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Using chlorophyll *a* fluorescence gains to optimize LED light spectrum for short term photosynthesis

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ABSTRACT

When changing from the traditional high pressure sodium (HPS) lamps to light emitting diode (LED) lamps there is a quite unexplored energy saving potential in the fact that they are far better suited for control, since both spectrum and light intensity can be adjusted. This work aims at finding a way to automatically adjust the spectrum of a LED lamp, equipped with several different types of LEDs, to maximize plant growth by feedback of a remote online measure correlated with growth.

A series of experiments were conducted on basil plants in order to examine whether remotely sensed steady-state chlorophyll fluorescence (F740) can be used for this purpose, and if its derivatives (fluorescence gains) w.r.t. applied powers change relative to each other for different light intensities and spectra.

A strong correlation between F740 and photosynthetic rate was indeed found. However, the order (w.r.t. LED type) of the fluorescence gains was only moderately affected by the light intensities and spectra investigated. The gain was highest w.r.t. red light (630 nm), though, when taking the electrical efficiencies of individual LED types into consideration, blue LEDs (450 nm) were equally, or even more efficient than the red ones.

An online controller to regulate optimal spectrum for basil appears to be unnecessary. However, the fluorescence gains could be used to adapt to changes in the efficiencies when crops and operating conditions change, or when the diodes degrade. The method also shows promise as a tool to find optimal light intensity levels as well as identifying plant stress.

1. Introduction

Modern greenhouses having lighting systems are large consumers of electricity. In Europe alone, the power consumption from greenhouse lamps is estimated to be 100–200 TWh per year (combining information from Eurostat (2015), Christensen and Larsson (2010), Persson (2012) and Stenberg (2012)). High pressure sodium (HPS) lamps are still dominating and the illumination is in general controlled manually by simply switching the lamps on and off. Changing to light emitting diodes (LEDs) entails an energy saving potential (Singh et al., 2015), due to higher electrical efficiency of many LEDs compared to HPS lamps. Ongoing research aiming at using LEDs for irradiance control to avoid light inhibition (Carstensen et al., 2016), to regulate electron transport rate (Van Iersel et al., 2016) and to match time of harvest to demand (Bånkestad and Wik, 2016) may decrease the illumination cost per produced plant even further. Yet another possibility to reduce the energy use might be to optimise the spectrum by changing the power split to diodes of different colors. The energy optimal split could potentially depend on a number of factors, such as plant species, required characteristics of the plant, available LED groups and their electrical efficiency.

In the seventies two large studies were conducted (McCree, 1972; Inada, 1976) in order to determine the photosynthetic efficiency of photons of different wavelengths using monochromatic light. McCree (1972) used leaves of 22 different species of crop plants and measured the action spectra, i.e. the CO_2 uptake rate for incident irradiance, and also the absorbance, for each wavelength (intervals of 25 nm). He concluded that the curves had similar shape for all species tested, which was later confirmed by Inada (1976) who did experiments on leaves from 33 different species. The concluded similarity, however, should not be interpreted as "equal", now that we have the possibility to modify the spectrum quite freely with advanced LED lamps. Fig. 1 shows normalized action spectra for incident quanta, derived from the experimental data given by McCree (1972). The two species selected, Pigweed (*Amaranthus edulis* Speg) and Castorbean (*Ricinus communis* L.),

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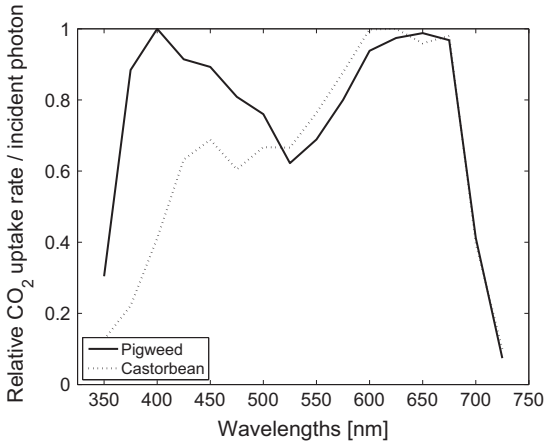


Fig. 1. Normalized action spectra for incident quanta for Pigweed and Castorbean. Derived from experimental data given by McCree (1972).

were the ones that deviated the most from the mean. These results clearly indicate that the most efficient light spectrum, in terms of CO_2 uptake rate, differs between different crops. The photosynthetic rate in a light combining sources that have different wavelengths may not necessarily equal the sum of the individual sources' photosynthetic rates. One example is the enhancement obtained when combining light of shorter wavelength (less than 680 nm; such as red light) with light of longer wavelength (greater than 680 nm; such as far-red light), the so called "Emerson effect" (Emerson, 1957), which is related to the excitation of the two photosystems. Further, the results likely differ if investigated at leaf level or at canopy level (Paradiso et al., 2011).

Many studies have been conducted that investigates the spectral effect on plant growth, commonly by comparing the impact that various light sources, and in particular their blue:green:red-ratio, has on the manually measured growth of a few plant species during their life cycles, e.g. Ouzounis et al. (2015), Cope et al. (2014), Massa et al. (2008), Kim et al. (2004), Dougher and Bugbee (2001). There are also studies on using LEDs as supplement to sunlight in greenhouses (Hernandez and Kubota, 2012; Hernandez and Kubota, 2014). However, to the best of our knowledge there are no studies reported on automatically finding the best spectrum based on a remote measure.

The present work aims at finding a way to automatically optimize the spectrum based on a remote measure of growth. Fig. 2 illustrates the idea, where the task is to distribute a predefined input electrical power, P_{tot}, on the available diodes so as to maximize the plant performance, y. Having a plant performance measure that can be measured remotely, a feedback controller could be sought that automatically adjusts the power towards the optimal spectrum.

The light energy absorbed by the leaf is either used to drive the photosynthesis, dissipated as heat or re-emitted as fluorescence (Maxwell and Johnson, 2000; Porcar-Castell et al., 2014). In the

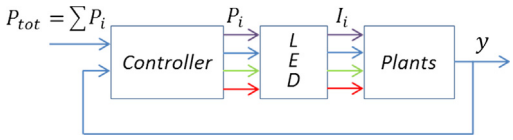


Fig. 2. In order to find an optimal spectrum, i.e. how to distribute the power P_{tot} among the different diode groups by feedback control, one needs to find a parameter of plant growth that could be measured remotely and online. In this study we investigate if chlorophyll fluorescence F740 could be a candidate measure y.

conducted experiments the photosynthetic rate, measured by an infrared gas analyser, is used as the reference of the "plant performance" to be maximized and steady-state chlorophyll fluorescence is the candidate signal to be used in the feedback loop, since it is remotely measurable and non-destructive. The fluorescence signal originates from chlorophyll *a* in photosystems I and II and is an emission of absorbed light energy with peak wavelengths around 685 and 740 nm (Papageorgiou and Govindjee, 2004). The fluorescence peak at 740 nm (F740) tends to give the strongest signal because the 685 nm peak (F685) is to a larger extent reabsorbed by chlorophyll *a* (Gitelson et al., 1998; Buschmann, 2007). In the analysis in this study only measurements of F740 are shown, since they correlated better with the photosynthetic rate than F685, and is not disturbed by light from the LEDs in the lamp having a peak at 660 nm.

A number of studies in the same research area, lighting control in horticulture by biological feedback, have also used chlorophyll flurescence as control signal, but in different ways. Carstensen et al. (2016) presents a novel method for probing photosynthetic status based on analysis of the dynamic chlorophyll fluorescence response to an excitation light. Detection of the chlorophyll fluorescence signal is made remotely on canopy level. Van Iersel et al. (2016) use PAM fluorometry on leaf level to measure quantum yield of photosystem II (Φ_{PSII}) and demonstrates that it can be used for controlling electron transport rate (ETR). Bånkestad and Wik (2016) evaluates the performance of an active proximal remote sensing system, measuring the ratio of red to far-red fluorescence (F685/F740), for the assessment of growth and biomass. In contrast, the present study explores the information in steady-state chlorophyll fluorescence F740 and how it relates to photosynthesis. Clearly, the properties of the chlorophyll fluorescence signal (intensity, spectrum, and dynamics) *in vivo* are controlled by many physical and physiological factors, making it attractive for biological sensing, but also challenging to interpret. The complexity of the signal increase with increasing scale, and more research is needed to expand the knowledge gained from leaf level measurements to canopy level and beyond (Porcar-Castell et al., 2014). This study addresses that particular challenge.

The relation between the amount of absorbed light, the amount of fluorescent light and the photosynthetic rate has been studied on both leaf level (Flexas et al., 2002) and canopy level (Guanter et al., 2014) and is dependent on plant health. For example, the *fraction* of fluorescence and photosynthesis is negatively correlated at low light intensity while it is positively correlated at high light intensity and stress (Maxwell and Johnson, 2000; Van der Tol et al., 2009). However, the *absolute* quantities of both fluorescence and photosynthesis are expected to increase with an increased incident light intensity, though the photosynthetic rate will eventually saturate (c.f. Fig. 4). With the hypothesis, that there is a positive correlation, not necessarily linear, between (the absolute quantity of) photosynthetic rate and steady-state fluorescence (under the conditions of interest here, i.e. well-irrigated and fertilized crops under moderate light conditions) the maximum photosynthetic rate for a predefined total power, P_{tot}, corresponds to the spectrum that maximises F740.

Assume that N different types of LEDs are avaliable. Then F740 depends on all the LED sources, i.e.

$$F740 = f(P_1, \ldots, P_N) \tag{1}$$

where P_i is the electrical power applied to the i:th group of LEDs. For a predefined total power P_{tot} we may write

$$P_N = P_{tot} - \sum_{i=1}^{N-1} P_i \tag{2}$$

Since the optimal spectrum is to maximise F740, the gradient of F740 should be zero with respect to all sources. Inserting (2) into (1) and differentiating gives

$$\frac{dF740}{dP_i} = \frac{\partial f}{\partial P_i} + \frac{\partial f}{\partial P_N}\frac{\partial P_N}{\partial P_i} = \frac{\partial f}{\partial P_i} - \frac{\partial f}{\partial P_N} = 0 \quad \text{for all } i \tag{3}$$

The last equality implies that the *fluorescence gains*, defined as $\partial F740/\partial P_i$ (i.e. $\partial f/\partial P_i$), should be equal for all LED groups i. Since we are only interested in how the fluorescence gains relate to each other, the actual relation between growth and F740 need not to be known. All that matters is that they are positively correlated to each other. 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 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. Motivated by this, a series of experiments manipulating the power to four different LED groups ranging from 420 nm to 630 nm were conducted on basil in order to investigate the following:

- 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 light intensity is changed?
- Do the magnitudes of the fluorescence gains ($\partial F740/\partial P_i$) change relative to each other when the spectrum is changed?

2. Materials and methods

Three different sets of experiments were conducted on basil plants:

(A) *No background light*
Stepwise increasing one LED group at a time.
(B) *Background light with blue to red (B:R) ratio 3:1 and 1:3*
Four intensity levels for each of the two spectra. Ramping one LED group at a time through each operating point.
(C) *High intensity background light*
Randomly changing one LED group at a time as a step change away from the operating point.

All experiments were carried out in a Styrofoam box ($w \times d \times h = 0.7 \times 0.7 \times 0.9$ m) with two LED lamps placed above (see Fig. 3). The light was detected by two spectrometers, one facing the lamps and one facing the plants. In the following, the conditions, instruments and setups are described in more detail.

2.1. Plants

Two trays with basil pots were placed in the box during the experiments. Fully grown commercially produced basil plants were used in experiment *(A)* and *(C)*, whilst in experiment *(B)* two different sets of plants grown in the lab were used. The first one was basil *Ocimum basilicum* 'Nufar' grown in 250 μmol m^{-2} s^{-1} for six weeks. The second set was basil *Ocimum basilicum* 'Genovese' grown in 160 μmol m^{-2} s^{-1} for four weeks.

2.2. Lamps

Two L4AS1 LED lamps (Heliospectra, Sweden), placed 0.9 m above the plants, were used as the only light source during the experiments.

The lamps contain diode groups having different peak wavelengths, of which we used 420, 450, 530, 630 and 660 nm. Table 1 shows relative (to LED450) electrical efficiencies of the individual diode groups, both in terms of radiant flux and photon flux. In the cases when LED 660 was used, it was held at a constant level since it overlaps with the fluorescence peak at 685 nm which initially was a candidate signal to investigate. The diodes are optically mounted in the lamp to provide a homogenous light at plant level. Due to heat generation from the lamp, the temperature in the box increased with light intensity. For light intensities lower than 500 μmol m^{-2} s^{-1} the temperature were in the range 25–28 °C, for incident light around 1000 μmol m^{-2} s^{-1} the temperature was about 30 °C, and for the one set with incident light just above 2250 μmol m^{-2} s^{-1} (see Table 4) the temperature reached 36 °C. This increase in temperature under high light needs to be considered when analysing the effect of incident light on photosynthesis.

2.3. Spectrometers

The incident light from the LED lamp was detected at canopy level by a Maya 2000 Pro Spectrometer (Ocean Optics, Dunedin, FL, USA) equipped with a 50 μm optical fiber and a cosine diffuser giving a field of view of 180°. The reflected and fluoresced light were also detected by another Maya 2000 Pro Spectrometer, but equipped with a 600 μm optical fiber with a field of view of about 25° in order to only measure green area. The fiber was placed between the two lamps, facing the plants. The fluorescence signal F740 was determined by integration over the wavelength interval 735–745 nm.

2.4. Infrared gas analyser

An infrared gas analyser, IRGA TPS-2 (PP systems, Haverhill, MA, USA) was used to determine the photosynthetic rate, in terms of CO_2 uptake per m^2 leaf area and s. The calculations are based on measurements of CO_2, O_2 and H_2O in a controlled air flow over a leaf sealed in a chamber, the leaf cuvette. The cuvette has a window which makes it possible to use the ambient light as light source (as in our case), but it is also possible to directly connect the leaf cuvette to the lamp (red and white LEDs) provided with the equipment. Fig. 4 shows photosynthetic light response curves of basil leaves from commercially produced plants. Three sample leaves and the IRGA's own light source were used in the measurement.

2.5. Experimental setup

As mentioned, three different setups were used in the experiments. In the first one, *(A)*, the photosynthetic rate and fluorescence were measured and compared when using one LED group at a time as the only light source. In the latter two, *(B)* and *(C)*, the deviation of the fluorescence was recorded while changing one LED group at a time around an operating point (background spectrum).

Experiment (A) The plants were exposed to light from one LED group at a time. LEDs with maximum intensity at 420, 450, 530 and 630 nm were used. The experiments were repeated 4 times, twice starting with the LED group of lowest wavelength and continued in the order of increasing wavelength, and twice in the opposite order. Ten light intensity steps were used for each LED, and the light was held constant for 12–15 min at each level in order to reach a steady state (see Fig. 5a). Both fluorescence and photosynthetic rate were measured throughout the experiments.

Experiment (B) Two spectra were chosen, one predominantly red (regR) having B:R ratio 1:3, and one predominantly blue (regB)

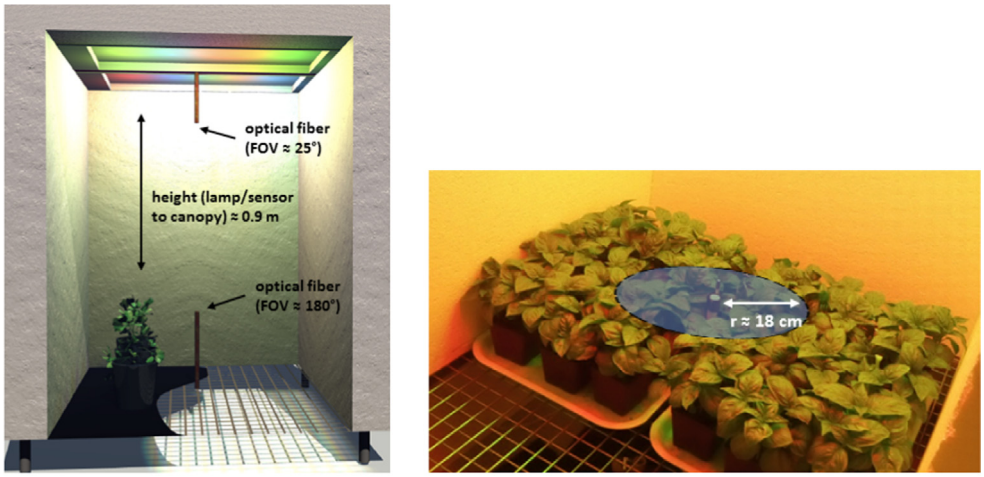


Fig. 3. (a) Styrofoam box (measurement unit) equipped with two lamps and two optical fibers for measuring incident light and canopy fluorescence; (b) two full trays of basil were placed in the unit for measurements, and the area viewed by the sensor had a radius of approximately 18 cm (the area in the image is for illustration).

Table 1
Relative electrical efficiencies of the individual LED groups in the L4AS1 lamp model (Heliospectra, Sweden) used in the study, as characterized by SP Technical Research Institute of Sweden.

LED group	Rel. radiant eff. (W/W)	Rel. photon eff. (μmol/J)
420	0.69	0.65
450	1.00	1.00
530	0.37	0.44
630	0.59	0.82

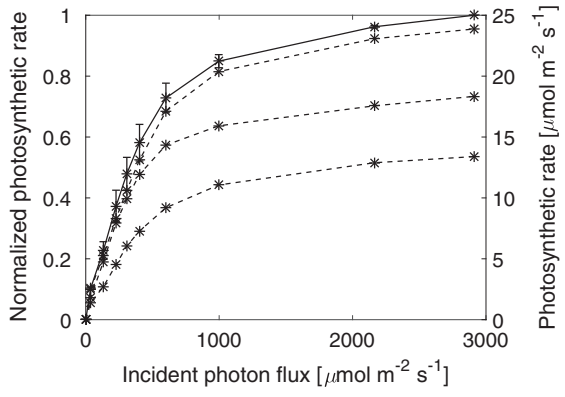


Fig. 4. Photosynthetic light response curves of basil leaves showing CO_2 uptake as a function of incident light (PAR). The dotted lines (right axis) show data from the three samples and the solid line (left axis) is the corresponding average of normalized data. The bars indicates ±1 standard deviation.

having a B:R-ratio 3:1, see Table 2. Four different intensity levels of each spectrum were used, thus 8 operating points in total. LEDs with maximum intensity at 420, 450, 530 and 630 nm were used and only one group of diodes was changed at a time. For regR an additional LED group was used, 660 nm, in order to reach the B:R-ratio 1:3, but it was held constant throughout each experiment.

Two sets of experiments were performed with slightly different setups, summarized in Table 3. One setup started with 15 min of dark adaption in order to get a reference value for the IRGA. Thereafter, one LED group at a time was changed from its value at the operating point. Fig. 5c depicts the schematic change of each LED group in the first set; starting with 15 min of constant light slightly lower than the operating point for the current LED group, followed by a 10 min ramp through the operating point. This scheme continued for each LED group in the order of increasing wavelength, directly one after another. The experiments were repeated three times at each operating point, of which one was in the reversed direction, i.e., starting with the LED group of highest wavelength (630) and with a decreasing ramp.

For the second setup the plateau level was held for 15 min, the ramp lasted 5 min, and the final value was held constant for 10 min (see Fig. 5d). Each experiment was repeated twice, one with increasing light (starting with LED 420) and one with decreasing light (starting with LED 630).

Experiment (C) The motive for this set was to see how the fluorescence gains were affected as the light intensities increased, and to introduce some randomness regarding the sequence of which LED group that was changed. The operating points used in this set of experiments have been divided into two groups; predominating red light (5 spectra) and predominating blue light (3 spectra), although the B:R-ratio varied more here than in *Experiment (B)*. Total PAR, B:R-ratio and the percentage of green light for the 8 operating points used are shown in Table 4.

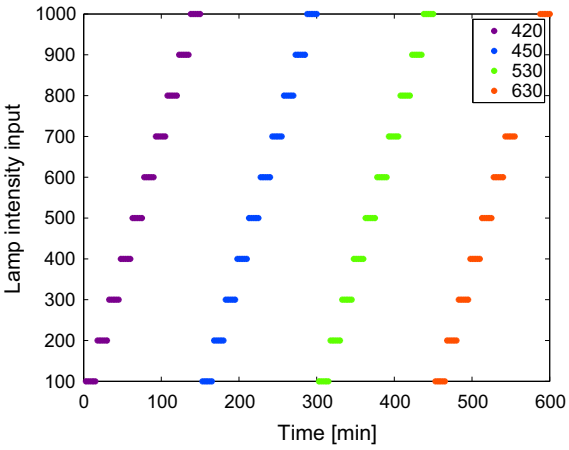
Randomly, one LED group at a time was deviated from the operating point. First by lowering the light and then by increasing it (the total intensity step was approximately 40 μmol m^{-2} s^{-1}) and at each level it was held constant for 10–15 min. An example is shown in Fig. 5b, for that particular operating point LED 630 was changed 12 times, LED 530 and 420 5 times each and LED 450 4 times.

3. Results and discussion

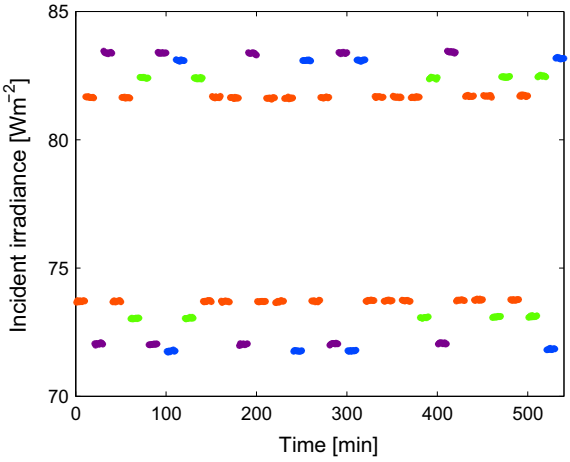
For the interpretation of the results it is important to keep in mind that for the intended use of the results (the method described in Introduction) it is only the *relative* quantities of each LED group compared to the others, within each experiment, that is important. This is also the strength of the approach since the method essentially becomes insensitive to environmental conditions and canopy structure.

3.1. (A) No background light

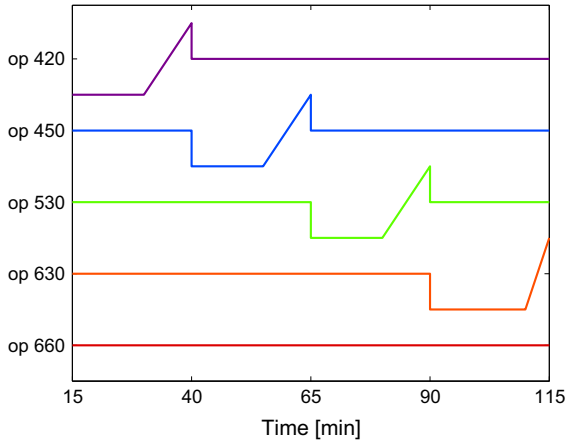
Both photosynthetic rate and fluorescence have an almost linear relationship with light intensity, at low to moderate light levels. Fig. 6 shows the relative fluorescence (F740) for all experi-



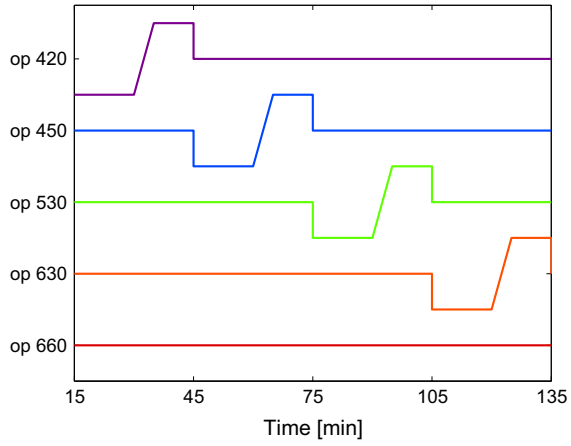
(a) Lamp input *Experiment (A)*.



(b) Measured incident irradiance *Experiment (C)*.



(c) Schematic changes for each LED, *Experiment (B)* 1st set.



(d) Schematic changes for each LED, *Experiment (B)* 2nd set.

Fig. 5. Lamp settings for the different experiments. (a) *Experiment (A)* Each LED steps from minimum to maximum lamp intensity in 10 steps. Lamp intensity input on the *y* axis refers to the setting in the lamp software. Second and fourth experiment had this setup, first and third experiment reversed the order of the LEDs. (b) *Experiment (C)* Total incident irradiance for one operating point. The color indicates which diode group that deviate from the operating point. (c) *Experiment (B)*, 1st set. Schematic changes for each LED around its operating point. Constant light in 15 min before the 10 min ramp. Twice starting with LED 420 and increasing ramps (as in the figure) and once in reversed order starting with LED 630 and decreasing ramps. (d) *Experiment (B)*, 2nd set. Schematic changes for each LED around its operating point. Constant light in 15 min, 5 min ramp, constant light 10 min. Once starting with LED 420 and increasing ramps (as in the figure) and once in reversed order starting with LED 630 and decreasing ramps. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Light intensities at the operating points in *Experiment (B)* measured during the experiments. PAR: 400–700 nm (μmol m^{-2} s^{-1}), blue: 400–500 nm, green: 500–600 nm, red: 600–700 nm.

Light regime	PAR	B:R	% Green
regR *i*	130	1:2.9	10
regR *ii*	195	1:2.7	10
regR *iii*	260	1:2.9	10
regR *iv*	540	1:2.9	10
regB *i*	195	3.1:1	18
regB *ii*	225	3.1:1	18
regB *iii*	270	3.1:1	18
regB *iv*	315	3.1:1	34[a]

[a] In order to increase total PAR in regB *iv* the green light (530 nm) had to be increased, since the blue LEDs had reached maximum capacity.

Table 3
The conditions for the 1st and 2nd set of *Experiment (B)*.

	1st set	2nd set
Background light	regR *i, ii, iii* regB *i, iii*	regR *iii, iv* regB *ii,iv*
Setup	plateau, ramp 15, 10 (min)	plateau, ramp, plateau 15, 5, 10 (min)
Replicates	3; up, down, up	2; up, down
Plant age	6 weeks	4 weeks
Growth light	250 μmol m^{-2} s^{-1}	160 μmol m^{-2} s^{-1}

ments versus the mean values of the relative photosynthetic rate (PN). Each dot is colored according to which LED group that was used. There is a strong correlation ($R^2 = 0.982$ for a first order curve fit and $R^2 = 0.996$ for a second order curve fit) between photosynthetic rate, measured on leaf level, and fluorescence at 740 nm, measured on canopy level, for the investigated light intensities (0–350 μmol m^{-2} s^{-1}). This is remarkable considering the complexity of the two de-excitation pathways and that measurements on canopy level are convoluted by e.g. canopy structure. It

Table 4
Light intensities at the operating points in *Experiment (C)* measured during the experiments. PAR: 400–700 nm (μmol m^{-2} s^{-1}), blue: 400–500 nm, green: 500–600 nm, red: 600–700 nm.

Light regime	PAR	B:R	% Green
regR *v*	320	1:3.1	10.2
regR *vi*	377	1:3.5	10.3
regR *vii*	895	1:2.7	10.6
regR *viii*	1134	1:8.3	6.4
regR *ix*	2276	1:2.5	10.1
regB *v*	243	2.7:1	20.0
regB *vi*	654	8.5:1	10.2
regB *vii*	955	3.5:1	23.6

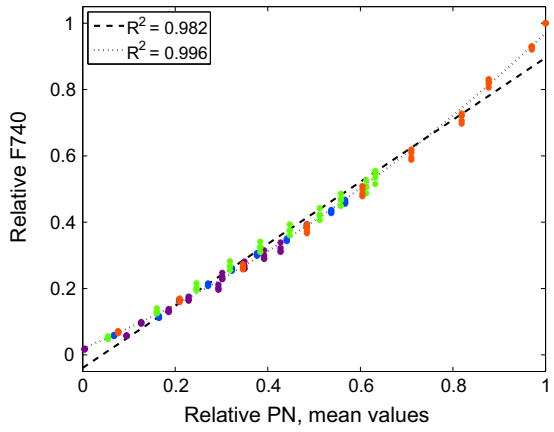


Fig. 6. Relative fluorescence, F740, versus mean relative photosynthetic rate, PN. Dashed line shows a first order curve fit ($R^2 = 0.982$) and the dotted line shows a second order curve fit ($R^2 = 0.996$). Each data point is colored according to which LED group that was used. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

is, at least partly, explained by the dominance of absorbed light on both fluxes (Guanter et al., 2014; Van Der Tol et al., 2014).

At the highest light intensities (250–350 μmol m^{-2} s^{-1}), where only red light is used, F740 increases somewhat faster than PN does, indicating a higher fluorescence emission efficiency relative to photosynthesis. This relative change is expected, the yield of photosynthesis decreases whilst the yield of fluorescence increases with increasing irradiance under low to moderate light levels (Maxwell and Johnson, 2000; Van der Tol et al., 2009), and it is also expected to be this small due to small variations in yields and the dominant effect of absorbed light (Van Der Tol et al., 2014). The data was obtained under light limiting conditions (c.f. Fig. 4) and therefore it is not possible to determine what happens with the relationship at light saturation.

The experiment was repeated four times. Fig. 7 shows the mean values ±1 standard deviation of (a) PN and (b) F740 versus incident photon flux. The standard deviation is smaller in the fluorescence data, which is one of the potential benefits of canopy level measurements, having an averaging effect, as compared to leaf level measurements. No difference could be observed as an effect of the order of the LED groups.

By using a second order curve fit the derivatives for each LED group have been calculated. Fig. 8 shows the derivative of (a) PN and (b) F740 versus incident photon flux. The derivatives are very similar in between LED groups and do not change much with light intensity, but there are indeed some small variations. As mentioned, based on the literature (Maxwell and Johnson, 2000; Van der Tol et al., 2009) we expect a small increase and small decrease in the yields/derivatives of fluorescence and photosynthesis, respectively, with light intensity. This was also observed, with the exception of LED450 and LED530 exhibiting a small increase in photosynthetic yield that we are unable to explain, but which may possibly be related to acclimation (Eberhard et al., 2008; Walters, 2005).

At the lowest light intensities the derivative is highest when changing LED 630, followed by 420, 450 and then 530, for both PN and F740. The data suggests that these relations change relative to each other, as light intensity increases. Assuming that the quantities are additive, Fig. 8a (photosynthetic yield) indicates that up to about 150 μmol m^{-2} s^{-1} it is most efficient to provide light from LED 630 and thereafter one should increase the light from LED 450. The lines in Fig. 8b (fluorescence gain) also intersect, but not at the exactly same light intensities. The conclusion when looking at the latter figure is that the most efficient light to use is LED 630 up to about 100 μmol m^{-2} s^{-1} and then use LED 420. Even though the conclusion slightly differs when using the leaf level photosynthetic yield and when using the canopy level fluorescence gain, both methods capture the fact that, at low light levels, LED 630 is the most efficient, followed by 420, 450 and 530. As a comparison one can calculate relative photosynthetic efficiency of the different LEDs based on McCree's data (McCree, 1972) by multiplying McCree's action spectrum of photosynthesis per incident quanta (average of all 22 species) with the spectrum of each LED normalized to the same photon flux. This operation yields the same order of LED efficiencies (i.e. LED 630 is most efficient, followed by 420, 450 and 530; data and calculations not shown).

All the analysis above was made with respect to the incident photon flux. One conclusion is that the (short term) photosynthetic rate for a given photon flux (μmol m^{-2} s^{-1}) does not differ much depending on which LED group that is used, i.e. the light spectrum. If comparing photosynthetic rate versus incident irradiance (W m^{-2}) instead, which would be of interest when aiming at finding the light spectrum, with a certain energy content, that maximizes the photosynthetic rate, the lines differentiate more (see Fig. 9a). In such case it is most efficient to use red diodes since light energy is inversely proportional to wavelength ($E = hc/\lambda$, where h is the Planck constant, c is the speed of light and λ is the wavelength).

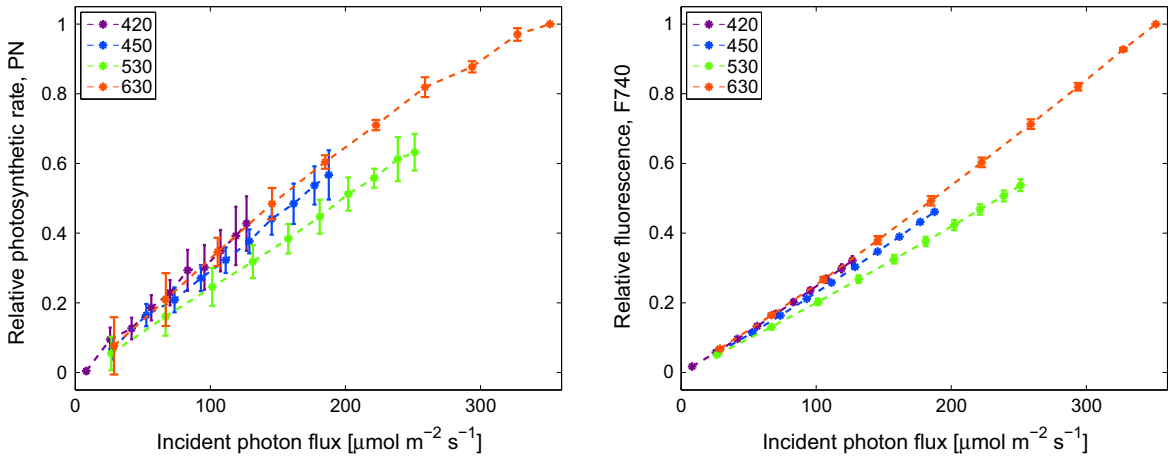
Another factor to take into consideration is the electrical efficiencies of the diodes in the LED lamp (see Table 1). Fig. 9b shows that the applied electrical power (for a particular photosynthetic rate) is lowest using LED 450 or 630 and highest using LED 530. Clearly, the relative order of the photosynthetic efficiencies of the LEDs are dominated by their electrical efficiencies. Note that the analysis are based on electrical efficiencies of this particular lamp; the relation is likely to be somewhat different in other LED lamps depending on LED specifications, electronics, temperature (cooling) and optics.

3.2. (B) Background light with B:R-ratio 3:1 and 1:3

The potential effects of different spectra were compared, using spectra with blue to red (B:R) ratio 1:3 and 3:1 (see Table 2), and four different intensity levels for each spectrum. At each operating point one LED group was changed at a time and the fluorescence gains (see Table 5 for abbreviations) were recorded.

The fluorescence gains when changing individual LED groups at each operating point are shown in Fig. 10, i.e. the derivative of F740 with respect to incident photon flux ($\partial F740/\partial q_i$),X incident irradiance ($\partial F740/\partial I_i$) and applied electrical power ($\partial F740/\partial P_i$).

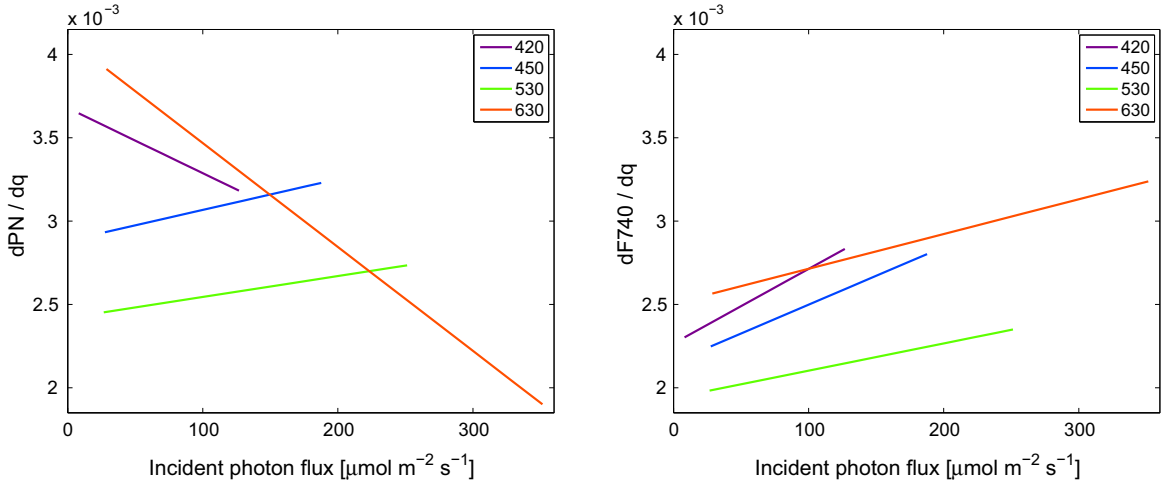
The fluorescence gains were lower for blue intense background light (compare left and right columns in Fig. 10), probably partly due to lower absolute fluorescence values (see Fig. 11), which might be related to less absorption of blue intense background



(a) Relative photosynthetic rate, PN, vs. incident photon flux.

(b) Relative fluorescence, F740, vs. incident photon flux.

Fig. 7. Results from *Experiment (A)*, i.e. using one LED group at a time. The experiment was repeated four times, the bars indicates ±1 standard deviation.



(a) dPN/dI vs. incident photon flux.

(b) Fluorescence gains vs. incident photon flux.

Fig. 8. Derivatives for second order curve fits to data in Fig. 7.

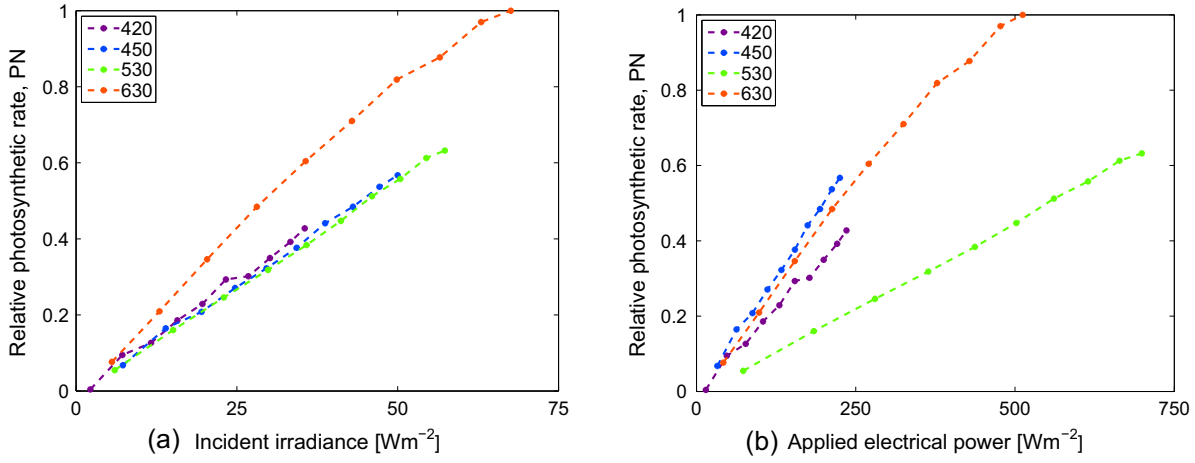


Fig. 9. Relative photosynthetic rate vs. (a) incident irradiance and (b) applied electrical power, in *Experiment (A)*. Per incident irradiance (a) LED 630 has the highest fluorescence gain while for applied electrical power (b) LED 450 and 630 are about equal, due to a high electrical efficiency of LED 450.

Table 5
Abbreviations of the fluorescence gains w.r.t. different quantities.

Derivative of F740 w.r.t.	Abbr.
Applied electrical power	$\partial F740/\partial P_i$
Irradiance	$\partial F740/\partial I_i$
Photon flux	$\partial F740/\partial q_i$

light as well as less canopy penetration causing less reabsorption of F685.

The derivatives relative to each other, when changing different LEDs, do not differ substantially as a consequence of neither the light intensity nor the light spectrum. It is interpreted as, independently of the operating point (in the interval investigated) the

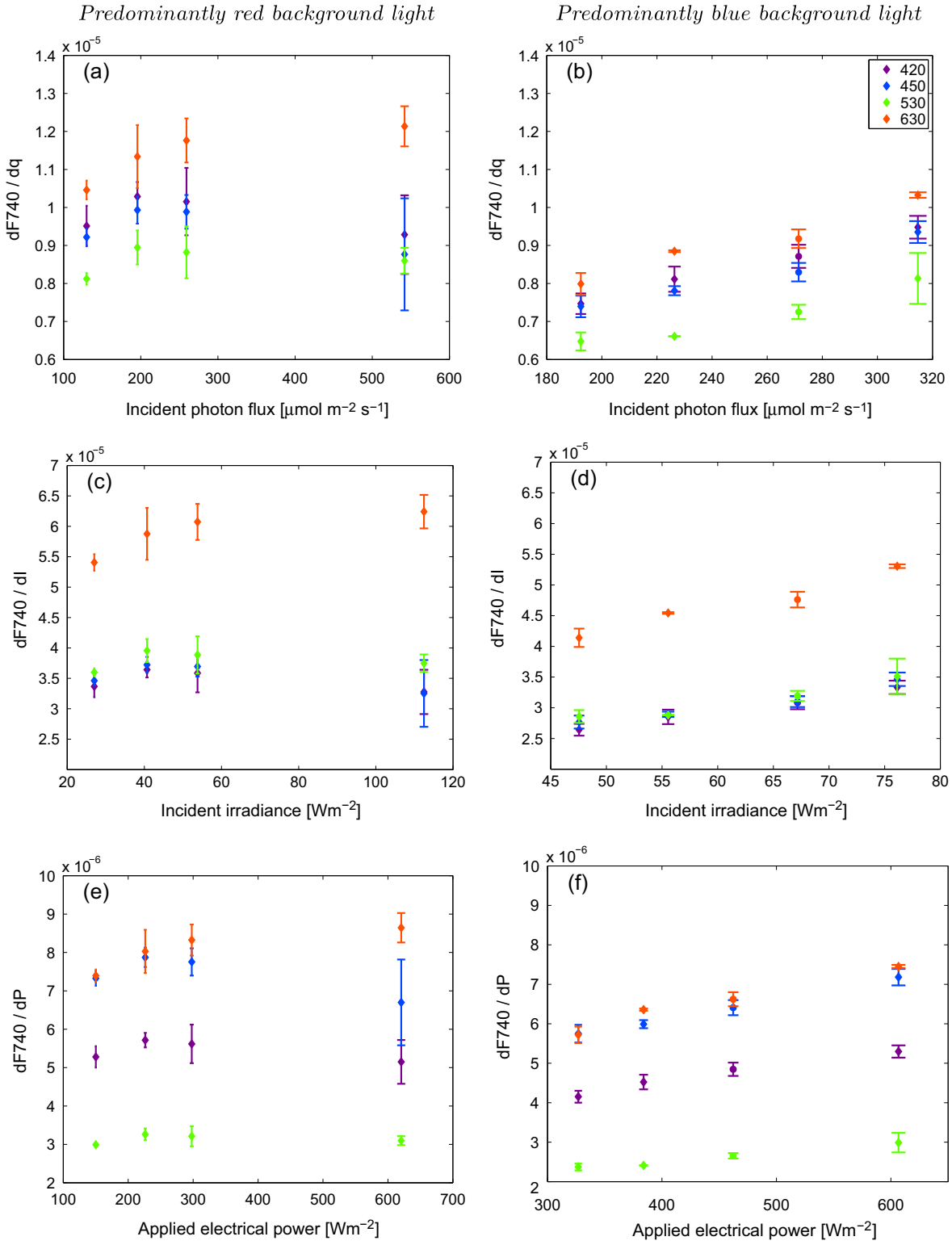


Fig. 10. Fluorescence gains in *Experiment (B)*. Left column: Predominantly red background light (B:R 1:3). Right column: Predominantly blue background light (B:R 3:1). (a,b) $\partial F740/dq_i$ vs. incident photon flux q, (c,d) $\partial F740/dI_i$ vs. incident irradiance I and (e,f) $\partial F740/dP_i$ vs. applied electrical power P. The bars indicates ±1 standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

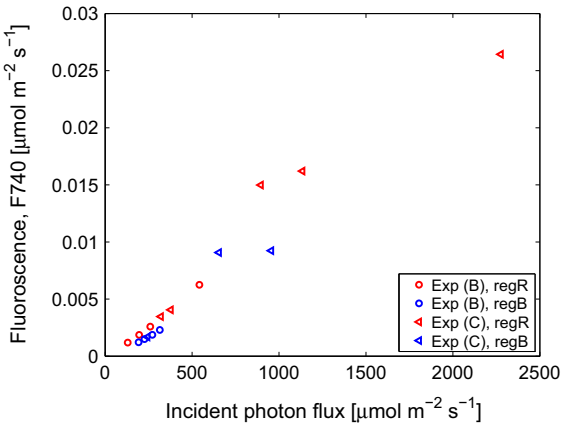


Fig. 11. F740 vs incident photon flux at the operating points in *Experiment (B)* (circles) and *Experiment (C)* (triangles). The color indicates whether predominantly red or blue background light have been used. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

same actions are needed to approach the optimal spectrum. Per incident photon flux, $\partial F740/\partial I_i$ is highest for LED 630 (red) and lowest for LED 530 (green) (see Fig. 10 a,b). This is in line with the result and conclusion from experiment *A*, i.e. changing cultivar and adding background light did not change the order of the derivatives. However, in this data there is one operating point above 500 µmol m^{-2} s^{-1} (see Fig. 10 a), for which there is no significant difference in $\partial F740/\partial I_i$ when changing LED 420, 450 or 530.

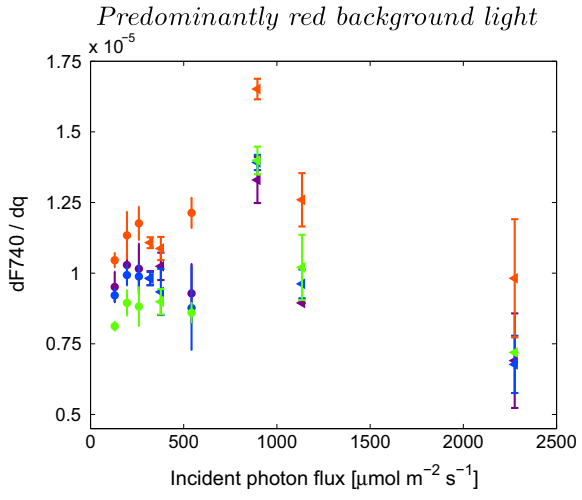
If taken into consideration that energy content is dependent on wavelength (see Fig. 10 c,d), LED 630 gives rise to a significantly higher fluorescence gain, while the other three diode groups have lower and similar gains. In the third row of plots the efficiencies of the different diode groups are taken into consideration. Same as in experiment *A*, fluorescence gain is highest (and about equal) for LED 630 and 450 (red and blue) and lowest for LED 530 (green).

3.3. (C) High intensity background light

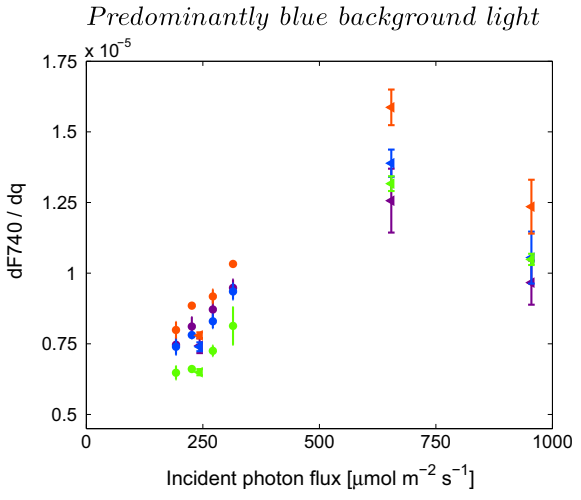
These experiments were conducted as a complement to *Experiment (B)* to investigate possible differences as the light intensity was further increased. The results are combined with the data from *Experiment (B)*. To be able to compare different sets of experiments normalization is needed since the level of the fluorescence (and consequently also its derivative) depends on the amount of biomass, the distance to the spectrometer etc. Fig. 11 shows the level of fluorescence for *Experiment (B)* and *(C)* where the three lowest light intensity levels from *Experiment (C)*, having the same magnitude as in *Experiment (B)*, have been used for normalization. As a result, all F740 values for *Experiment (C)* were multiplied by a factor (2.13) in order to get in the same region as in *Experiment (B)*.

In Fig. 12 the fluorescence gains versus incident photon flux for this experiment, together with the data from *Experiment (B)*, are shown. The gains do not continue to increase for the highest light intensities. We also note that the gain remains highest for LED 630. For light intensities lower than 500 µmol m^{-2} s^{-1} LED 530 generates the lowest gain but for higher light intensities LED 530 has increased relative to the others and there is no significant difference in the fluorescence gains for LED 420, 450 and 530.

The data set includes light intensities expected to saturate photosynthesis. Based on light response curves on leaf level (see Fig. 4), the plants move from light limitation to light saturation at around 800 µmol m^{-2} s^{-1}, which indeed coincides with the transition from increasing to decreasing fluorescence gains. This relationship has been reported by others (Van Der Tol et al., 2014) and is included in models of leaf chlorophyll fluorescence (Van der Tol et al., 2009; Vilfan et al., 2016). The decline in fluorescence emission efficiency has been correlated with an increase in non-photochemical quenching (NPQ), often attributed to reactions of the xantophyll cycle (Demmig-Adams and Adams, 1992; Rosema et al., 1998). However, the exact mechanisms of NPQ is still an area of active research. In simple and general terms, under low light most of the absorbed light energy is dissipated through photochemical quenching and with increasing irradiance the yield of photosynthesis decreases while the yield of fluorescence increases (Maxwell and Johnson, 2000; Van der Tol et al., 2009). Moving to high irradiance levels dissipation of excess energy through NPQ kicks in and the yields of the other two de-excitation pathways decrease. Further on, the peak in fluorescence yield seems to correlate with light saturation of photosynthesis (Van Der Tol et al., 2014). Our data suggests that this is indeed the case also on canopy



(a) *Experiment (B)*, circles, 4 operating points and *Experiment (C)*, triangles, 5 operating points.



(b) *Experiment (B)*, circles, 4 operating points and *Experiment (C)*, triangles, 3 operating points.

Fig. 12. The fluorescence gains vs. incident photon flux, for (a) predominately red background light and (b) predominately blue background light. The same information as in Fig. 10a,b (i.e. the circles which are from *Experiment (B)*) with added information from *Experiment (C)*, i.e. the triangles. The bars indicates ±1 standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

level. A weakness in our measurements, however, is that the temperature in the unit increased significantly at the highest irradiance levels and heat stress has been shown to decrease fluorescence yield, as well as photosynthetic yield (Ač et al., 2015). Thus, it is not possible to disentangle the effect of high light and high temperature on fluorescence yield at the highest irradiance levels, but it is likely to be synergistic (Rosema et al., 1998). Zeaxanthin formation in the xantophyll cycle (de-epoxidation), which increases NPQ and has been linked to both light saturation and high temperature (Gilmore and Yamamoto, 1992), is probably one of the mechanisms involved. Interestingly, the fluorescence at high irradiances are affected by other stressors as well, most commonly by a decline in fluorescence yield (Ač et al., 2015). Also, the irradiance at which the peak in fluorescence yield occurs decreases with increasing stress (Flexas et al., 2002; Van der Tol et al., 2009). As such, measurements of the fluorescence gain parameter, as described in this paper, can potentially be used to derive the light saturation point and/or identify plant stress on canopy level.

4. Conclusions and future work

In this study we have investigated the possibility of using remotely sensed fluorescence (gain) as a feedback signal for energy optimizing the spectrum of a LED lamp w.r.t. short term photosynthetic rate in basil.

With experiments using one LED group at a time (*Experiment (A)*) we have shown that for the considered light intensities (0–350 μmol m^{-2} s^{-1}) there is a strong and nearly linear correlation between steady-state fluorescence around 740 nm, F740, and photosynthetic rate, PN, for basil plants (see Fig. 6). This indicates that F740 can be used as a relative measure of PN for the investigated light intensities. More experiments are needed to characterize the relationship in more detail, including photosynthesis measurements on canopy level, light saturating conditions as well as different levels and types of plant stress.

For a given incident photon flux, the short term photosynthetic rate was not very sensitive to which LED group that was used. But some variations were indeed noted and the relative gains did change somewhat as the intensities varied (see Figs. 7 and 8). For a given incident irradiance (W/m^2) though, the differences were more significant. LED 630 (having the longest wavelength) is the most efficient, since an energy quanta contains more photons the longer the wavelength. When including the efficiencies of the different diodes in the analysis, which is the relevant quantity for minimizing the energy consumption, LED 450 and 630 were found to be equally efficient in driving photosynthesis (see Fig. 9).

Experiments with different background light show that the fluorescence gains $\partial F740/\partial P_i$ are not substantially affected by neither the background spectra considered (B:R ratio 1:3 and 3:1) nor the light intensity. The only significant shift noted, was when the total incident light exceeded 500 μmol m^{-2} s^{-1}. The fluorescence gain, $\partial F740/\partial q$, for green LEDs (530 nm) was then slightly increased relative to the others, resulting in similar gains for LED 420, 450 and 530, though still lower than for LED 630 (see Fig. 12).

These experiments suggest that the fluorescence gains could be used to find optimal spectrum in terms of maximizing photosynthetic rate for a given applied electrical power; but since the relation between the fluorescence gains seems to be rather static with respect to light intensity and spectrum (see Fig. 10e and f) an online controller might be unnecessary for optimizing the power split to the different diodes. Note, however, that this conclusion is based on, and limited to, measurements of short term changes in photosynthesis on leaf level (and fluorescence on canopy level) of healthy basil plants grown under low to moderate light intensities. It could be interesting to investigate other operating conditions in future work. Under the studied conditions the controller would increase LED 450 and 630 until they saturate. If higher light intensity is needed it would provide light from LED 420 and only turn on LED 530 if all the others were already saturated. This indicates that specified bounds of the spectrum to ensure healthy and morphologically well developed plants need to be determined beforehand. Instead of having an online controller, it should be possible to use this method for calibrating the spectrum to different plant species and to identify if and how the efficiencies of the diodes change relative to each other over time, or as a function of different operating conditions.

Data from the high intensity background light experiment show that there is a transition from increasing to decreasing fluorescence gains around the light saturation point of photosynthesis, and with support from the literature we speculate that the method can potentially also be used for finding the optimal light level as well as identify stress on canopy level. However, the applicability of the method for this purpose needs to be investigated further; it was not an objective in this study.

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