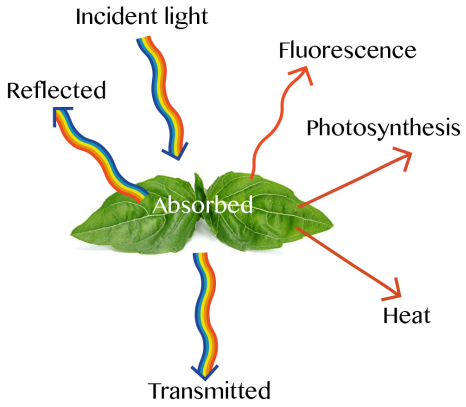

Figure 1.2: Light that hits the leaf is either absorbed, reflected or transmitted. The absorbed light is used for photosynthesis, reemitted as heat or reemitted as fluorescence.

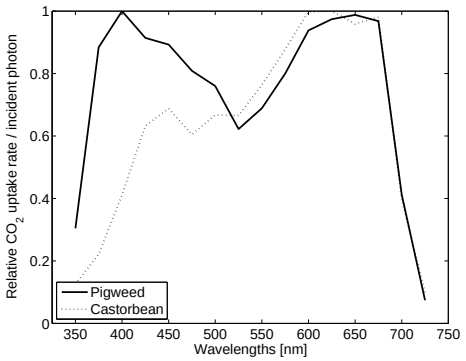

Figure 1.3: Normalised action spectra for incident quanta for Pigweed and Castorbean. Derived from experimental data given by McCree (1972).

1.2.4 Light quality impact on plant growth

The light quality, or spectrum, affects the plants in different ways. For example, in general UV-light (280–400 nm) inhibits cell elongation and can cause sunburn, blue light (400–520 nm) enhance the photosynthesis, green light (520–610 nm) is mostly reflected, red light (610–750 nm) enhance the photosynthesis and impact the photoperiodism while far-red (750–1000 nm) stimulates cell elongation, influence flowering and germination (Table 8.1 in Durner, 2013). However, different plant species are more or less sensitive to light quality. For a review of the many studies on this topic see Olle and Viršile (2013) and Ouzounis et al. (2015).

In the seventies two large studies were conducted in order to determine the photosyn-

thetic efficiency of photons of different wavelengths, the so-called action spectra (Inada, 1976; McCree, 1972). They concluded that the shape of the action spectra is similar for all species tested (22 and 33 different species), with one peak in red light and one lower peak in blue light. However, the similarities should not be interpreted as being equal. Figure 1.3 shows normalised action spectra for incident quanta, derived from the experimental data given by McCree (1972), showing the two species deviating the most from the mean.

Several restrictions, inferred by the experimental conditions used by McCree, apply when interpreting the McCree action spectra. The experiments were performed on cut leaves which could possibly have imposed stress to the leaves with a decreased photosynthetic efficiency as a result. Furthermore, the efficiency of individual wavelengths depend on whether it is investigated at leaf level or at canopy level (Paradiso et al., 2011), due to their different penetration depth. More importantly, McCree used narrow wavelength bands (25 nm), hence the McCree action spectra applies only to the use of single wavelengths. The combined effect of different wavelengths, however, is not necessarily the sum of the quantum efficiency of individual wavelengths. One example of this is the enhancement obtained when combining light of wavelengths below 680 nm with light of wavelengths above 680 nm (far-red light). This is called the "Emerson effect" (Emerson, 1957), which is due to the simultaneous excitation of the two interconnected photosystems operating in series. Furthermore, the light intensities applied by McCree were rather low, implying that photosynthesis was light limited, meaning that a high fraction of absorbed photons were expected to yield photosynthesis. At high light intensities, photosynthesis becomes light saturated, meaning that the photosynthetic efficiency of photons in general will decrease (Lawlor, 2001). How this affects the relative quantum efficiency of different wavelengths is however not well studied.

Chapter 2

Spectrum optimisation

In this section the theory and hypotheses that our research is based upon is presented. Then follows the research questions and description of the experiments that have been conducted.

2.1 Theory and hypotheses

If growth efficiency of plants varies over time and with the exposed light spectrum, we have the potential to use automatic control methods to decide their illumination. In this thesis our goal is to maximise plant growth for a given, constant illumination power, P_{tot} (see Figure 2.1), which means that we need a measure of the growth. We have studied the potential of using steady-state chlorophyll *a* fluorescence at 740 nm, F740, for that purpose. The advantage is that fluorescence response is fast, remotely measured on canopy level and non destructive to the plants. Therefore, fluorescence is a promising candidate to be used as a biological feedback in a closed loop aiming at finding the optimal spectra for maximised plant growth.

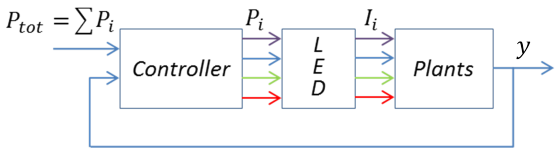

Figure 2.1: In order to find an optimal spectrum, i.e. how to distribute the power P_{tot} among the different diode groups by feedback control, one needs to find a quantity of plant growth that can be measured remotely and online. In this study we investigate if steady-state chlorophyll *a* fluorescence at 740 nm, F740, can be a candidate measure y. P_i is the power to each LED group, and I_i is the corresponding intensity of the emitted light.

The fraction of absorbed light that is used for photosynthesis, re-emitted as heat, or re-emitted as fluorescent light will vary, for example as a function of plant status and

illumination intensity. This has been studied both on leaf level (Flexas et al., 2002) and on canopy level (Guanter et al., 2014). For example, the relative level of fluorescence is negatively correlated with photosynthesis (i.e. desirable plant growth) at low light intensities, while it is positively correlated at high light intensities and under stress (Maxwell and Johnson, 2000; Tol et al., 2009). For healthy plants exposed to light with only small variations in intensity, a reasonable assumption is that the photosynthetic rate and fluorescence are positively correlated (also indicated by our experiments, see Figure 6 Paper 1 and Figure 2 in Paper 3). Furthermore, under such an assumption, the maximum photosynthetic rate for a predefined total power, P_{tot}, corresponds to the spectrum that maximises the fluorescence, F740.

Assume that N different LED colours are available. Then F740 depends on all the LED sources, i.e.

$$F740 = f(P_1, ..., P_i, ..., P_N) \tag{2.1}$$

where P_i is the electrical power applied to the i:th group (colour) of LEDs and f is a scalar function. For a predefined total power P_{tot} we may write

$$P_N = P_{tot} - \sum_{i=1}^{N-1} P_i. \tag{2.2}$$

As our assumed optimisation goal is to maximise F740, the gradient of F740 should be zero with respect to all sources, assuming that a (global) maximum exists. Differentiating Eq. (2.1) w.r.t. P_i, and using Eq. (2.2), give

$$\frac{dF740}{dP_i} = \frac{\partial f}{\partial P_i} + \frac{\partial f}{\partial P_N}\frac{\partial P_N}{\partial P_i} \tag{2.3a}$$

$$= \frac{\partial f}{\partial P_i} - \frac{\partial f}{\partial P_N} = 0 \quad \text{for all } i. \tag{2.3b}$$

The last equality implies that the *fluorescence gains*, defined as $\partial F740/\partial P_i$ (i.e. $\partial f/\partial P_i$), should optimally be equal for all LED groups i. As we are only interested in how the fluorescence gains relate to each other, the actual relation between growth and F740 need not be known, as long as they are positively correlated to eachother. The control task then fits to a combination of extremum seeking control (see Trollberg et al., 2014 and references therein) to track the fluorescence gains, and self optimizing control (Skogestad, 2000) to aim for equal gains (Wik et al., 2014). In principle, when not being at the optimum, the controller would increase the power to the LEDs with the highest gain and reduce the power to the one(s) with the lowest gain.

2.1.1 Research questions

The work within this thesis has mainly been experimental. The aim has been to evaluate the possibility of using fluorescence gains as a biological feedback for spectrum optimisation for plant growth. In particular, we have studied the following research questions:

- Is remotely measured F740 a sufficiently good measure of photosynthetic rate?
- Do the magnitudes of the fluorescence gains ($\partial F740/\partial P_i$) change relative to each other when the illumination intensity and/or spectrum are changed?

2.2 Experiments

The main objective of the experiments was to estimate the fluorescence gains (according to Equation 2.3), for different LED colours (i) and at different background light levels (operating points). This was done by changing the light to one LED colour at a time and using the approximation

$$\frac{\partial F740}{\partial P_i} \approx \frac{\Delta F740}{\Delta P_i}. \tag{2.4}$$

In the equation presented above, the power consumed by the LEDs is the input signal, which is the important quantity when performing the energy optimisation. However, we mainly present results using quantum flux (q, $\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$) as input, i.e. measuring the fluorescence gains as

$$\frac{\Delta F740}{\Delta q_i}. \tag{2.5}$$

This can easily be transformed to power consumed per area, knowing the wavelength (λ_i) and efficiency (η_i) of the diodes, according to

$$E_i = \frac{hc}{\lambda_i} \quad \text{and} \quad \Delta P_i = \eta_i \cdot \Delta q_i \cdot E_i \cdot N_A, \tag{2.6}$$

where E is the energy of one photon, h is Planck's constant, c is the speed of light and N_A is Avogadro's constant, i.e. numbers (of photons) per mole.

The output signal was the fluorescence, more specifically the photon flux in the wavelength span 735-745 nm. In some experiments also the photosynthetic rate was measured as an output signal, to be used as a reference quantity. The aim was to prove that the fluorescence correlates to photosynthetic rate within the current settings.

2.2.1 Experimental setup

The experimental analysis has been performed on plants commonly grown in greenhouses; basil (Paper 1 and 2), cucumber and Simpson lettuce (Paper 2 and 3), and also Galiano lettuce , dill and tomato (Paper 2). All experiments have been performed in a controlled environment, with respect to light, temperature and humidity. To ensure an even light distribution the experimental unit (Figure 2.2) was confined by white styrofoam blocks or reflective curtains (silver/white Diamond Diffusion Foil, Easy Grow, UK).

Spectrometer

In the experiments, the light has been measured using spectrometers. One spectrometer was installed at canopy level facing the lamp, for measurement of incident light, and the other one was installed at the height of the lamp facing the plants, thus measuring the reflected and fluoresced light on the canopy level. From the spectrometer data it is possible to calculate the illumination flux for any wavelength interval, i.e. $\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$.

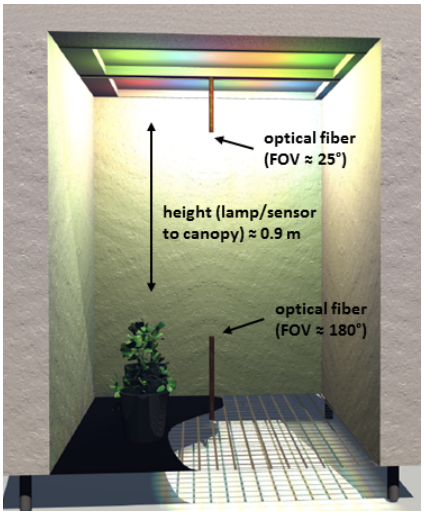

Figure 2.2: Experimental unit; delimited by reflective walls, lamp at the top and two spectrometers measuring incident light and reflected/fluorescet light.

Infrared gas analyser

In a few sets of experiments, the photosynthetic rate has also been measured (Paper 1 and 3), using an infrared gas analyser (IRGA). This is done on one leaf at a time, in contrast to the fluorescence that is measured on canopy level. The leaf is sealed in a chamber, known as a leaf cuvette, that has a window ensuring that the incident light is identical to that of the neighbouring plants. There is a controlled air flow through the cuvette, where the carbon dioxide, oxygen and water moisture is measured and used to determined the photosynthetic rate, defined as carbon dioxide uptake per leaf area and second.

Light scheme

The light strategies can be divided in two different types. Either one LED colour at a time was used (Paper 1 and Paper 2) in the absence of background light or, at different background light levels (various spectra and intensities) one LED colour at a time was deviated from the current operating point (Paper 1 and Paper 3). When one LED colour at a time was used the light was either held at a constant level for a long time (up to 15 min) in order to ensure that steady-state is reached, or slowly ramping the light not awaiting for steady-state. The purpose of the ramping was to evaluate if it was possible to decrease the experimental time without changing the results. For the sets where the IRGA is used though, longer times and larger intensity steps is needed in order to get an acceptable signal as the noise level is higher than for the fluorescence signal.

For experiments with different background light, the aim was to evaluate if, or how, the background light quality and quantity affected the fluorescence gains caused by excitation of different LED colours. In Paper 1 operating points having blue to red (B:R)

ratio 1:3 and 3:1 were investigated at four different intensity levels within the range 100-500 $\mu mol\, m^{-2}\, s^{-1}$. In addition, some more extreme levels were also investigated. In Paper 3 the green to blue (G:B) ratio was held constant at 1:2, whereas the amount of red light was changed in five different spectra from 50% up to 100% of the total quantum flux. This was done for five different intensity levels in the range 160-1000 $\mu mol\, m^{-2}\, s^{-1}$. Different schemes were investigated, but always sequentially changing one LED colour at a time to measure the fluorescence gain, i.e. the change in fluorescence divided by the change in incident light caused by a certain LED colour, at the specific operating point.

Chapter 3

Summary of included papers

This chapter provides a brief summary of the papers that constitute the basis for this thesis. Full versions of the papers are included in Part II. The papers have been formatted to increase readability and to comply with the layout of the rest of the thesis.

Paper 1

> L. Ahlman, D. Bånkestad and T. Wik, Using chlorophyll *a* fluorescence gains to optimize LED light spectrum for short term photosynthesis, *Computers and Electronics in Agriculture*, vol. 142, pp. 224-234, 2017.

A series of experiments were conducted on basil plants in order to examine whether remotely sensed steady-state chlorophyll *a* fluorescence at 740 nm, F740, can be used as a control parameter in a feedback loop, aiming at adjusting the incident light spectrum for maximal plant growth. A second goal was to investigate if the derivatives of the chlorophyll fluorescence w.r.t. applied powers (fluorescence gains) change relative to each other for different light intensities and spectra. Using one LED group at a time, a high correlation between chlorophyll *a* fluorescence at 740 nm, remotely measured at canopy level, and photosynthetic rate, PN, measured at leaf level, was found. This indicates that F740 can be used as a relative measure of PN, at least for the spectra and (low) light intensities being investigated. For different operating points, having a wide variety of spectra and intensity levels, the fluorescence gains when changing each of the different LED groups were compared. The relative order of the gains was remarkably consistent. With respect to incident photons the gain was highest for red light followed by blue and lowest for green light. Investigating with respect to applied powers the efficiency of the individual diodes is a dominant factor.

Paper 2

L. Ahlman, D. Bånkestad and T. Wik, LED spectrum optimisation using steady-state fluorescence gains, *Acta Horticulturae*, vol. 1134, pp. 367-374, 2016.

In this article six different plant species were included in the investigation; besides basil also tomato, cucumber, dill and two types of lettuce. Two different methods have been compared, either holding a constant light level for 9 minutes (in order to reach steady-state fluorescence) before changing to next light level, or slowly increasing the light from a low to a higher level. The fluorescence gains, $dF740/dq$ ($\Delta F740/\Delta q$), were calculated for the two methods and for all LED groups. Differences in the mutual relation of the fluorescence gains are obtained for different plant species, even though the similarities are more pronounced. The largest difference between the species were the relative difference between LED 400 and LED 530. Cucumber got the lowest gain for LED 400 and the lettuces and dill got the lowest gain for LED 530. For the second method, the ramp experiments, the experiments were conducted on four of the plant species. The two methods essentially give the same relative gains, but ramping tends to slightly overestimate the output from the blue LEDs.

Paper 3

L. Ahlman, D. Bånkestad and T. Wik, The correlation between changes in photosynthetic rate and changes in canopy level chlorophyll fluorescence in various background light and excitation of different LED colours, *To be submitted*, 2018.

Since our previous results indicated that red LEDs were most efficient per quanta, we wanted to evaluate the mutual relation of the fluorescence gains when the proportion of red light in the background spectrum increased. This was done on experiments on cucumber and lettuce. Spectra from 50% up to 100% red light were evaluated at intensity levels ranging from 160-1000 $\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$. At each light level an excitation was added sequentially by each of the six LEDs that was included in the investigation. An additional aim was to evaluate the correlation between fluorescence and photosynthesis at various background light levels. The experiments were conducted on cucumber and lettuce.

The relative efficiency of the different LED colours in terms of the fluorescence gains are in agreement with the action spectrum (i.e. the photosynthetic rate as a function of wavelengths) for cucumber and lettuce, found by McCree, 1972. The mutual relation between the fluorescence gains corresponding to the different LED colours did not change significantly with background light quality, but it was found to be species dependent. Furthermore, the relative size of the gains did change due to light quantity, but only up to the level where the photosynthesis light curve saturates. This opens up for using fluorescence gains to find the light level where the photosynthesis saturates.

Chapter 4

Concluding remarks and future challenges

Steady-state chlorophyll *a* fluorescence and short term photosynthetic rate have a strong correlation. This was concluded from experiments on basil, cucumber and lettuce, where these quantities were measured in parallel at a number of different light regimes. The light was generated using LED lamps having a number of different diode colours. The light regimes were either generated using one LED colour at a time, or using a combination of many, to generate various background light. We have not only shown a strong correlation between the absolute value of fluorescence and the absolute value of the photosynthetic rate, but we can also closely relate the mutual relation of the fluorescence gain and the photosynthetic rate gain. That means that the relation between an increase caused by an excitation of one LED colour compared to the increase caused by an excitation of another LED colour, is preserved when measuring fluorescence instead of gas exchange. Due to this correlation, it should be possible to use fluorescence gains to optimise the light spectrum for short term photosynthetic rate, as we suggested in Chapter 2.1.

On the other hand, our experiments show that the mutual relations between fluorescence gains of different LED colours, do not change substantially with background light spectrum. The consequence is that if implementing such a controller it will first maximise the energy input to the LED colour that causes the highest fluorescence gain, then increase the energy input to the LED colour having the second highest fluorescence gain, and so on until the total desired intensity has been reached, generally resulting in no power to the LEDs with lowest gains, unless there are spectrum constraints motivated by other reasons than short term growth. It should be noted that most figures presented are per energy quanta, but in order to energy optimise, the input should be the amount of consumed energy. In such case the individual efficiency of each LED colour will be a critical factor. The energy efficiency differs with background conditions, for example temperature, and different diodes are more or less sensitive to the temperature. This, and the fact that the diodes degrade with time, open up for using our method, i.e. comparing the mutual fluorescence gains, in order to identify degradation of individual LEDs over

time and consequently perhaps change the used power split between the different colours.

As the background light intensity increases, we noticed some changes in the mutual relation of the fluorescence gains, but only up to a certain light level, where eventually no further changes occur. Measurements on cucumber, show that this light level correlates to light saturation for photosynthesis. This opens up for the possibility of using the fluorescence gain signals to identify where the light curve for photosynthesis saturates.

4.1 Future work

We believe that the fluorescence gain can be an interesting quantity to monitor, due to its strong correlation to photosynthesis and the fact that it can be measured fast and remotely on canopy level. However, the initial idea of an online controller for continuously optimising the light spectrum, seems to be unnecessary. Instead the fluorescence gain might be used for identifying the light intensity level where photosynthesis saturates, or to identify diverse degradation of the different LED colours. Furthermore, it would be interesting to monitor the fluorescence gains over a whole growth cycle, in order to investigate whether any changes in the mutual relation of the fluorescence gain can be identified as a function of plant health. Possible stress factors to be investigated are light inhibition, salt stress, biotic stress and drought.

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