

OPTIMISATION OF LIGHT SPECTRAL QUALITY TO IMPROVE PLANT GROWTH AND DEVELOPMENT

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Abstract

The many advantages of light-emitting diode (LED) grow lamps in terms of programmability and energy efficiency make them an attractive replacement for old lighting systems. However, when replacing conventional broad-waveband 'white' lighting with narrow-waveband LED lighting it is important to ensure that the system is able to produce plants of an equal or higher quality and yield. In order to choose suitable light 'recipes', i.e. combinations of wavelengths for optimal plant growth, we must first understand how light quality affects plant growth and development. The following work explores the role of each part of the light spectrum, both in natural sunlight in canopy environments in the field and glasshouse, and in an artificial light environment in controlled environment growth rooms.

It was determined that, blue, green, red and far-red wavebands are all instrumental in the provision of positional signals to the leaf which enable optimisation of plant physiology to light conditions, especially in the canopy environment. A new hypothesis for the role of the blue:green light ratio in whole-canopy water use efficiency is presented. Wavelength-specific effects were observed in hypocotyl elongation, leaf expansion, photosynthetic stoichiometry and absorption of different wavelengths, root architecture, photosynthetic capacity, whole-plant morphology (in terms of biomass partitioning between leaves, stems and roots) and fresh weight yield of stems and leaves. In the glasshouse, it was determined that monochromatic red LED lighting is a suitable alternative for conventional high-pressure sodium light, as

it boosted leaf photosynthetic capacity as well as leaf area, fresh and dry weight yield, and was the most efficient light source in terms of 'crop per watt'.

Finally, further applications of the work such as those for commercial horticulture and farming in closed plant production systems are presented, and it is concluded that LED lighting is likely to have a crucial role in the future of global food security.

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Abbreviations

3 – PGK	3 – phosphoglycerate kinase
A	Carbon assimilation/ photosynthesis
A _{max}	Maximum rate of carbon assimilation/ photosynthesis
<i>A. sativa</i>	<i>Avena sativa</i>
<i>A. thaliana</i>	<i>Arabidopsis thaliana</i>
ABA	Absciscic acid
ADP	Adenine disphosphate
A _{max}	Maximum photosynthetic capacity
ANOVA	Analysis of Variance
ATP	Adenine triphosphate
B	Blue (light)
B:G	Blue to green (light ratio)
Chl <i>a</i>	Chlorophyll <i>a</i>
Chl <i>b</i>	Chlorophyll <i>b</i>
CDL	Critical day length
CO ₂	Carbon dioxide
COP1	CONSTITUTIVELY PHOTOMORPHOGENIC 1
CRY	Cryptochrome
CRY1	Cryptochrome 1
CRY2	Cryptochrome 2
Cv.	Cultivar
Cv. L.G.	<i>Lactuca sativa</i> L. cv. Lobjoits Green
Cv. Match	<i>Lactuca sativa</i> L. cv. Match
DNP	Day neutral plant
ETR	Electron transport rate
FAD	Flavin adenine dinucleotide
FADH	Flavin adenine dinucleotide hydrogen

FKF1	Flavin-kelch-Fbox-1
FR	Far-red (light)
FT	FLOWERING LOCUS T
G	Green (light)
G3P	Glycerate-3-phosphate
G:R	Green to red (light ratio)
GA	Gibberellic acid/ Gibberellin
gs	Stomatal conductance
H ⁺	Hydrogen ion
H ₂ O	Water
HIR	High irradiance reaction
HPS	High-pressure sodium
HY5	LONG HYPOCOTYL 5
IRGA	Infra-red gas analysis
J	Joules
J _{max}	Maximum rate of electron transport
kJ	Kilojoules
LAI	Leaf area index
LCP	Light compensation point
LDP	Long day plant
LED	Light-emitting diode
LFR	Low-fluence response
LHC	Light harvesting complex
LHCI	Light harvesting complex one
LHCII	Light harvesting complex two
LKP2	LOV-kelch-protein-2
Lm	Lumens
LRC	Light response curves
MH	Metal halide

NADP ⁺	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NPQ	Non-photochemical quenching
<i>O. sativa</i>	<i>Oryza sativa</i>
O ₂	Oxygen
PAM	Pulse-amplitude modulated
PAR	Photosynthetically active radiation
PCR	Photosynthetic carbon reduction (cycle)
PDL	Plant detectable light
PFD	Photon flux density
Pfr	Far-red light absorbing phytochrome
PFT1	PHYTOCHROME AND FLOWERING TIME 1
PGA	Phosphoglycerate/ phosphoglyceric acid
PHOT	Phototropin
PHOT1	Phototropin-1
PHOT2	Phototropin-2
PHY A	Phytochrome A
PHY B	Phytochrome B
PHY	Phytochrome
PIFs	Phytochrome interacting factors
PPFD	Photosynthetic photon flux density
Pr	Red-light absorbing phytochrome
PSI	Photosystem I
PSII	Photosystem II
R	Red (light)
R:FR	Red to far-red (light ratio)
R:G	Red to green (light ratio)
RB	Red + blue (light)
RBG	Red + blue + green (light)

RBGFR	Red + blue + green + far-red (light)
Rd	Dark respiration
RH	Relative humidity
ROS	Reactive oxygen species
Rubisco	Ribulose - 1,5 - biphosphate carboxylase enzyme
RuBP	Ribulose - 1,5 - biphosphate
RUE	Radiation use efficiency
SAS	Shade avoidance syndrome
SDP	Short day plant
SEM	Standard error of means
TFL	Tubular fluorescent lamps
TPDL	Total plant detectable light
UV-A	Ultraviolet - A
UV-B	Ultraviolet -B
<i>V. faba</i>	<i>Vicia faba</i> cv. Wizard
V _{cmax}	Maximum rate of carboxylation
VLF	Very low fluence
VLFR	Very low fluence response
W	Watts
WT	Wild type
WUE _i	Intrinsic water use efficiency
ZTL	Zeitlupe
λ	Wavelength
ϕ PSII	Photochemical yield/ efficiency of photosystem II

Table of contents

Abstract.....	1
Acknowledgements	3
Abbreviations.....	4
Table of contents	8
List of Figures.....	12
List of Tables	12
1 – Introduction	25
1.1 Artificial lighting in research and horticulture.....	12
1.2 Conventional light sources Vs LED.....	26
1.3 Plant sensing of light.....	30
1.4 The photosynthetic photoreceptors.....	31
1.5 Mechanism of photosynthesis.....	36
1.6 Carbon assimilation rate.....	41
1.7 Non-photochemical quenching and chlorophyll fluorescence.....	42
1.8 Light absorption profile through the leaf	44
1.9 Non-photosynthetic photoreceptors.....	49
1.10 UV-B light sensing mechanism	51
1.11 UV-A/ Blue and green light sensing mechanism	51
1.12 Red/ far-red light sensing mechanism.....	53
1.13 Light mediated physiological responses.....	54
1.14 Light requirement for response.....	55
1.15 Germination.....	56
1.16 Skotomorphogenesis to photomorphogenesis	57
1.17 Root growth.....	58
1.18 Photoacclimation of the chloroplasts.....	59
1.19 Guard cell development and stomatal aperture	61
1.20 Shade avoidance	64
1.21 Adaptation of morphology and protection and repair of the photosynthetic apparatus under high light	68
1.22 Conclusions, aims and hypotheses	71
2 Materials and Methods	74
2.1 Chapter 3 - Spectral variation in natural light environments	74

2.1.1	Growth environment and plant material	74
2.1.2	Equipment	75
2.1.3	Data collection	77
2.1.4	Data analysis	78
2.2	Chapter 4 - A comparison of growth, development and physiology of <i>Coriandrum sativum</i> grown under different combinations of blue, green, red and far-red light in controlled environment growth rooms.....	82
2.2.1	Growth environment	82
2.2.2	Plant material	87
2.2.3	Data collection	87
2.3	Chapter 5	101
2.3.1	Growth environment	101
2.3.2	Plant material	105
2.3.3	Data collection	105
2.4	Statistical analysis	109
3	Spectral variation in natural light environments.....	110
3.1	Introduction:.....	110
3.2	Methodology and hypotheses.....	115
3.3	Results.....	117
3.1.1	Sunlight spectrum above the canopy in the field and glasshouse	117
3.1.2	Changes in level of plant detectable light and spectra within crop canopies	120
3.3.1	Changes in light ratios through the canopy profile	125
3.3.2	Changes in PAR and leaf gas exchange.....	128
3.3.3	Determining cause of changes in stomatal conductance.....	130
3.4	Discussion	132
3.4.1	Green light gradient as a positional signal for increased water use efficiency	133
3.4.2	Far-red light primes leaves to absorb green light for better energy distribution throughout the canopy	136
3.4.3	Mimicking light quality in natural light environments to create ideal morphologies.....	137
3.5	Conclusions.....	138
4	A comparison of growth, development and physiology of <i>Coriandrum sativum</i> grown under different combinations of blue, green, red and far-red light in controlled environment growth rooms.....	140
4.1	Introduction:.....	140
4.2	Aims and hypotheses.....	145

4.3	Methodology	147
4.4	Results.....	148
4.4.1	Germination assays.....	148
4.4.2	Growth, development and physiology	149
4.4.3	Biomass partitioning.....	169
4.4.4	Comparison of absorption spectrum and chlorophyll content	173
4.4.5	Visualisation of vein networks in <i>C. sativum</i> cv. Cruiser grown under combinations of red, blue and green light	178
4.4.6	Investigation into the differences in fresh-weight yield of cv. Standard and cv. Cruiser grown under the B light recipe	181
4.5	Discussion	183
4.5.1	Overview of novel observations	183
4.5.2	Germination.....	185
4.5.3	Stem elongation.....	185
4.5.4	Leaf expansion	187
4.5.5	Stomatal distribution and water use efficiency.....	189
4.5.6	Root development	191
4.5.7	Efficiency of photosystem II and electron transport	192
4.5.8	Carbon assimilation rate and photochemistry	193
4.5.9	Leaf chlorophyll content and absorption spectra.....	196
4.5.10	Assimilate portioning and yield	201
4.6	Conclusions.....	202
5	Red LED lamps boost photosynthesis and fresh-weight yield for more crop per watt than conventional HPS	205
5.1	Introduction	205
5.2	Aims and hypotheses.....	211
5.3	Methodology	213
5.4	Results.....	214
5.4.1	Measured light levels.....	214
5.4.2	Physiological effects	217
5.4.3	Crop yield at harvest	230
5.4.4	Cost analysis.....	235
5.5	Discussion	237
5.5.1	The benefit of supplementary light.....	237
5.5.2	Comparison of growth and physiology under conventional HPS and new LED lighting.....	238
5.5.3	Red LED light for increased 'crop per watt'	239

5.6	Conclusions	240
6	General discussion	241
6.1	Summary of findings	241
6.2	Perspectives on green light	244
6.3	Improvements and further work	246
6.4	Future research.....	247
6.4.1	UV-B light for the improvement of nutrition, pest and disease resistance	247
6.4.2	Light for control of flowering.....	249
6.4.3	Light recipes tailored to growth stage.....	252
6.4.4	'Smart' lighting systems.....	253
6.5	Further applications of optimal light recipe research	255
6.5.1	Vertical farming	255
6.5.2	Growth of crops in space	257
6.6	LEDs for the future of horticultural lighting	258
6.7	Final conclusions.....	259
7	References	261

List of Figures

Figure 1.1: The spectrum of high pressure sodium (HPS), fluorescent tube (FT), metal halide(MH) and blue+green+red light-emitting diodes (RGB LED) lights as measured with the Fieldspec HH pro spectroradiometer (ASD, Boulder Colorado). Photon flux density (PFD) measured in $\mu\text{mol m}^{-2} \text{s}^{-1}$ is shown for wavelengths from 325-1075nm.

MH, HPS and FTs are broad spectrum white light sources which also emit large amounts of infra-red wavelengths (>750nm) - heat. LEDs are steady state, narrow waveband emitting devices which can be used to target individual photoreceptors. 26

Figure 1.2: The difference spectrum – photon flux density (PFD) at wavelengths of 325-1075nm - when comparing an old fluorescent bulb (usage approx. 1 year for 12 hours per day) to a new fluorescent bulb in adjacent controlled environment growth rooms used for horticultural research at the University of Nottingham Sutton

Bonington campus. Lights were measured at the distance that they each provided $30\mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR with the FieldSpec HH 2 spectroradiometer (ASD). Output in the lower region of the spectrum, specifically the areas around 606nm, deteriorated in the old bulb, resulting in a fall from 10.8 to 8.9 in red (600-699) to far-red (700-750) light ratio. 29

Figure 1.3: The molecular structure of Chlorophyll a (left) and Chlorophyll b (right).

Chlorophyll a differs only in that the circled CHO group is replaced by a CH_3 group.

Adapted from Rabinowitch (1969). 32

Figure 1.4: The absorption spectra of chlorophyll a (chl a) and chlorophyll b (chl b) from leaves of *Spinacea oleracea* isolated and suspended in 80% acetone, measured at 5nm intervals (green lines), absorption spectra of β carotene, lutein and zeaxanthin suspended in acetone, as measured by Kopczynski et al (2005) (orange/red lines) and absorption of an intact *Coriandrum sativum* leaf as measured with the

FieldSpec HH 2 spectroradiometer (ASD, Boulder Colorado) and integrating sphere (Li-Cor, Illinois) (black line).	34
Figure 1.5: Schematic diagram of the inner structure of the chloroplast (Taiz and Zeiger, 2010).....	36
Figure 1.6: Schematic diagram of the photosynthetic complexes involved in the light-dependent reactions of photosynthesis (Hopkins and Hüner, 2009).	37
Figure 1.7: The Z-Scheme configuration of the photosynthetic electron transport chain shows linear electron transport converting photonic energy to chemical energy in the form of adenine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH). PSII uniquely is able to donate further electrons through the splitting of water molecules. Based on the model by Walker (2002).	39
Figure 1.8: The photosynthetic carbon reduction cycle (and its organic carbon compound products), split into the three main stages with the detailed steps of carboxylation and reduction. Based on information in Hopkins and Hüner, 2009.	40
Figure 1.9: The photonic energy in photosynthetically active radiation (PAR) absorbed by chlorophyll may take one of three competitive pathways. 1) It may be channelled into photochemistry, termed 'photochemical quenching'. 2) It may be dissipated as heat, termed 'non-photochemical quenching' (NPQ). 3) It may be re-emitted as light, termed 'chlorophyll fluorescence'. Based on information from (Maxwell and Johnson, 2000; Hopkins and Hüner, 2009).....	43
Figure 1.10: Schematic depiction of penetration of monochromatic blue, green and red light through the vertical profile of a leaf illuminated from above the adaxial surface. Blue light is quickly absorbed by multiple pigments in the top layers of leaf cells, whereas red light may penetrate further due to mainly being absorbed by just the chlorophylls. Green light may reach the bottom layer of cells due to the chlorophyll's weak absorption in the green (the 'green window') and scattering of	

green light within the leaf (the 'detour effect'). Based on data from Nishio et al (1993); Vogelmann and Han (2000); Evans and Vogelmann (2003).	46
Figure 1.11: Action spectrum of photosynthesis based on the quantum yield for CO ₂ fixation an absorbed light basis of cucumber leaves developed under a sunlight spectrum. Red is most efficient at driving photosynthesis on an absorbed light basis, followed by green then blue light. Adapted from Hogewoning et al (2012).	47
Figure 1.12: The different wavelengths/ colours in the portion of the electromagnetic spectrum detectable by plants and the photoreceptors which they activate (based on information from Shropshire et al., 1961; Ahmad et al., 2002; Kim et al., 2007; Carvalho et al., 2010; Heijde and Ulm, 2012; Banaś et al., 2012). Numbers represent wavelength ranges in nanometers.	50
Figure 2.1: The LI-6400XT portable photosynthesis machine measuring head with sun and sky attachment.	76
Figure 2.2: The FieldSpec HH 2 spectroradiometer connected to the laptop via USB (a) and in standalone mode with RCR attachment (b).	77
Figure 2.3: Spreadsheet for the conversion of watts per meter squared at 1nm intervals between 325-1075 to photon flux density using the equation as described above in section 2.4.	80
Figure 2.4: The 30 watt LED floodlamps from above, below and in the growth area.	82
Figure 2.5: Adjustable output multi-waveband LED lamp as designed and built by members of the Faculty of Engineering, University of Nottingham, UK.	83
Figure 2.6: The spectra of the LED lamps as measured with the FieldSpec HH 2 spectroradiometer and converted from Wm ⁻² to photon flux density (PFD).	85
Figure 2.7: Demonstration (on lettuce) of the use of the Walz JuniorPAM chlorophyll fluorometer for measurement of rapid light curves of chlorophyll fluorescence.	90

Figure 2.8: (a) The FieldSpec HH 2 spectroradiometer connected to the integrating sphere and laptop. (b) The black plug, white plug and their configuration for (c) R _{ld} and R _{ls} , (d) R _{lr} and T _{lr} and (e) T _{il} measurements.	92
Figure 2.9: Selection and cropped leaf image in ImageJ for determination of leaf area inside GFS-3000 cuvette.	96
Figure 2.10: Photograph (a) and schematic (b) of the glasshouse set-up. Benches were arranged in a south-facing glasshouse in Leicestershire, UK. One 400 watt HPS lamp was suspended 167 cm above bench 1, two 30 watt blue LED lamps suspended 60 cm above bench 3 and two 30 watt red LED lamps suspended 66 cm above bench 4. Bench 2 was left as a sunlight only control.	101
Figure 2.11a: Spectra of the supplementary lights as measured in the hours of darkness (night), on a dull day at midday (cloudy day) and on a bright day at midday (bright day) with the ASD Fieldspec HH 2 spectroradiometer at plant height directly under the lamps (no lamps for sun control).....	103
Figure 2.12: Walz MONITORING-PAM Chlorophyll fluorometers for the continuous measurement of ϕ PSII, ETR and NPQ of lettuce leaves.	107
Figure 3.1a: The solar spectrum as measured as photon flux density (PFD) on a clear, bright summer day in the field and the glasshouse with the FieldSpec HH 2 spectroradiometer (ASD). Readings averaged from 5 measurements taken between 11am and 1pm. Average total photosynthetically active radiation (PAR - λ 400-700nm) in the field was $824 \mu\text{mol m}^{-2} \text{s}^{-1}$ and average total plant detectable light (TPDL - λ 325-750nm) was $1049 \mu\text{mol m}^{-2} \text{s}^{-1}$. In the glasshouse, average total PAR was $434 \mu\text{mol m}^{-2} \text{s}^{-1}$ and average TPDL was $544 \mu\text{mol m}^{-2} \text{s}^{-1}$	118
Figure 3.2: (a) Light spectra at three canopy levels in mature canopies of <i>V.faba</i> , <i>A. sativa</i> and <i>O. sativa</i> as measured with the Fieldspec HH 2 spectroradiometer (ASD). ‘Top’, ‘middle’ and ‘base’ refer to positions in the canopy profile 10cm from the	

tallest point, at the middle point and 10cm from the ground respectively. N=15 for V. faba and A. sativa and n=10 for O. sativa. UV-A = 325-399nm; Blue = λ 400-499nm; Green = λ 500-599nm; Red = λ 600-699nm; Far-red = λ 700-750nm.	120
Figure 4.1: (a) Commercial glasshouse potted coriander growth, Langmead Herbs, UK. (b) Potted coriander as sold in TESCO supermarkets, UK.	143
Figure 4.2: Coriander seedlings 10 days (a) and 20 days (b) after sowing in the glasshouse. Each seed case produces 2 seedlings which have a pair of cotyledons. The next leaf is the first 'true leaf' with the distinctive lobed coriander shape.	144
Figure 4.3: For each light recipe (see chapter 2.1.1), (a) germination success as calculated as the percentage of a possible 12 seedlings of cv. Standard to emerge from the soil within 15 days and (b) the average number of days taken to the first germination event (i.e. any seedling emerging through the soil surface). Results are a mean of 3 runs of the experiment. Bars show standard error of means.	148
Figure 4.4: Photos of example plants (C. sativum cv. Standard) grown under each light recipe 7 (a/b), 14 (c/d), 21 (e/f) and 28 (g) days after the first plant germinated. Bars in (g) show scale of 5cm.	150
Figure 4.5: Height of the supported plant (a) and true leaf (not including the cotyledons) number (b) of plants grown under each light recipe as measured 7, 14, 21 and 28 days after germination. Measurements were of 8 plants under each light recipe in 3 runs of the experiment (n=24). Bars show standard error of means.	151
Figure 4.6: Aerial photo of representative fully expanded first true leaves of C. sativum cv. Standard grown under each light recipe.	153
Figure 4.7: Average first true leaf area (cm ²) of C. sativum cv. Standard grown under each light recipe. Results are a mean of 8 fully expanded leaves under each light recipe in 3 runs of the experiment (n=24). Bars show standard error of means and letters represent statistical differences (P<0.05).	153

Figure 4.8: (a) Stomatal density per 1 mm² on abaxial and adaxial leaf surface of the third true leaf of *C. sativum*. cv. Standard grown under each light recipe and (b) the ratio of abaxially located stomata to adaxially located stomata. Leaf surface impressions were taken from each side of three leaves in each light recipe in three runs of the experiment (n=9). Bars show standard error of means and letters represent statistical differences (P<0.05)..... 154

Figure 4.9: Rapid light response curves of chlorophyll fluorescence were taken on the last fully expanded leaf of *C. sativum* cv Standard. grown under each light recipe with the Walz JuniorPAM chlorophyll fluorometer, 14-15 days post germination. Results are from a mean of 3 experiments (n=9). The figure shows (a) electron transport rate (ETR), corrected for absorption of photosynthetically active radiation (R = 0.53887; B = 0.541865; RB = 0.55522; RBG = 0.541141; RBFR = 0.549186; RBGFR = 0.479432) as measured with the FieldSpec HH2 spectroradiometer (ASD) and integrating sphere (LI-COR) as can be seen in Table 4.1 and (b) quantum yield on photosystem II (ϕ PSII) under increasing light levels. 156

Figure 4.10: Chlorophyll a:b ratio (a) and total chlorophyll content per cm² (b) of the third true leaf of *C. sativum* cv. Standard grown under each light recipe. Results are a mean of samples from 3 runs of the experiment (n=9). Bars show standard error of means and letters represent statistically significant differences (P=<0.05). 158

Figure 4.11: Spot measurements of in situ photosynthesis (A) taken on the last fully expanded leaf of plants under each light recipe 21 days after germination using the Licor-LI6400XT with sun and sky clear plastic window. Graphs show photosynthesis per cm⁻² (a) and adjusted for leaf area to show whole leaf photosynthesis (b) under 150 μ mol m⁻² s⁻¹ of the light in which they were grown. Bars show standard error of means and letters represent significant differences (P<0.05). Results are a taken from a mean of three experiments (n=12)..... 159

Figure 4.12: Photosynthetic light response curves measured with the GFS-3000 (Walz) showing (a) the carbon assimilation rate (A) of *C. sativum* cv. Standard grown under each light recipe and (b) these values corrected for absorption of PAR (400-750nm). (Absorption values: R = 0.53887; B = 0.541865; RB = 0.55522; RBG = 0.541141; RBFR = 0.549186; RBGFR = 0.479432) as measured with the FieldSpec HH2 spectroradiometer (ASD) and integrating sphere (LICOR) as can be seen in Table 4.1. Bars show standard error of means. Results are a taken from a mean of three experiments (n=9). 161

Figure 4.13: (a) Dark respiration (RD), (b) light compensation point (LCP) and (c) maximum rate of carbon assimilation of the third true leaf of *C. sativum* cv. Standard grown under each light recipe 21-24 days after germination. Parameters were calculated by entering the photosynthetic light response curve values (Fig 4.12) into the model described by (Prioul and Chartier, 1977). Results are taken from a mean of three experiments (n=9). Bars show standard error of means and letters represent statistically significant differences ($P < 0.05$). 163

Figure 4.14: Intrinsic water use efficiency (WUE_i) of leaf three of *C. sativum* cv. Standard grown under each light recipe, as calculated with the data from the Walz GFS-3000 photosynthetic light response curves (Fig. 4.12). WUE_i = carbon assimilation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) divided by stomatal conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$). Results are taken from a mean of three experiments (n=9). Bars show standard error of means. 164

Figure 4.15: Photosynthetic CO_2 response curves were measured with the GFS-3000 (Walz) to show carbon assimilation under increasing carbon dioxide concentrations (a) of the third true leaves of *C. sativum* cv. Standard grown under each light recipe 24-28 days after germination. V_{cmax} (b) and J_{max} (c) were then calculated by entering the values into a two-segment model (Bernacchi, 2013) which used the equations of

Farquhar et al., 1980. Results are taken from a mean of three experiments (n=9). Bars show standard error of means and letters represent statistically significant differences (P<0.05).....	165
Figure 4.16: Fresh weight (a), dry weight (b), leaf area (c) and leaf:stem fresh weight ratio (d) of <i>C. sativum</i> cv. Standard grown under each light recipe for 28 days after germination. Results are a mean from 8 plants in 3 experiments (P=24). Bars show standard error of means and different letters represent statistical differences (P<0.05).....	167
Figure 4.17: Pots of <i>C. sativum</i> cv. Cruiser 21 days after sowing (a) and individual plants washed from the soil (b) for the R, B, RB and RBG light recipes.	169
Figure 4.18: Total fresh weight and above ground fresh weight (a), total leaf area and leaf to stem ratio (b), fresh weights of leaves, stems and roots (c) and fresh weight biomass partitioning between leaves, stems and roots (d) of <i>C. sativum</i> cv. Cruiser seedlings 21 days after sowing under the different light recipes. Results are from a mean of at least 4 plants in 3 separate experiments (n=12+). Bars show standard error of means and letters indicate significant differences between groups (P<0.05).	170
Figure 4.19: Total root network length (a) average diameter of the taproot (b) and example root scans (c) of <i>C. sativum</i> cv. Cruiser seedlings 21 days after sowing under the different light recipes. Results are from a mean of at least 4 plants in 3 separate experiments (n=12+). Bars show standard error of means and letters indicate significant differences between groups (P<0.05). Images were acquired in high resolution with a flat-bed scanner and analysed in the WinRhizo root measuring program (Arsenault et al., 1995).	172
Figure 4.20: The absorption spectrum between 325 and 750nm of the adaxial (a) and abaxial (b) surfaces of the fully expanded third true leaf of <i>C. sativum</i> cv. Cruiser	

(n=4) and the adaxial (c) and abaxial (d) surfaces of the fully expanded third true leaves of <i>C. sativum</i> cv. Standard (n=1) grown under the different light recipes as measured with the FieldSpec HH 2 spectroradiometer (ASD) and integrating sphere with broad-wavelength white light illuminator (LI-COR).	174
Figure 4.21: Percentage of total absorption between 400 and 750nm made up of each waveband (blue = 400-499nm; red = 600-699nm; green = 500-599nm) of adaxial (a) and abaxial (b) leaf surfaces of the third true leaf of <i>C. sativum</i> cv. Cruiser (n=4) and the adaxial (c) and abaxial (d) leaf surfaces of the third true leaf of <i>C. sativum</i> cv. Standard (n=1) grown under the different light recipes. Bars show standard error of means and letters indicate significant differences between groups ($P<0.05$).	175
Figure 4.22: Chlorophyll a:b ratio (a) and total chlorophyll content per cm^2 (b) of the third true leaf of <i>C. sativum</i> cv. Cruiser grown under each light recipe (n=4). Bars show standard deviation from the mean and letters represent statistically significant differences ($P<0.05$).....	177
Figure 4.23: The fully expanded first true leaf from a plant in each light recipe (R, B, RB and RBG) cleared of pigment to allow for visualization of the vein network. Images were scanned at 1200dpi with a ruler for scale (a). Expanded, high resolution images (not to scale) of the individual B (b), R (c), RB (d) and RBG (e) light recipe leaves are also shown for better image clarity.....	180
Figure 4.24: Photosynthetic light response curves measured with the LI-6400XT (LICOR) showing the carbon assimilation rate (A) of the third true leaves of <i>C. sativum</i> cv. Cruiser grown under each light recipe (n=4).	181
Figure 4.25: Roots of <i>C. sativum</i> cv. Standard grown under the B light recipe imaged with a flat-bed scanner at high resolution 21 days after sowing.	182
Figure 5.1: (a) Daily average PAR ($\text{kJ m}^{-2} \text{d}^{-1}$) as measured by the Sutton Bonington Met Station from a rooftop over the two 45-day growth periods in Summer 2014.	

Filled in data points indicate Moni-PAM (Walz) data was also recorded on this day (see Fig 5.1b.). Results indicate a high level of variation between bright and cloudy days over the growth period. 215

Figure 5.2: (a) Average daily photochemical yield of photosystem II (ϕ PSII), (b) electron transport rate through PSII (ETR, corrected for absorbance) and (c) level of non-photochemical quenching (NPQ) as measured with Walz MONITORING-PAM chlorophyll fluorometers in two cultivars lettuce – L.G. (L. sativa L. cv. Lobjoits Green) and Match (L. sativa L. cv. Match) – grown under a sunlight only control, and with either $50\mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. Letters indicate significant differences between groups ($P<0.05$). 219

Figure 5.3: (a) Minimum daily photochemical yield of photosystem II (ϕ PSII), (b) maximum daily electron transport rate through PSII (ETR, corrected for absorbance) and (c) daily maximum level of non-photochemical quenching (NPQ) as measured with Walz MONITORING-PAM chlorophyll fluorometers in two cultivars lettuce – L.G. (L. sativa L. cv. Lobjoits Green) and Match (L. sativa L. cv. Match) – grown under a sunlight only control, and with either $50\mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. Letters indicate significant differences between groups ($P<0.05$). 222

Figure 5.4: (e) Combined data from all treatments combined in order to determine average light level required to saturate photosystem II. 226

Figure 5.5: natural sunlight levels at plant height over a typical bright day, and the amount of supplementary light that would need to be added to reach a target level of $755\mu\text{mol m}^{-2} \text{s}^{-1}$ for near maximum saturation of photosynthesis..... 227

Figure 5.6: Relative electron transport rate (ETR) as measured by the Walz JuniorPAM chlorophyll fluorometer in of two cultivars of lettuce – L.G. (L. sativa cv.

Lobjoits Green) and Match (L. sativa L. cv. Match) – grown under a sunlight only control, and with either 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. Bars show standard error of means. 228

Figure 5.7: Average leaf area (cm^2), fresh weight (g) and dry weight (g) 45 days after sowing of two cultivars of lettuce – L.G. (L. sativa cv. Lobjoits Green) and Match (L. sativa L. cv. Match) – grown under a sunlight only control, and with either 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. For dry weights, fresh plant material was dried for 1 week at 80°C. N=4 (run 1) or n=8(run 2). Bars show standard error of means and letters represent statistically significant differences ($P<0.05$). 230

Figure 5.8: Total percentage change in leaf area, fresh weight and dry weight of plants of each cultivar in each run under each supplementary light treatment as compared to the sunlight control 45 days after sowing of two cultivars lettuce – L.G. (L. sativa L. cv. Lobjoits Green) and Match (L. sativa L. cv. Match) – grown under a sunlight only control, and with either 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. N=4 (run 1) or n=8(run 2). For dry weights, fresh plant material was dried for 1 week at 80°C. 234

List of Tables

Table 2.1: The percentage of total photon flux density (325-750nm) made up by each waveband (UV-A/blue 325-499nm; green 500-599nm; red 600-699nm; far-red 700-750nm) in each light recipe. Peaks of LEDs: Red (R) = 665nm; Blue (B) = 455nm; G = 525nm; Far-red (FR) = 745nm.....	86
Table 2.2: The ratios of each waveband (UV-A/blue 325-499nm; green 500-599nm; red 600-699nm; far-red 700-750nm) in each light recipe.....	86
Table 2.3: Configuration of integrating sphere components for each measurement.....	92
Table 2.4: Absorbance at 455nm and 460nm of the adaxial sides of leaves of two cultivars of lettuce, cv. Match and cv. Lobjoits Green (L.G.) as measured with the Fieldspec HH 2 spectroradiometer (ASD, Boulder Colorado) in conjunction with an integrating sphere (LI-COR, Nebraska).....	108
Table 3.1: Light ratios in full sunlight in the field and glasshouse. UV-A = λ 325-399nm; Blue = λ 400-499nm; Green = λ 500-599nm; Red = λ 600-699nm; Far-red = λ 700-750nm. Measurements were taken on a clear, bright summer day with the FieldSpec HH 2 spectroradiometer (ASD). Readings averaged from 5 measurements taken between 11am and 1pm. Standard error of means (SEM) are also presented.....	119
Table 4.1: Average absorption values (and standard error of means – SEM) between 400 and 750nm from the adaxial (AD) and abaxial (AB) side of leaves of <i>C. sativum</i> cv. Cruiser (n=4) from each light recipe as measured with the FieldSpec HH 2 spectroradiometer (ASD) and integrating sphere (LICOR).	

Absorption values from cv. Standard (n=1) are also presented for purposes of comparison.....173

Table 5.1: A simple cost analysis was carried out based on the cost per plant of providing supplementary light divided by the average increase in fresh weight in run 1 and 2 combined. This was scaled up using the information in chapter 2.1.3 to determine how many plants could have been grown in the area under 1 HPS lamp or 2 red or blue LED lamps illuminated with $50\mu\text{mol m}^{-2} \text{ s}^{-1}$ of supplementary PAR. Based on current electricity prices (EoN, Sept 2016), the cost per kilowatt hour (kWh) of electricity was assumed to be £0.13. The energy in kilowatt-hours is equal to the power in watts (W), times the time-period in hours divided by 1000. Lamps were run for a total of 16 hours per day for 35 days and therefore time period in hours equated to 560.....235

1 – Introduction

1.1 Artificial lighting in research and horticulture

In research, artificial lighting is commonly used as the sole source of light in controlled environment growth rooms or tissue culture labs (Yeh and Chung, 2009). Controlled environments enable researchers to gain accurate, repeatable results through tight control of parameters such as temperature, humidity, carbon dioxide concentration and light level in order to isolate causal factors. Lights have also been used in similar ways in glasshouses to determine the effects of supplementing natural sunlight (Garcia-Victoria and Warmenhoven, 2012).

In commercial glasshouses where plants are produced for eventual sale growth lamps are often used to supplement natural light, to extend day length to extend the growing season (Heuvelink *et al.*, 2006) or to bring about the onset of particular plant developmental events such as flowering (Yanagi *et al.*, 2006). Also, the idea of urban, indoor or ‘vertical’ farming with supplementary or completely artificial lighting is becoming increasingly popular (Despommier, 2010, 2013). Such systems allow the year-round production of food in places where conventional farming practices would not be possible.

1.2 Conventional light sources Vs LED

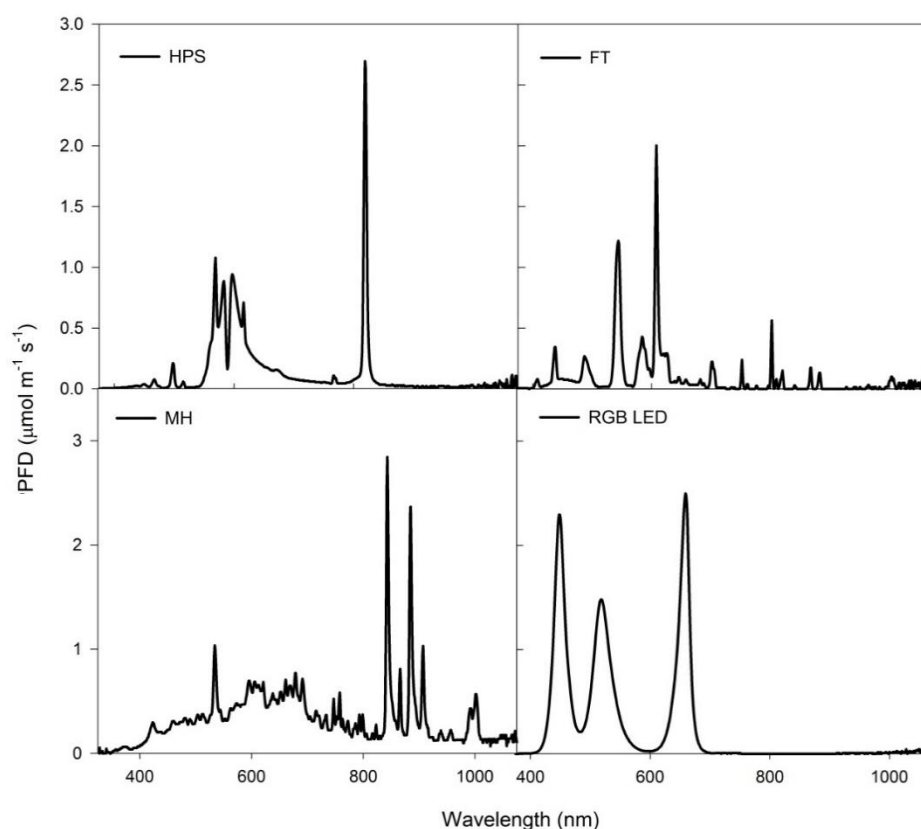


Figure 1.1: The spectrum of high pressure sodium (HPS), fluorescent tube (FT), metal halide(MH) and blue+green+red light-emitting diodes (RGB LED) lights as measured with the Fieldspec HH pro spectroradiometer (ASD, Boulder Colorado). Photon flux density (PFD) measured in $\mu\text{mol m}^{-2} \text{s}^{-1}$ is shown for wavelengths from 325-1075nm. MH, HPS and FTs are broad spectrum white light sources which also emit large amounts of infra-red wavelengths (>750nm) - heat. LEDs are steady state, narrow waveband emitting devices which can be used to target individual photoreceptors.

It could be argued that conventional forms of artificial lighting used for research and horticulture, i.e. tubular fluorescent lamps (TFL), metal halide (MH), high-pressure sodium (HPS) lamps are highly inefficient. They produce a

broad spectrum of light (Fig. 1.1) including many wavelengths that cannot be utilised by the plant for growth (Pinho *et al.*, 2012). Light emitting diodes (LEDs) however are narrow band devices (Fig. 1.1) which are now commercially available in wavebands suitable for plant growth (LUMILEDS, 2016) and being continuously developed to produce a wider range of colours ranging from UV-B (Ren *et al.*, 2007; Lui *et al.*, 2014) all the way into the far-red (Yeh and Chung, 2009). LEDs give the unique option of controlling not only light quantity, (being able to switch between 0 and 100% output almost instantaneously) but also spectral composition. Optimal light 'recipes' (bespoke combinations of narrowband wavelengths) may be developed to exactly meet the plants' light requirements ensuring energy is not wasted on producing inefficient wavelengths (Morrow, 2008; Yeh and Chung, 2009; Massa *et al.*, 2010)

In fact, LEDs are set to surpass conventional forms of lighting in terms of photon flux efficacy ($\mu\text{mol/J}$) and luminous efficacy (lm/W) by the year 2020, and already have a higher electrical efficiency for producing photosynthetically active radiation (PAR) (Pinho *et al.*, 2011) – light which may be utilised by the plant for photosynthesis (Zhu *et al.*, 2008). LEDs have been shown to be up to 3 times more efficient at lighting an area of canopy than some commonly used lighting (Morrow, 2008). As LEDs emit very little radiant heat this allows them to be placed nearer to biological material than conventional lighting (Massa *et al.*, 2010), ensuring that more light is intercepted by the canopy, further increasing the overall energy efficiency of the system and decreasing power costs.

The long operational life of the LEDs ensures that the system will be functional for a long period of time before the lights need to be replaced. LED lights are solid state and do not burn a filament to produce light, greatly increasing their functional lifetime compared to traditional light sources (Yeh and Chung, 2009). Studies by Kim *et al.*, (2007) have shown that LEDs can remain operational at their original maximum light output capacity for at least 10,000 hours, and possible output is known to decrease by just 30% of the original maximum output over 50,000 hours (Morrow, 2008). That means that lamps could be run for 12 hours per day on full power for over 27 months at full original output, and over 11 years before deteriorating to only 70%. Output of conventional bulbs would deteriorate up to 50% over 5 years. The minimal maintenance required for an LED lighting system could greatly cut down on bulb procurement, disposal and fitting replacement costs. LEDs have the added advantage that they do not contain the mercury found in TFL lamps (Toda, 2006) or any other hazardous substances, and are therefore also cheaper and easier to dispose of.

The longevity and highly controllable spectral output of LEDs also means that they could provide a standard light quality for plant research purposes. Current research often simply differentiates between the type of applied light sources e.g. fluorescent tubes or metal halide bulbs. However, there is often much variation in spectral output between light sources even of the same type, especially when new lights are mixed with older ones (Fig. 1.2). As light quality is known to have a huge impact on plant physiology (Carvalho *et al.*, 2010), which will be discussed in detail later, a more fixed spectral quality provided by

LEDs could allow researchers to rule out physiological variation caused by this variation in light quality.

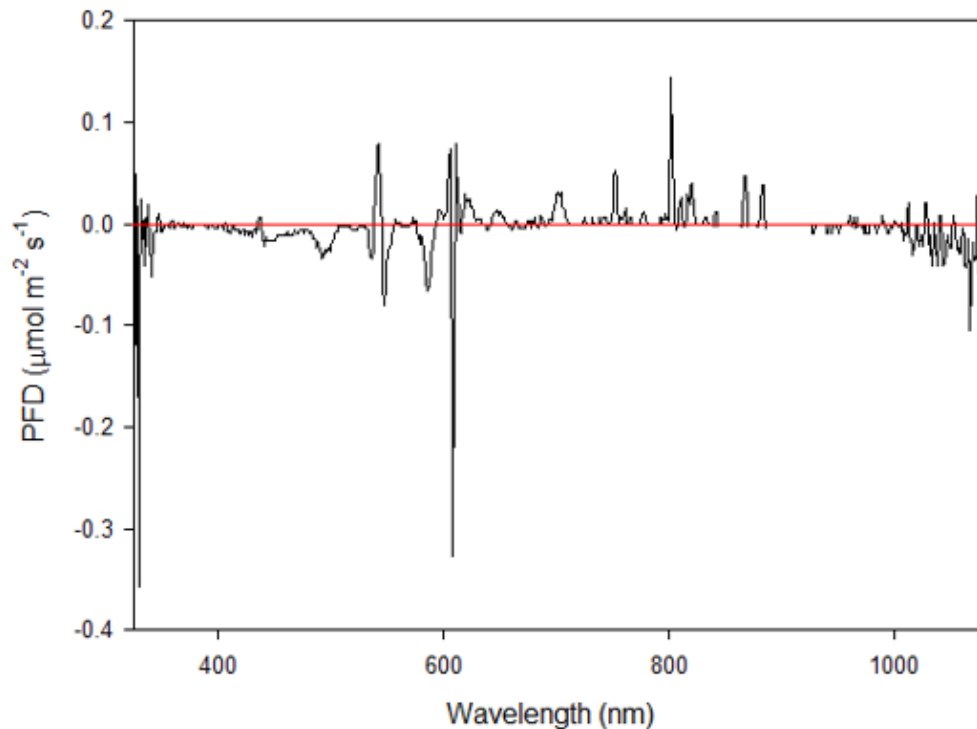


Figure 1.2: The difference spectrum – photon flux density (PFD) at wavelengths of 325-1075nm - when comparing an old fluorescent bulb (usage approx. 1 year for 12 hours per day) to a new fluorescent bulb in adjacent controlled environment growth rooms used for horticultural research at the University of Nottingham Sutton Bonington campus. Lights were measured at the distance that they each provided $30\mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR with the FieldSpec HH 2 spectroradiometer (ASD). Output in the lower region of the spectrum, specifically the areas around 606nm, deteriorated in the old bulb, resulting in a fall from 10.8 to 8.9 in red (600-699) to far-red (700-750) light ratio.

In summary, the switch from conventional artificial lighting to LEDs could save large amounts of energy and therefore greatly reduce the costs to growers and the environment. LEDs also provide the opportunity for fine control of growth

and development. However, the yield and quality achieved with conventional lighting systems must be surpassed, or at least maintained, if LED lighting systems are to be adopted by growers. As current horticultural light sources provide the plant with a broad range of wavelengths (Fig. 1.1), it is crucial to understand which of these wavelengths the plant needs and in what quantity before removing unnecessary wavelengths to improve energy efficiency. The 'recipe' of wavelengths in an LED light source is very important and must be carefully considered. To develop a light recipe that contains the right combination of wavelengths to produce plants of the same or a higher quality than conventional light sources, we need to first understand how plants sense and use light.

1.3 Plant sensing of light

Plants perceive light as individual portions of the electromagnetic spectrum (Carvalho *et al.*, 2010). Light is electromagnetic radiation made up of photons (or quanta) of light which may be thought of as individual particles. These particles travel in waves at varying energies when emitted from a light source, and therefore light can be considered both particle and wave (Greiner and Müller, 1994). As depicted in figure 1.12, more energised photons travel at shorter wavelengths (wavelength defined as the distance between two peaks in the wave) and are closer to the UV-B end of the spectrum, whilst less energised photons travel at longer wavelengths and are closer to the far-red end of the spectrum (Hopkins and Hüner, 2009). As discovered by Newton, (1704), the 'white' light that we see is therefore actually made up of a

combination of many wavelengths or 'colours' of light. Pigment proteins which are activated when they absorb light of specific wavelengths, usually at a threshold intensity and/or duration of exposure, are known as photoreceptors (Fankhauser and Chory, 1997). The wavelengths visible to the cones of the human eye (the visible light spectrum) lie between approximately 400 and 700nm in the 'blue' to 'red' area of the spectrum whilst plant photoreceptors are able to sense wavelengths between approximately 320 and 750nm, from the 'UV-B' through to the 'far-red' (Carvalho *et al.*, 2010).

1.4 The photosynthetic photoreceptors

It is well known that plants utilise light in the process of photosynthesis in order to assimilate carbon from the atmosphere, allowing them to metabolise and grow, ultimately converting solar energy to edible biomass (Zhu *et al.*, 2008).

The light energy channelled into photosynthesis is mainly absorbed by the green pigment chlorophyll (Zhu *et al.*, 2008). Chlorophyll has a complex structure containing a high number of conjugated double bonds (Fig. 1.3), allowing it to absorb strongly in the visible light wavelengths (Rabinowitch, 1969; Fig. 1.4).

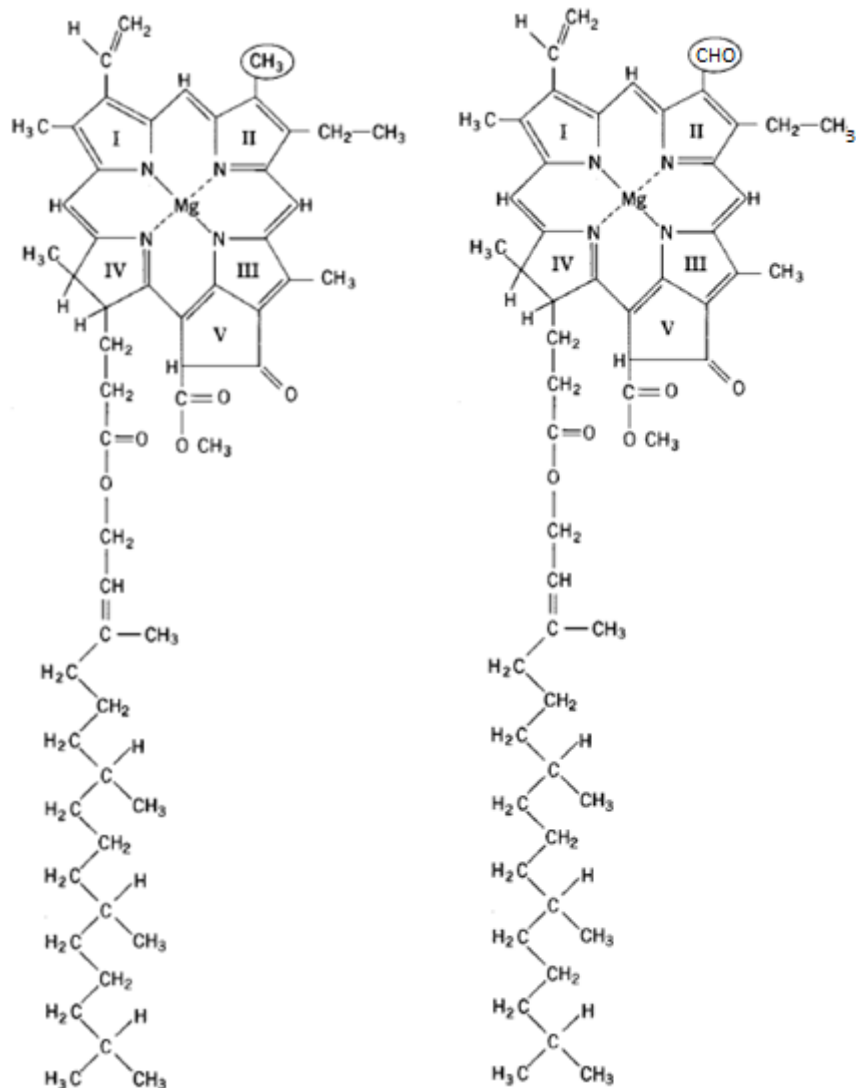


Figure 1.3: The molecular structure of Chlorophyll a (left) and Chlorophyll b (right). Chlorophyll a differs only in that the circled CHO group is replaced by a CH₃ group. Adapted from Rabinowitch (1969).

The wavelengths of light that may be absorbed and utilised in photochemistry are known as PAR and lie between 400 and 700nm (Zhu *et al.*, 2008). However, chlorophyll absorbs different wavelengths of PAR with varying efficiency (Zhu *et al.*, 2008; Hogewoning *et al.*, 2010). Found in the green tissues, two types of chlorophyll – chlorophyll a (Chl *a*) and chlorophyll b (Chl *b*) - are contained the chloroplasts of all higher land plants. The only difference between them is that

a CH_3 group in Chl *a* is substituted for a CHO group in chl *b* (Fig. 1.3; Rabinowitch, 1969). This small change in structure shifts the absorption spectrum, narrowing the area of weak absorption efficiency in the green area between the blue and red (Fig. 1.4). In solution, chl *a* has absorption peaks at approximately 437nm in the blue area of the spectrum and 672nm in the red area, whilst the chl *b* blue peak lies nearer 472nm and the red peak at 653nm (Fig 1.4; Evans and Anderson, 1987).

When suspended in solution, chlorophyll *a* and *b* absorb maximally in the red (λ 600-699 nm) and blue (λ 400-499 nm) regions of the spectrum and more weakly in the green. Chlorophyll however *in planta* does not exist in isolation but in conjunction with other pigments and cellular structures. For example, the flavonoids, anthocyanins and carotenoids (discussed in more detail below) all affect absorption in the green area of the spectrum. Despite the low extinction coefficient of chlorophyll in the 500-599nm region, the optical properties of the leaf as a whole (detailed in Merzlyak *et al.*, 2009) result in the red-shifted, more relevant whole leaf absorption spectrum shown in Fig. 1.4.

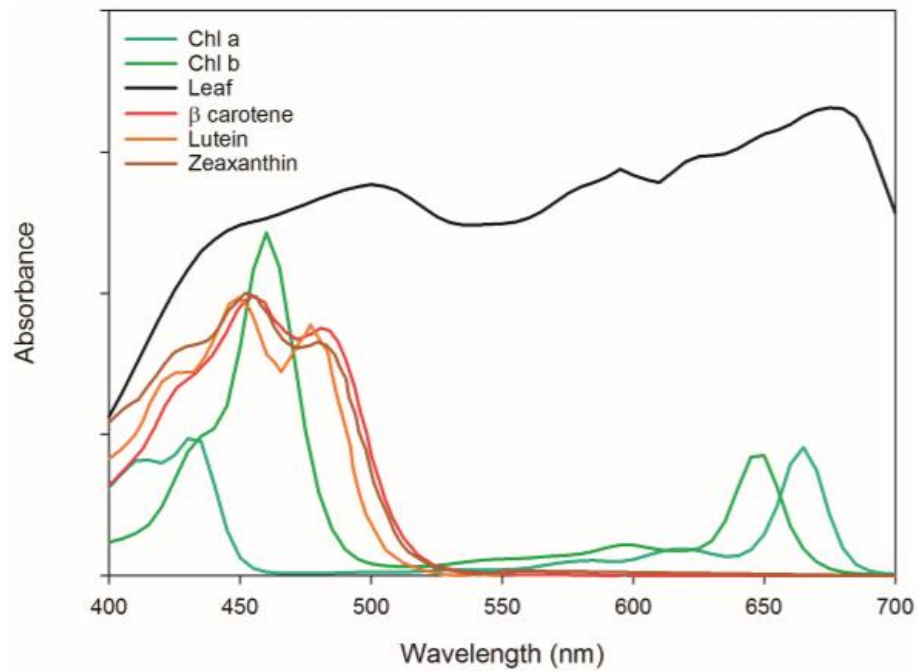


Figure 1.4: The absorption spectra of chlorophyll a (chl *a*) and chlorophyll b (chl *b*) from leaves of *Spinacea oleracea* isolated and suspended in 80% acetone, measured at 5nm intervals (green lines), absorption spectra of β carotene, lutein and zeaxanthin suspended in acetone, as measured by Kopczynski et al (2005) (orange/ red lines) and absorption of an intact *Coriandrum sativum* leaf as measured with the FieldSpec HH 2 spectroradiometer (ASD, Boulder Colorado) and integrating sphere (Li-Cor, Illinois) (black line).

Carotenoids are a family of lipid soluble coloured pigments present in all plants and alga, mostly in chloroplasts and chromoplasts. The most common of these pigments, the carotenes and xanthophylls, are red to orange in colour and together their absorbance range extends into the green region (400 – 550nm, peaking at around 470nm), effectively covering the poorest region of chlorophyll absorbance (500-550nm). In comparison with chlorophyll they are less abundant with ratios of carotenoid to chlorophyll varying between 0.1 to

0.5 depending on species and conditions (Murchie and Horton 1998, Murchie et al 2002). The most abundant carotene is β -carotene and the principal xanthophylls are zeaxanthin, violaxanthin and lutein. The most well characterised function of xanthophyll carotenoids is perhaps their essential photoprotective role in high light (Murchie and Niyogi, 2011; Ruban *et al.*, 2012) which is discussed later. However, their absorption spectra (extending into the green) is often used to make the argument that the xanthophylls serve an accessory role in light harvesting within the blue and green regions, passing energy to chlorophyll to be used in photosynthesis (Hopkins and Hüner, 2009; Walla *et al.*, 2014). Energy transfer from carotenoids to chlorophyll has been demonstrated *in vitro* (e.g. Cerullo *et al.*, 2003) and been found to be important in some algae (Hofmann *et al.*, 1996; Hashimoto *et al.*, 2015) and higher plant light harvesting complex II (LHCII) (Gradinaru *et al.*, 2000; Croce *et al.*, 2003; Dall'Osto *et al.*, 2014). Transfer seems to happen with varying efficiency, for example lutein will transfer 70-80 % of the energy to drive photochemistry (Dall'Osto *et al.*, 2014) but other pigments produced by the xanthophyll cycle may transfer much less and dissipate the rest in the photo-protective mechanism of non-photochemical quenching (NPQ) (Akimoto *et al.*, 2005, Murchie and Harbinson 2015) which is discussed further below. *In vitro* studies using LHCII showed that energy transfer from carotenoid to chlorophyll is highly efficient and contributed 10 – 20 % of total energy absorbed by this chromophore (Gradinaru *et al.*, 2000).

1.5 Mechanism of photosynthesis

Chlorophyll along with the accompanying carotenoid pigments is arranged within the photosynthetic reaction centres of photosystem II (PSII) and photosystem I (PSI) and their associated light harvesting antenna complexes (LHCII and LHCI respectively). These are located in the energy transducing thylakoid membranes of cytoplasmic organelles called chloroplasts (Allen, 2003).

Here photons of PAR (of any wavelength, as photons enter the reaction at the same energy level) are captured and passed to chlorophyll, (Hillier and Babcock, 2014) causing the excitation of an electron to enter a higher energy state where the energy is immediately passed to a neighbouring chlorophyll molecule by resonance transfer (in the antenna) or released to the electron transport chain (ETC) (Allen, 2003, Hillier and Babcock, 2014).

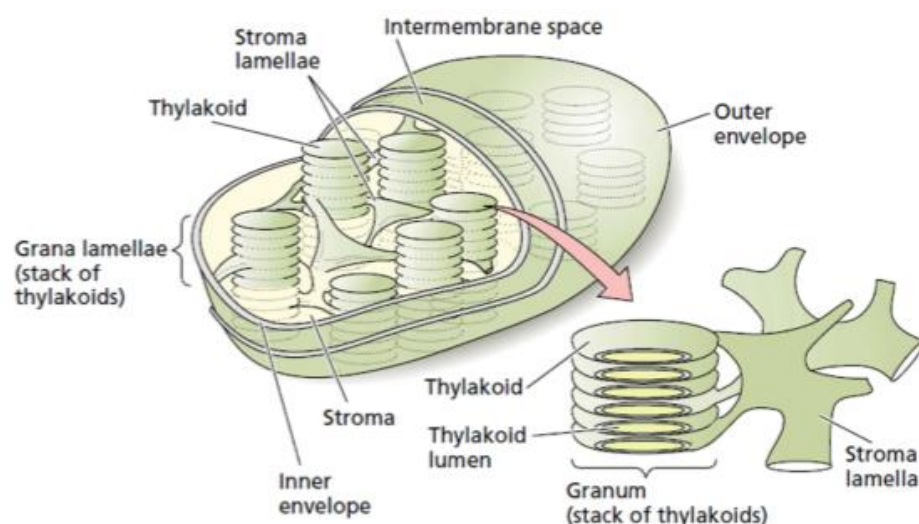


Figure 1.5: Schematic diagram of the inner structure of the chloroplast (Taiz and Zeiger, 2010).

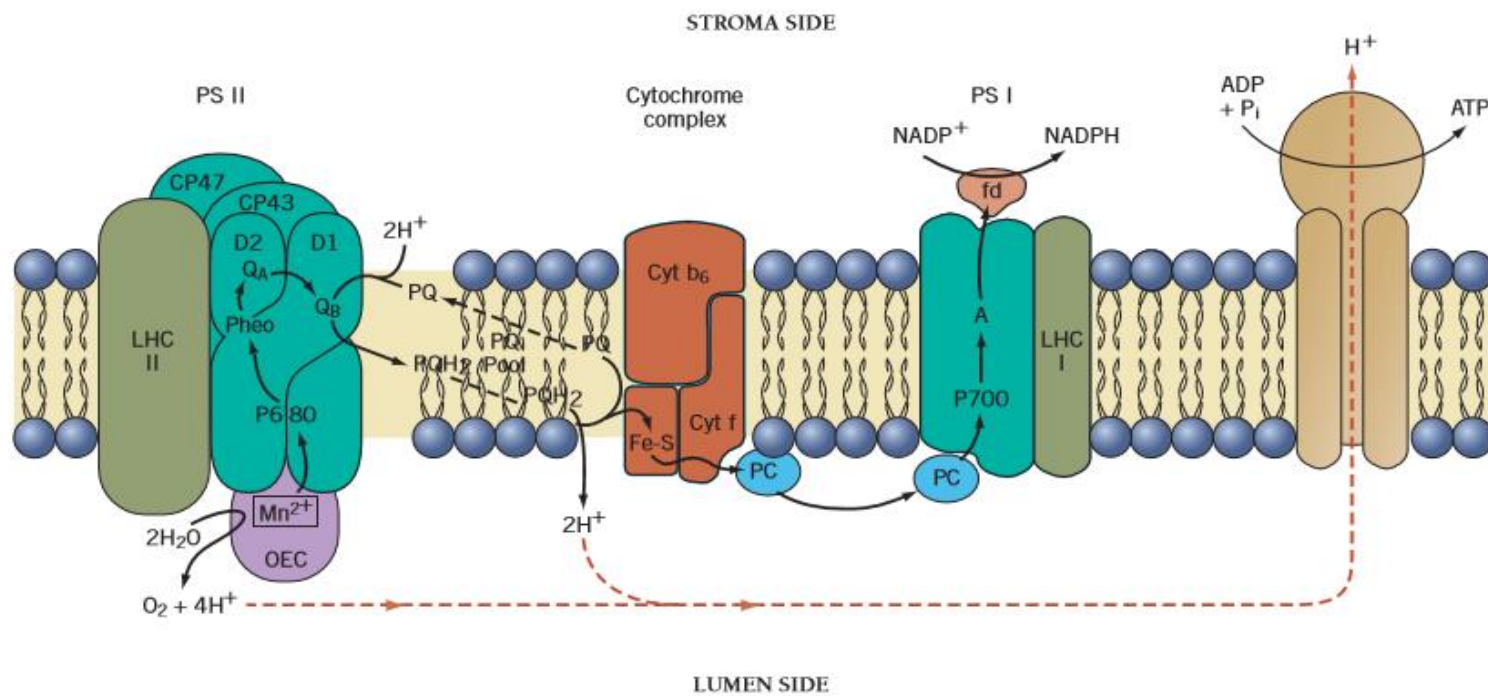


Figure 1.6: Schematic diagram of the photosynthetic complexes involved in the light-dependent reactions of photosynthesis (Hopkins and Hüner, 2009).

Uniquely, PSII is able to donate further electrons to the ETC through photolysis - the photonic energy powered dissociation of hydrogen ions from water molecules. Figures 1.6 and 1.7 show the arrangement of the photosynthetic apparatus and a schematic of energy transfer along the ETC known as the Z-scheme respectively.

Upon entering the electron transport chain after PSII, electrons are transferred to the first stable electron acceptor, plastoquinone Q_A and then the second acceptor plastoquinone Q_B and so on to each consecutive plastoquinone electron acceptor. An electron acceptor may only hold one electron at any one time, and must pass the first one along to a downstream acceptor before it becomes oxidised or 'open' and can accept another. This binding and unbinding of an electron to (or the reduction and oxidation of) successive plastoquinone molecules is a chain of reactions which leads to, connected by the cytochrome *b₆f* complex, PSI. Here nicotinamide adenine dinucleotide phosphate ($NADP^+$) molecules are converted nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) molecules. Meanwhile, the hydrogen ions (H^+) formed in photolysis at PSII and in the ETC are pumped into the lumen, where they form a proton gradient which causes the phosphorylation of adenine disphosphate (ADP) to make adenine triphosphate (ATP).

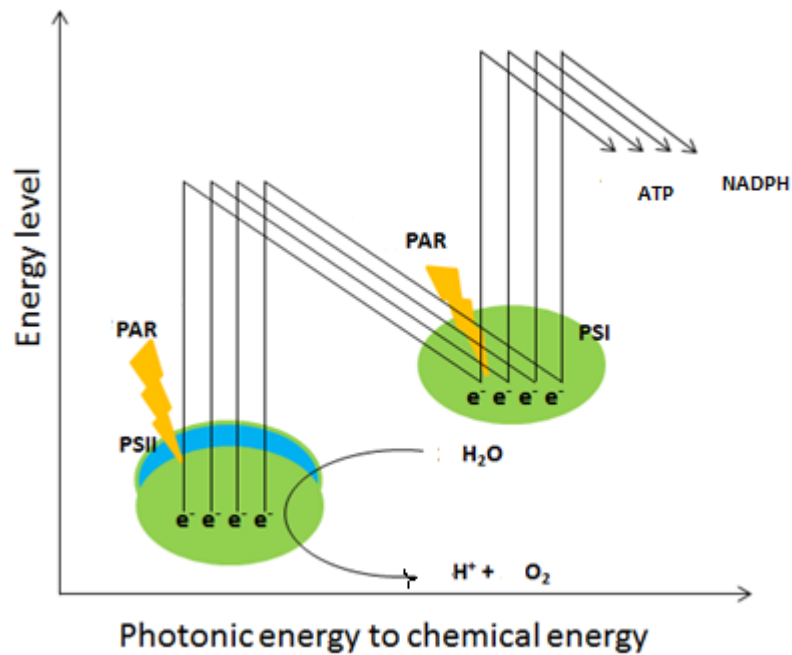


Figure 1.7: The Z-Scheme configuration of the photosynthetic electron transport chain shows linear electron transport converting photonic energy to chemical energy in the form of adenine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH). PSII uniquely is able to donate further electrons through the splitting of water molecules. Based on the model by Walker (2002).

In the presence of sufficient light and water, the electron transport chain generates energy rich ATP and NADPH molecules (Zhu *et al.*, 2008). However, the actual conversion of inorganic carbon in the form of carbon dioxide from the atmosphere to organic carbon molecules which are essential for plant growth and metabolism, for example starch and sucrose (Raines, 2011) depends on the stromal reactions. Here the NADPH and ATP produced in the ETC are used in the 'Photosynthetic Carbon Reduction (PCR) cycle', also known

as the 'Calvin-Benson cycle' for the scientists who discovered the pathway, which is catalysed by the enzyme rubisco (Hopkins and Hüner, 2009).

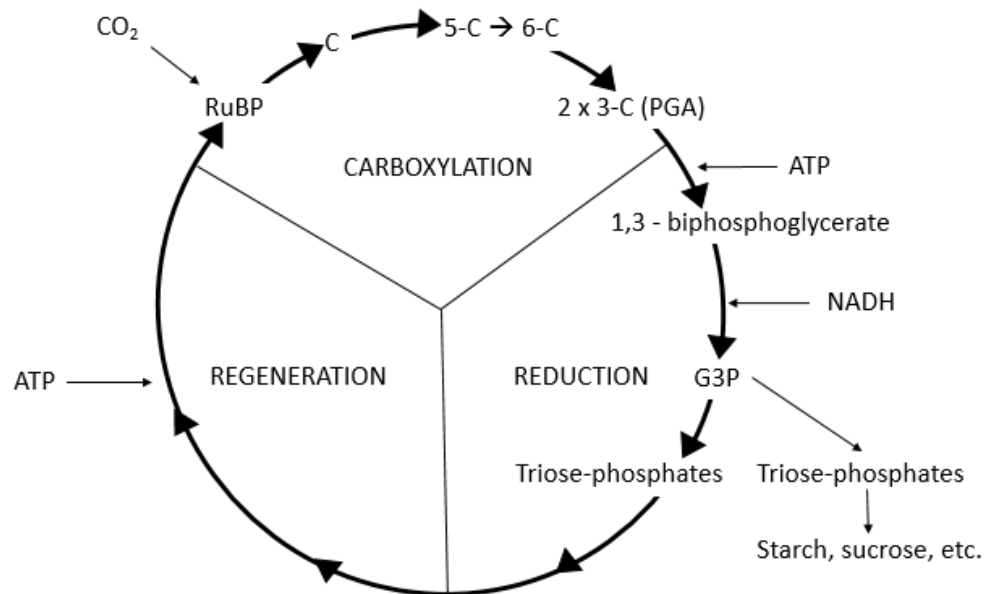


Figure 1.8: The photosynthetic carbon reduction cycle (and its organic carbon compound products), split into the three main stages with the detailed steps of carboxylation and reduction. Based on information in Hopkins and Hüner, 2009.

The PCR cycle consists of three main stages (Fig. 1.8). The first is 'Carboxylation', where carbon dioxide molecules are pulled into the cycle by the carbon acceptor Ribulose - 1,5 - biphosphate (RuBP) mediated by RuBP carboxylase enzyme (Rubisco) converting a 5-carbon molecule to an unstable 6-carbon intermediate. The addition of water then hydrolyses this intermediate to two 3-carbon molecules, known as 3-phosphoglycerate or phosphoglyceric acid (PGA). Next is the 'Reduction', phase which involves the ATP and NADPH molecules. Mediated by the enzyme 3-phosphoglycerate kinase (3-PGK), 6 ATP molecules each break the chemical bond with one of their phosphates, converting them to ADP and reducing 6 PGA molecules to 1,3-

biphosphoglycerate. Further reduction takes place when the enzyme NADP-glyceraldehyde- 3- phosphate- dehydrogenase mediates the conversion of 6 NADPH molecules and 6 1,3-biphosphoglycerate to 6 NADP+ molecules and 6 glyceraldehyde-3- phosphate (G3P) molecules. G3P is the main organic carbon output of the PCR cycle. From here it can be metabolised into triose-phosphates and sugars such as sucrose and starch. Finally, the 'Regeneration' phase consumes 3 more ATP in order to covert some of the triose-phosphate produced back into RuBP, so that the cycle may continue (Hopkins and Hüner, 2009; Taiz and Zeiger, 2010).

1.6 Carbon assimilation rate

On a basic level, photosynthetic rate depends on a simple supply and demand principle. That is, how fast the photosynthetic 'substrates' - water and carbon dioxide catalysed by PAR - can be delivered to the photosynthetic cells, and then how fast the chemical reactions which process these substrates into organic carbon compounds are (Farquhar and Sharkey, 1982). The rate of supply is always determined by the least available substrate at any given time (Blackman, 1905) which can also be affected by plant physiological status, such as the aperture of stomata and the ability of the roots and vasculature system to provide water. Demand is more complex; its rate depending on the amount of open electron acceptors in the electron transport chain and the rate of the reactions in the PCR cycle (Farquhar *et al.*, 1980) and even the rate of export of fixed carbon from the chloroplast and cells. Carbon assimilation rate can be measured directly by measuring gas exchange between the leaf to the atmosphere (Johnson and Murchie, 2011).

1.7 Non-photochemical quenching and chlorophyll fluorescence

Once photonic energy is absorbed by chlorophyll it may then take one of three competitive pathways. Much of the energy is channelled into the electron transport chain and the reactions of photosynthesis as described above, but the plant will not always have the capacity to use all of the energy in this way. When electron acceptors in the ETC are saturated i.e. all reduced or 'closed' and the light harvesting complexes (LHCs) continue to absorb PAR this results in the production of 'excess excitation energy' (Aro *et al.*, 1993). This extra energy must be quickly dissipated in order to avoid its transfer to oxygen causing the formation of highly damaging reactive oxygen species (Walla *et al.*, 2014). Most of the excess excitation energy is dissipated as heat in a highly complex pathway involving the reversible interconversion of the photoprotective carotenoids violaxanthin and zeaxanthin and the regulatory protein PsbS (Niyogi *et al.*, 2005; Hubbart *et al.*, 2012), in what is known as the xanthophyll cycle (Davison *et al.*, 2002). This is referred to as non-photochemical quenching. However, a tiny amount of energy - usually only 1-2% of the total light absorbed - is re-emitted as light. This re-emitted light energy is termed 'chlorophyll fluorescence' (Maxwell and Johnson, 2000; Murchie and Lawson, 2013)..

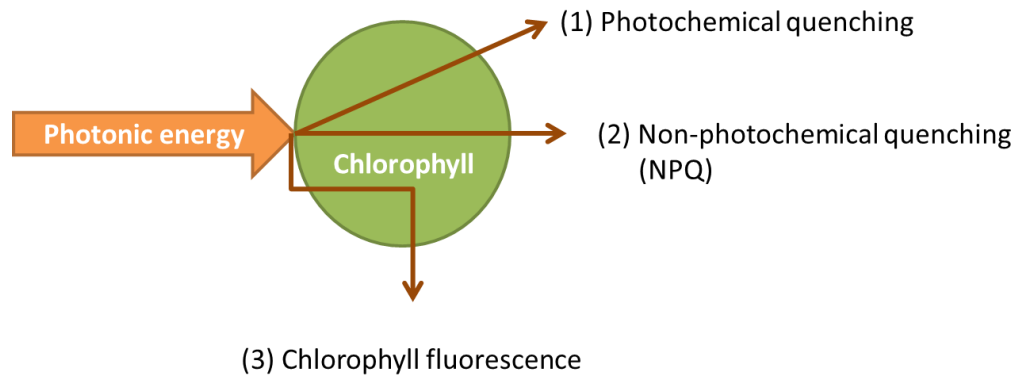


Figure 1.9: The photonic energy in photosynthetically active radiation (PAR) absorbed by chlorophyll may take one of three competitive pathways. 1) It may be channelled into photochemistry, termed 'photochemical quenching'. 2) It may be dissipated as heat, termed 'non-photochemical quenching' (NPQ). 3) It may be re-emitted as light, termed 'chlorophyll fluorescence'. Based on information from (Maxwell and Johnson, 2000; Hopkins and Hüner, 2009).

As the three energy pathways can be seen to be in competition, the amount of energy channelled into each one depends on the amount channelled into the other two. I.e. if photosynthesis decreases thermal dissipation and/or re-emission as light must increase to deal with the excess energy. The yield (proportion) of re-emitted chlorophyll fluorescence is therefore a good indicator of the state of photosystem II (PSII) because this is the first reaction centre in the electron transport chain (Figs. 1.6; 1.7) and the only one that emits fluorescence at room temperature. Importantly, chlorophyll fluorescence analysis permits the routine separation and quantification of photochemical quenching and non photochemical quenching. NPQ is an important protective and regulatory process that maintains homeostasis and prevents damage to the chloroplast but also reduces quantum efficiency.

Measurement of chlorophyll fluorescence is therefore a popular method of determining the quantum efficiencies of photosynthesis and further, dissecting the underlying processes (Murchie and Lawson, 2013).

1.8 Light absorption profile through the leaf

The photosynthetic pigments absorb light in the red (500-599nm), green (400-499nm) and blue (300-399nm) with varying efficiency (Fig. 1.4; 1.11. However, once absorbed these enter photosynthesis at the same energy level (McEvoy and Brudvig, 2006) meaning that at the most basic level, providing either red, green or blue photons to drive photosynthesis will allow the plant to metabolise and grow. As conversion of electrical power to photons within LED systems increases in efficiency with increasing wavelength (Tennessen *et al.*, 1995) and red light has the longest wavelength, providing monochromatic red LED light could be proposed to be the most energy efficient way of driving plant growth with artificial lighting. However, the next step in creating a suitable light recipe is to determine which other wavelengths of light, and in what quantity, are needed for optimal plant growth and development.

First, it is important to consider that due to the physical and biological properties of the leaf (Terashima *et al.*, 2009) different proportions of each wavelength will ultimately reach the chloroplasts located in different parts of the leaf profile (Fig. 1.10). The main leaf photosynthetic pigments are concentrated into the chloroplasts and, on a microscale, are not spread evenly across the leaf surface (Terashima *et al.*, 2009). Thus, the transmission of photons through the vertical leaf profile can be thought of as the passing of

particles through the mesh of a sieve (Rühle and Wild, 1979). Chlorophyll *a* and *b* being the most abundant pigments in the chloroplasts of green plants provide the 'sieve' with the tightest mesh (see section 1.18 for discussion on acclimation of the chlorophyll *a/b* ratio). As discussed above, chlorophyll absorbs relatively weakly in the green absorbing mostly in the blue and red regions of the spectrum (Fig 1.4, Evans and Anderson, 1987), with up to 80% of all green light thought to be transmitted through the chloroplast (Terashima *et al.*, 2009), effectively widening the mesh of the sieve and allowing more green photons to pass deeper into the mesophyll (Fig. 1.10). This 'green window' (Terashima *et al.*, 2009), coupled with the highly refractive properties of leaf tissue, means that green photons can reach chloroplasts much deeper into the mesophyll than red and blue photons (Sun *et al.*, 1998).

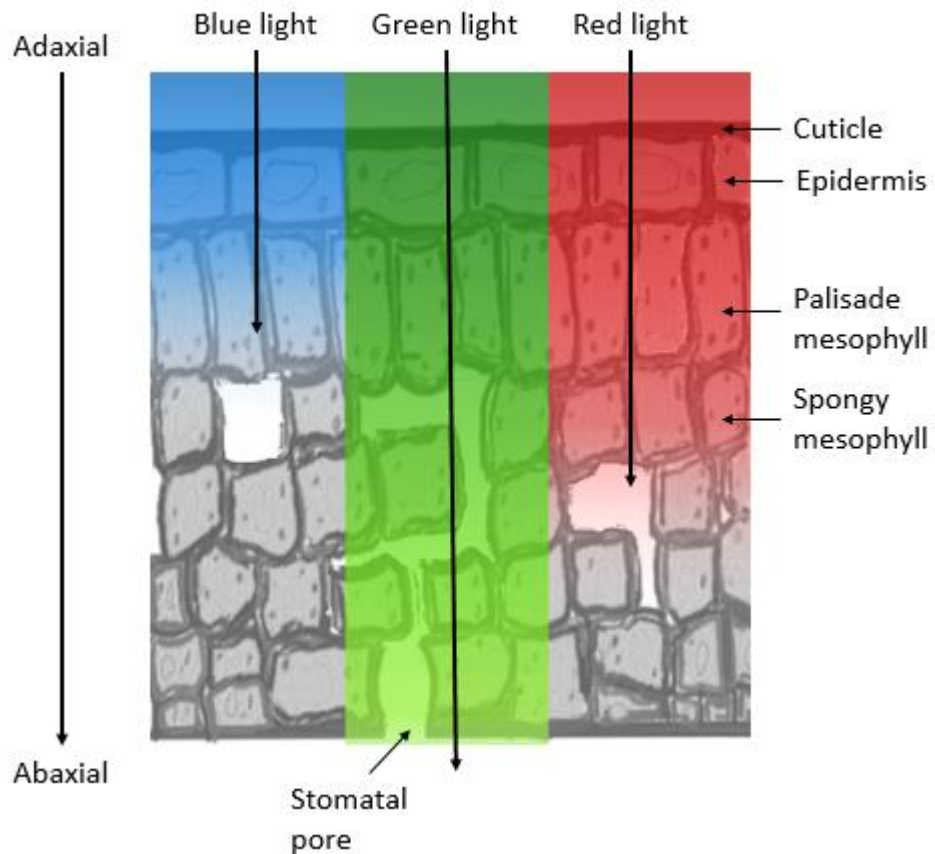


Figure 1.10: Schematic depiction of penetration of monochromatic blue, green and red light through the vertical profile of a leaf illuminated from above the adaxial surface. Blue light is quickly absorbed by multiple pigments in the top layers of leaf cells, whereas red light may penetrate further due to mainly being absorbed by just the chlorophylls. Green light may reach the bottom layer of cells due to the chlorophyll's weak absorption in the green (the 'green window') and scattering of green light within the leaf (the 'detour effect'). Based on data from Nishio *et al* (1993); Vogelmann and Han (2000); Evans and Vogelmann (2003).

Scattering of green light inside the leaf brings about the 'detour effect' (Vogelmann, 2008), whereby the path of light through the vertical leaf profile is lengthened and therefore photons have a higher chance of hitting and being

absorbed by chloroplasts on their passage through the leaf. This means that green light absorbed by the leaf is able to drive photosynthesis with a quantum efficiency greater than blue light and similar to red light (Fig. 1.11).

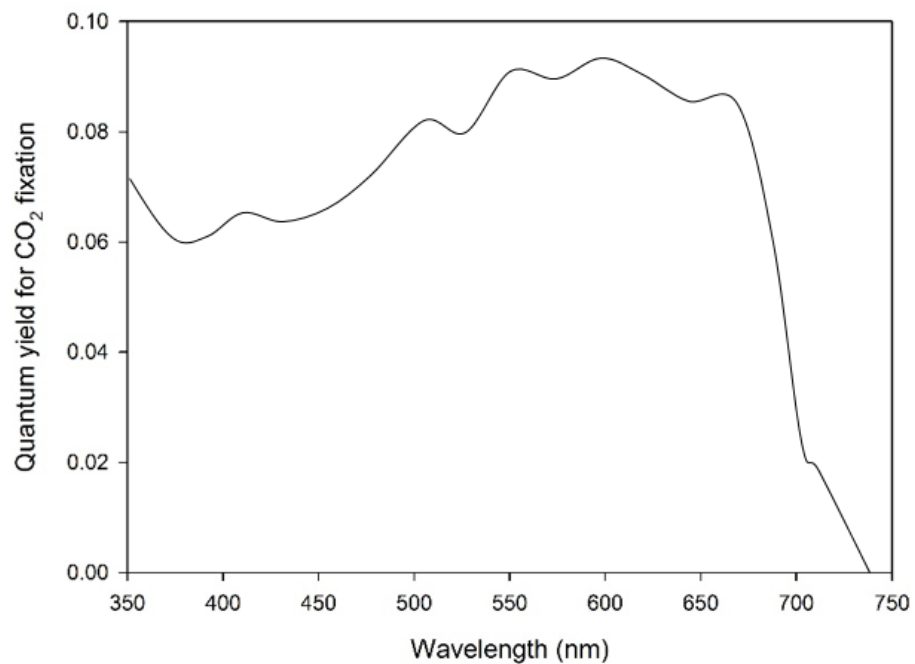


Figure 1.11: Action spectrum of photosynthesis based on the quantum yield for CO₂ fixation on an absorbed light basis of cucumber leaves developed under a sunlight spectrum. Red is most efficient at driving photosynthesis on an absorbed light basis, followed by green then blue light. Adapted from Hogewoning et al (2012).

Evans and Vogelmann (2003) tracked blue ($\lambda 450 \pm 91\text{nm}$) and green ($\lambda 567 \pm 24\text{nm}$) light absorption and carbon fixation through the vertical profile of a *Spinacia oleracea* leaf by imaging chlorophyll fluorescence and measuring ¹⁴C in transverse leaf sections. When illuminating with $150\mu\text{mol m}^{-2} \text{s}^{-1}$ from the adaxial surface, blue and green light were maximally absorbed at $65\mu\text{m}$ and $110\mu\text{m}$ deep respectively, with 50% of the light being absorbed at depths of

120 μm and 230 μm respectively. The ^{14}C profile showed that carbon fixation driven by blue light mainly occurred adjacent to the leaf surface, whilst fixation driven by green light occurred throughout the leaf, declining after a depth of 200 μm . This pattern of absorption is consistent with earlier findings by Vogelmann and Han, 2000, who profiled absorption of brief flashes of 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ monochromatic blue ($\lambda 450 \pm 50\text{nm}$), red ($\lambda 650 \pm 10\text{nm}$) and green ($\lambda 550 \pm 50\text{nm}$) light from the adaxial to abaxial surface of *Spinacia oleracea* using chlorophyll fluorescence and found maximum absorption to be at depths of 50, 100 and 150 μm respectively. To support this, Nishio *et al* (1993) concluded as much as 90% of red and blue light can be absorbed by the chloroplasts in the top 20% of the leaf profile (Nishio *et al.*, 1993). However, the same study also concluded that the sharp decline in red and blue light penetration from the top to the bottom of the leaf does not correlate with the carbon fixation gradient through the leaf.

Although the accessory pigments such as carotenoids function in light harvesting they do so with varying efficiency (see section 1.4). This has been used to argue that blue light is less efficient on a per unit photon and per unit energy basis at driving photosynthesis than red light and green light (Fig. 1.11; McCree, 1972; Hogewoning *et al.*, 2012). This is compounded by the fact that when absorbed by chlorophyll alone, blue light results in a lower photochemical or *energetic* efficiency than red despite driving photosynthesis with equal *quantum* efficiency (Zhu *et al.*, 2008).

This all indicates that photosynthesis deep within the vertical leaf profile, where blue and red photons are scarce, must be partly driven by green photons. This hypothesis is supported by observations by Terashima *et al* (2009) who found that supplementing saturating white halogen light with monochromatic green light (λ 550nm $\pm$ 30nm) increased leaf photosynthesis in *Helianthus annuus* with a greater efficiency than supplementary monochromatic red light (λ 641-690nm), further substantiating the theory that green light can drive photosynthesis deep in the mesophyll layers where other wavelengths have less reach. It therefore appears that it would be beneficial to add green light to a light recipe in order to most efficiently drive photosynthesis. The question is how much and at what point during canopy development.

1.9 Non-photosynthetic photoreceptors

The other plant photoreceptors besides chlorophyll must be considered. Although the chlorophylls (along with carotenoids) are the most abundant pigments, plants have a range of other photoreceptors that do not channel light energy into photochemistry (Fig. 1.12). These non-photosynthetic photoreceptors instead have the function of allowing the plant to sense its light environment (quantity, quality, direction and duration of light) so that it may adjust its growth and/or developmental pattern accordingly (Carvalho *et al.*, 2010).

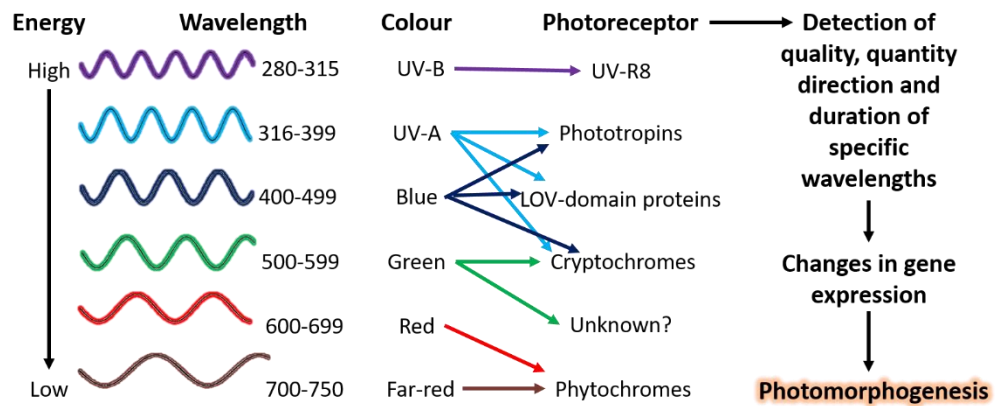


Figure 1.12: The different wavelengths/ colours in the portion of the electromagnetic spectrum detectable by plants and the photoreceptors which they activate (based on information from Shropshire et al., 1961; Ahmad et al., 2002; Kim et al., 2007; Carvalho et al., 2010; Heijde and Ulm, 2012; Banaś et al., 2012). Numbers represent wavelength ranges in nanometers.

The collective term for this suite of light-induced changes which occur over a plant's life-cycle is photomorphogenesis. The quality or spectral composition, i.e. the different wavelengths of light that a plant is exposed to has a key role in this process (Carvalho *et al.*, 2010). The interactive network of non-photosynthetic photoreceptors, each of which detects light at specific set of wavelengths, has a crucial role in almost every aspect of growth and development; from germination all the way through to seed production (Carvalho *et al.*, 2010). Once a non-photosynthetic photoreceptor is activated it triggers a signal transduction pathway, resulting in changes in gene expression and ultimately biochemical and/or physiological changes that may be localised to the site of perception but may also occur in distant organs or plant-wide due to complex hormone signalling and transport of protein messenger molecules (Bou-Torrent *et al.*, 2008a). In the model plant

Arabidopsis thaliana, downstream of photoreceptor activation, the LONG HYPOCOTYL 5 (HY5) transcription factor has been shown to act as the link between the plant light and hormone signalling pathways (Cluis *et al.*, 2004).

1.10 UV-B light sensing mechanism

The photoreceptor UVR8 detects levels of UV-B light (280-315nm). UVR8 exists as an inactive homodimer until activated by UV-B light, upon which it dissociates and the monomers rapidly accumulate in the cell nucleus (Rizzini *et al.*, 2011). UV-B light is sensed not by a conventional chromophore attached to the homodimer but by a cross-dimer tryptophan 'pyramid', causing the breakdown of the salt-bridge network which holds the inactive molecule together (Christie *et al.*, 2012). Once in the nucleus it interacts with the CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) protein and causes changes in gene expression. Spontaneous re-dimerisation occurs after a number of hours and stops this UVR8-COP1 interaction, halting signalling (Heijde and Ulm, 2012). UV-B light sensing and its subsequent effects on plant physiology are not discussed further in this chapter as they were not explored as part of the experimental work carried out in the main thesis. For a comprehensive review of the effects of UV-B light on plant growth and development see Jenkins, (2014).

1.11 UV-A/ Blue and green light sensing mechanism

UV-A/Blue light (316-499nm) is detected by several photoreceptors in higher plants. The LOV-domain photoreceptors (named for their property of always reacting to light, oxygen or voltage) include two families of sensors of environmental blue light intensity found in higher plants. These are 1) the

Phototropins (PHOT) and 2) Zeitlupe (ZTL), Flavin-kelch-Fbox-1 (FKF1) and LOV-Kelch-Protein-2 (LKP2) (Pudasaini and Zoltowski, 2013; Suetsugu and Wada, 2013). The phototropins phototropin 1 (PHOT1) and phototropin2 (PHOT2) are the best characterised and each contain a flavin mononucleotide (FMN) chromophore which allows them to be activated by UV-A (315 – 399nm) and blue (400-499nm) light (Briggs and Christie, 2002).

The cryptochrome photoreceptors also absorb UV-A (315-399nm), blue (400-499nm) and green (500-599nm) light (Bouly *et al.*, 2007). The best studied plant cryptochromes are cryptochrome 1 (CRY1) and cryptochrome 2 (CRY2), in *Arabidopsis thaliana* (*A. thaliana*), which are thought to be expressed ubiquitously in all cell-types throughout the plant (Tóth *et al.*, 2001). Both CRY1 and CRY2 bind to two distinct chromophores - a Flavin adenine dinucleotide (flavin/ FAD) chromophore and a pterin chromophore. The flavin molecule exists in one of three redox states (Fig. 1.13), each of which absorbs photons of different wavelengths (Kao *et al.*, 2008). Flavin is produced by the plant as a fully oxidised, biologically inactive FAD. Upon absorbing UV-A/blue light, the FAD is converted to a neutral semiquinone radical FADH[•], or the anion FAD^{•-}, activating the cryptochrome and leading to UV-A/blue light responses. The FAD^{•-} form of flavin may however absorb green light, causing it to be converted to a fully reduced hydroquinone FADH⁻ or FADH₂ (Bouly *et al.*, 2007; Banerjee *et al.*, 2007; Sellaro *et al.*, 2010). This reduction returns the cryptochrome to a biologically inactive state and typically reverses the UV-A/blue light responses (Talbott *et al.*, 2002).

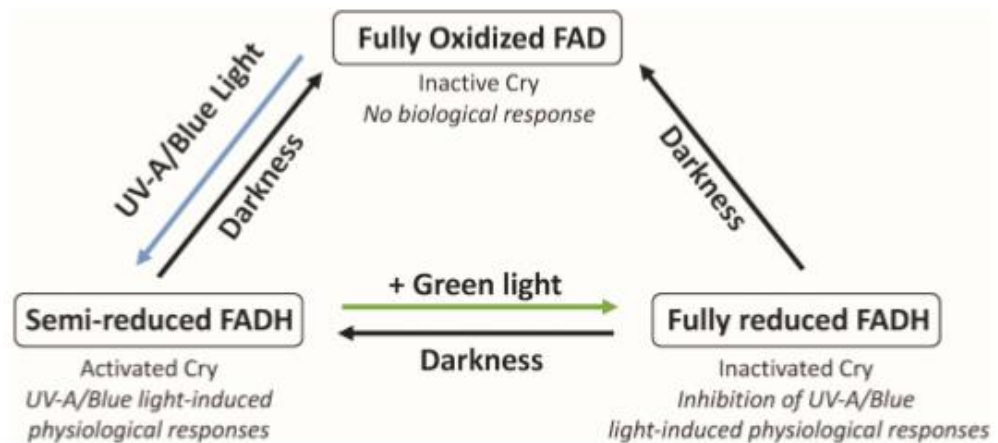


Figure 1.13: The Photo-conversion between the different redox states of the flavin chromophore on the cryptochrome photoreceptor (based on work by Banerjee et al., 2007; Bouly et al., 2007; Sellaro et al., 2010). The biologically inactive chromophore of cryptochrome (FAD) is converted to a semi-reduced FADH upon absorption of UV-A/blue light, triggering UV-A/blue light responses. However upon absorption of green light, FADH is further reduced to FADH₂, inactivating cryptochrome and causing reversal of the UV-A/blue light responses.

1.12 Red/ far-red light sensing mechanism

Light in the red (600-699nm) and far-red (700-750nm) portions of the electromagnetic spectrum is specifically detected by a family of photoreceptors known as the phytochromes (encoded by PHY genes). Phytochromes are known to be encoded by a family of genes and have individual and overlapping functions. Monocots such as rice have 3 phytochrome encoding genes, PhyA, PhyB and PhyC (Takano *et al.*, 2005), whilst dicots such as *Arabidopsis thaliana* have 5, PhyA, PhyB, PhyC, Phy D and PhyE (Bou-Torrent *et al.*, 2008). Phytochrome A (PHYA) is the sole group 1 phytochrome, which senses far-red

light, whilst the other phytochromes (group 2) are thought to sense only red light (Quail, 1998).

Found in the majority of plant cells (Sharrock and Mathews, 2006), phytochromes mediate a range of both developmental and physiological responses to the intensity and duration of these long wavelength photons. Early light response experiments suggest that the phytochrome absorption peaks lie between 640-660nm in red light and 720-740 in far-red light (Shropshire et al., 1961). The phytochromes sense light with the phytochromobilin chromophore. Phytochromobilin may exist as one, of two forms which are freely interconvertible in response to R and Fr light exposure. This means that phytochromes may exist in one of two interchangeable, spectrally distinct forms. These are known as Pr and Pfr (Nagatani, 2010). The Pr form is biologically inactive, but will readily be converted to the biologically active Pfr form following exposure to red light. Upon light conversion, Pfr rapidly migrates to the nucleus, where it interacts with various phytochrome interacting factors (PIFs) (Castillon *et al.*, 2007) to set off a complex transcription cascade. This will ultimately lead to biochemical and/or morphological changes within the plant. In this way, the phytochromes act as long-wavelength photon activated protein-kinases (Deshaies and Meyerowitz, 2000), allowing plants to sense their red/far-red light environment, and adjust their growth and development accordingly.

1.13 Light mediated physiological responses

Plants are sessile and therefore must adapt to their environment by changing their biochemistry and morphology to grow out of unfavourable irradiance

situations. Their ability to sense their light environment (as described above) combined with the fact that the plant developmental program continues to be extremely flexible and adaptive even after germination (Depuydt and Hardtke, 2011), means that mature plants of the same species may have highly variable morphologies, depending entirely upon the light conditions under which they have been grown.

1.14 Light requirement for response

The intensity of the wavelength, 'fluence rate' or 'photon flux density' and duration of exposure that is required to cause a photomorphogenic response may differ according to the type of photoreceptor and the response to be initiated. Plant responses to light can be placed in one of three categories, indicating the amount of light required to trigger the response. Very low fluence (VLF) responses, such as the activation of transcription factors for chemicals involved in photosynthesis, require cumulative photon flux densities (PFDs) of the specific wavelength to be as little as $0.001 \mu\text{mol m}^{-2} \text{s}^{-1}$, or the equivalent of being exposed to just a few seconds of moonlight. Low fluence responses (LFR), for example germination of some seeds, require PFDs of $1\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$ or more, delivered as one intense flash or multiple less intense flashes in quick succession. Finally, high irradiance reactions (HIR) such as the inhibition of hypocotyl elongation are most common and require prolonged exposure to fluence rates of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ or more (Fankhauser and Chory, 1997; Yanovsky *et al.*, 1997). VLFRs and LFRs show reciprocity, i.e. an increase in the fluence rate and/or time of exposure will cause a proportionate increase in the strength of the response. HIRs however work differently and do not show

reciprocity. They have no proportional increase in response to increasing fluence but accumulated photons must reach and stay at a certain level for the response to occur (Fankhauser and Chory, 1997; Yanovsky *et al.*, 1997).

1.15 Germination

Higher plants exhibit the innate property of seed dormancy, allowing them to delay germination until conditions will be most favourable for growth. This is partly controlled by genetics but greatly influenced by external environmental factors such as temperature, moisture and light (Finch-Savage and Leubner-Metzger, 2006). Many plant species will germinate in total darkness but a large number have a red-light requirement in order to initiate the process. The phytochromes are the only photoreceptors which have been shown to be directly involved in inducing the light-dependent seed germination process (Hennig *et al.*, 2002). For example, in *Arabidopsis thaliana* (*A. thaliana*), phytochrome A (PHYA) and phytochrome B (PHYB) have been shown to be the main control as they mediate the LFR germination response to UV-A all the way through to far-red light (Casal and Sánchez, 1998; Finch-Savage and Leubner-Metzger, 2006). As discussed in section 1.12 the phytochromes do have absorption peaks and action spectrum peaks in the red/far-red parts of the spectrum, but they also absorb weakly in the other parts of the spectrum (Lin, 2000; Finch-Savage and Leubner-Metzger, 2006). The phytochromes ultimately induce germination by causing the increased production of the phytohormone gibberellin (GA), which acts antagonistically against another phytohormone, abscisic acid (ABA) to break seed dormancy (Oh *et al.*, 2007).

By contrast, in seeds of *Lolium rigidum* (ryegrass), blue (400-499nm) and green light with action spectrum peaks at 510 and 550nm, have been shown to additively induce maintenance of seed dormancy. This is thought to be either cryptochrome mediated but working differently in seeds than growing plants (as green light most often reverses cryptochrome mediated blue light responses – see section 1.11), or mediated by another, unidentified green light photoreceptor (Goggin *et al.*, 2008; Goggin and Steadman, 2012).

1.16 Skotomorphogenesis to photomorphogenesis

When a seedling emerges from the soil into the light, it must change its developmental process from dark growth, known as skotomorphogenesis, to growth in the light, termed photomorphogenesis (Leivar *et al.*, 2008). The first stage of this change is the process of de-etiolation; loss of the apical hook, cotyledon expansion, maturation of chloroplasts, root elongation and the arrested growth of the hypocotyl (detailed in Bou-Torrent *et al.* 2008). Before plants sense light, in order to use the energy in their seed reserves efficiently, energy is channelled into rapid elongation of the hypocotyl, in order to break through the soil as soon as possible, upon which more resources may be gained in the process of photosynthesis. The apical hook allows the plant to push upwards through the soil without damage to the cotyledon/s, which are folded within (Josse and Halliday, 2008). Both cryptochromes and phytochromes are involved in the process of seedling de-etiolation. It has been shown that upon exposure to light PHYA and/or PHYB become activated and this causes the degradation of the PIFs which repress the de-etiolation genes in

skotomorphogenesis, bringing about the changes required for photomorphogenesis to begin (Leivar *et al.*, 2008).

Unlike blue, red and far-red light, which all facilitate the process of de-etiolation (Parks *et al.*, 2001), pulses of green light (514-554nm) have been shown to accelerate hypocotyl elongation in dark-grown seedlings rather than inhibiting it. This response persists in *cry*-, *phy*- and *phot*- photoreceptor mutant backgrounds (Folta, 2004; Dhingra *et al.*, 2006) suggesting another unknown photoreceptor is mediating this response. This response to green light may be beneficial when a plant germinates below dense foliage (see section 1.20 below).

1.17 Root growth

Root growth and development is also directly influenced by the above soil light environment. Although light sometimes influences germination (Hennig *et al.*, 2002), it most often can only penetrate a few millimetres into the soil (Lee *et al.*, 2016). However, there is a growing pool of evidence that light induces signalling molecules in plant stems/ shoots to travel down into the root tissues. For example, light activation of PHYA and PHYB in the shoots of *A. thaliana* has been shown to cause a redistribution throughout the seedling of the phytohormone auxin, triggering the emergence of lateral roots (Salisbury *et al.*, 2007). Recent work by Lee *et al.* (2016) has also demonstrated efficient conduction of light itself through the vascular system of shoots of *A. thaliana* through to the roots. Like the aerial (above soil) portions of the plant, the roots contain photoreceptors, which have been shown to be activated in an experimental setting where the roots are directly exposed to specific

wavelengths of light. The evidence suggests that cryptochromes and phototropins respond to blue light to mediate primary root growth (Canamero *et al.*, 2006) whilst the phytochrome sensing of red and far-red light mediates primary root elongation (Correll and Kiss, 2005) and both help mediate gravitropism i.e. the downward orientation of root growth. Lee *et al.* (2016) confirmed that, in soil-grown *A. thaliana*, red stem-piped light directly activated root PHYB promoting accumulation of the transcription factor HY5 to cause root growth and gravitropism. This suggests that blue light travels to the roots in a similar manner to promote the cryptochrome and phototropin responses seen in experimental settings.

1.18 Photoacclimation of the chloroplasts

Photoacclimation is the alteration of amounts of photosynthetic components in response to a short or long term change in irradiance or spectral quality (Anderson *et al.*, 1995; Walters, 2005). For example, plants adjust photosystem stoichiometry and antenna size to optimise electron transport for the light conditions in which they are grown. Chloroplasts acclimated to low light intensities will often enlarge the antenna size of light harvesting complex II (LHCII), producing more peripheral LHCII (LHCIIb) per reaction centre in order to capture more light (Anderson *et al.* 1995, Ballottari *et al.*, 2007). Since LHCII has a higher proportion of chlorophyll b than other complexes, leaves grown in shaded/ low light conditions will often develop a lower chlorophyll a:b ratio than those grown at higher irradiances.

However, chloroplasts also acclimate to light quality which is partly driven by the fact that PSII and PSI have differing absorbance profiles. Shaded leaves or

chloroplasts which receive a high proportion of far-red light can 'over-excite' PSI in relation to PSII. Hence, they tend to increase the relative proportion of PSII to PSI centres in order to capture enough of the depleted 'PSII light' to help match PSI excitation by the abundant 'PSI light', better balancing photosystem excitation and optimising photosynthetic efficiency (Chow *et al.*, 1990; Anderson *et al.*, 1995; Murchie and Horton, 1998; Murchie *et al.*, 2002; Walters, 2005). A similar principle holds for state transitions which transfers LHCI between PSII and PSI (Bellafiore *et al.*, 2005). This (probably redox regulated) set of processes which continually maintains quantum efficiency may also be affected by green wavelengths at lower canopy positions.

Recent work by Hogewoning *et al* (2012) confirms that leaves grown in an artificial shade light spectrum (with the highest intensity light being above $\lambda 680\text{nm}$ i.e. enriched in far-red) have a higher quantum yield for CO_2 fixation when exposed to PSI -exciting wavelengths than plants grown in an artificial sunlight spectrum or a spectrum enriched in blue light of the same intensity. Chlorophyll a:b ratios were lowest in the shade light grown plants but these plants also had the lowest PSI:PSII ratio. Antenna size of the LHCs of the shade spectrum plants were similar to those of the sunlight spectrum grown plants, confirming that the acclimation processes mediated by overall light intensity and light quality are distinct. This supports previous observations that photosystem stoichiometry is dynamic and maintains quantum yield (Anderson *et al.*, 1995).

1.19 Guard cell development and stomatal aperture

Stomata are composed of two specialised guard cells found on the epidermis of the green tissues of the plant, which open and close pores which are the gateways for gas exchange between the inner leaf and the external environment. They are therefore a crucial component of the plant photosynthetic apparatus (Lawson, 2009). Light is known to be one of the main stimuli (along with carbon-dioxide and developmental programmes) for the initiation of production of stomata. Kang *et al.*, (2009) demonstrated that blue, red and far-red light – via the photoreceptors CRY1 and CRY2, PHYB and PHVA respectively – additively promote the production of and maturation of stomata. To do this, blue, red and/or far-red light cause the degradation of the constitutively expressed photomorphogenesis repressor gene COP1, preventing it from suppressing the proteins needed to initiate stomatal production.

Light (along with atmospheric carbon dioxide concentration and plant water balance) also controls stomatal aperture. There are two distinct guard cell reactions to light – the red light response and the blue light response (Lawson, 2009; Chen *et al.*, 2012; Busch, 2014). The origin and form of the signal for the guard cell response to red light is still unknown, and therefore the exact mechanism of red light induced stomatal opening is under debate. It is uncertain whether the guard cells sense and respond to red light directly, if the signal originates from the mesophyll and is mediated via the vapour phase to the guard cells or if the signal is produced in response to one of the products of photosynthesis itself (reviewed in Lawson, 2009; Busch, 2014). However, it

is known that the intensity of red light required for stomatal opening is much greater than that required for blue light mediated stomatal opening (Sharkey and Raschke, 1981; Gorton *et al.*, 1993). The stomata are highly sensitive to blue light, requiring up to ten times less blue light than red light to achieve maximum aperture (Sharkey and Raschke, 1981) and also, unlike red light, responding rapidly to short (<30 seconds) pulses of blue light (Zeiger *et al.*, 1987).

The mechanism for the blue light response is relatively well documented (Mao *et al.*, 2005; Chen *et al.*, 2012). In brief, the cryptochromes work in conjunction with the phototropins, which are also located in the guard cell plasma membrane, to mediate blue light induced stomatal opening (Mao *et al.*, 2005; Chen *et al.*, 2012). In the presence of small amounts (<5 $\mu\text{mol m}^{-2} \text{s}^{-1}$) of UV-A/blue light, cryptochrome and phototropin act additively to suppress COP1 and ABA production respectively, allowing the activation of guard cell plasma membrane H^+ -ATPases. These H^+ -ATPases pump H^+ to the outside of the plasma membrane, resulting in a positive (outside) to negative (inside) electrical potential gradient that causes the influx of K^+ ions into the membrane. The osmotic potential of the guard cell membranes is therefore reduced and encourages the passive movement of water into the guard cell, increasing turgor and exposing the stomatal pore for gas exchange (Mao *et al.*, 2005; Chen *et al.*, 2012).

As described above in section 1.11, green light deactivates cryptochrome (Bouly *et al.*, 2007), therefore removing the signal for suppression of ABA

production in the guard cells and promoting a decrease in stomatal aperture. Longer-wave blue and green wavebands have been found to have a role in the modulation of stomatal aperture in a wide range of plants including *Vicia faba*, *Pisum sativum*, *Commelina communis*, *Nicotiana tabacum*, *Nicotiana glauca* and *Arabidopsis thaliana* (Frechilla *et al.*, 2000; Talbott *et al.*, 2002, 2006). Frechilla *et al* (2000) found that wavelengths between 490-580nm reversed blue-light induced stomatal opening in detached epidermal strips, consistent with the findings by Talbott *et al* (2002). In both studies, under continuous light the degree of reversal was light intensity dependent with full reversal when green light was twice that of blue light. The action spectrum for this green-light response showed a maximum at λ 540nm with minor response at λ 490nm and λ 580nm (Frechilla *et al.*, 2000). Further work demonstrated that the same response can be observed in intact leaves of *A. thaliana* (Talbott *et al.*, 2006) and work by Kim (2004) also concluded that green-light (λ 500-599nm) exposure reversibly decreases stomatal conductance in lettuce. Talbott *et al* (2006) determined that stomata of the *npq1* mutant, which lacks zeaxanthin, did not react to changes in the blue-green light ratio. Green light (λ 550 $\pm$ 5nm) has also been reported to antagonise UV-B (λ 287nm) induced stomatal opening in epidermal peels of *Vicia faba* and, like blue-green reversible stomatal opening, UV-B-green reversible stomatal opening is not observed in the *npq1* mutant (Isinger *et al.*, 2003), suggesting that zeaxanthin could also be involved in the regulation of stomatal aperture by green light.

By contrast, green light has been found to induce a small degree of stomatal opening when it is the only source of light, although the response is smaller

than that observed under blue or red light. Monochromatic green light (540nm) induces stomatal opening in epidermal peels of *Vicia faba* (Isinger *et al.*, 2003) and also opening of abaxial (but not adaxial) stomata in intact, attached leaves *Helianthus annuus* (Wang *et al.*, 2008). Perhaps this type of stomatal response has evolved so that, in the absence of a blue light signal, the leaf may take advantage of the green light which has penetrated through the canopy, particularly green light which has been reflected back up to illuminate the underside of the leaf, for photosynthesis. This suggests two possibilities: first that green light activates two photoreceptors – cryptochrome, to act antagonistically against blue light, and a putative unknown photoreceptor, to promote a small amount of stomatal opening allowing photosynthesis and growth when green light is the only light available. Secondly, the possibility of photosynthetic regulation either by photosynthate level or redox state of the chloroplast.

1.20 Shade avoidance

Plants have developed a set of physiological responses to light quality which allow them to detect neighbour proximity and to compete with other plants in the vicinity, maximising photosynthesis when light is at a premium. Due to the differing absorption profiles of different portions of the light spectrum though the leaf and canopy (see section 1.8 and chapter 3 for more information) plants shaded by other vegetation and/or in dense canopies are exposed to lower light levels enriched with far-red and green wavelengths. They develop symptoms such as stem and petiole elongation and upward leaf orientation, collectively termed 'shade avoidance syndrome' (Bou-Torrent *et al.*, 2008).

Shade avoidance syndrome (SAS) due to an increased proportion of far-red (700-750nm) light exposure has been well documented (for a comprehensive review see (Franklin and Whitelam, 2005). When the red to far-red light ratio (R:FR) falls this causes a decrease in activation of PHYB which in turn leads to decreased branching and reallocation of storage organ assimilates into rapid stem elongation (Tao *et al.*, 2008). This response seems much like that of the rapidly elongating hypocotyl during skotomorphogenesis, where the plant's main priority is to reach the light so that it may begin to photosynthesise and survive, only in skotomorphogenesis this means breaking through the soil and in SAS it means breaking through the canopy. In fact, like in de-etiolation, in low R:FR light ratios the PIFs, which usually suppress transcription of the genes preventing rapid stem elongation, are degraded enabling growth into better light environments (Tao *et al.*, 2008).

It is becoming increasingly apparent that green light is an additional signal for activating the shading response, enabling plants to adapt to a low light environment within a canopy. Increasing the green light ratio in a constant background of blue and red light has been shown to activate some of the same responses that would be seen in phytochrome mediated far-red light induced shade avoidance syndrome. Zhang *et al.*, (2011) found that the addition of increasing amounts of green light caused shading symptoms in *A. thaliana*, including the elongation of petioles and the upward orientation of leaves, even when the addition of this green light increased the over-all intensity of the growth light. Plants grown under higher irradiances with a high proportion of green light developed a more shaded, low-light acclimated morphology than

those grown under a lower fluence rate with no green light. In addition, Sellaro *et al.*, (2010) found that *A. thaliana* hypocotyl length, another symptom of shading, increased proportionally to decreases in the blue to green light ratio. Mullen *et al.*, (2006) found that leaves in *A. thaliana* grew in a more upright position in response to monochromatic green light ($\lambda 550\pm 25\text{nm}$) and this persisted in phytochrome and cryptochrome mutant backgrounds. Zhang *et al.*, (2011) also found that green-light induced SAS occurred in both cryptochrome and phytochrome *A. thaliana* knockdown mutants. The same study used microarray analysis to measure the concentrations of shade-induced gene transcripts (those that accumulate in the plant in far-red-light induced SAS) under varying levels of supplementary green light. In wild-type plants there was no change/ a small decrease in these transcripts, but in cryptochrome knockdown mutants transcripts built up exactly as they would in far-red SAS. This indicates that cryptochrome inhibits the effects of green light induced SAS (perhaps through the absorption of UV-A/blue light and the subsequent responses) and that there appears to be an unknown photoreceptor which mediates acclimation to a green light enriched light environment.

Until recently, the phytohormones that change levels in response to green-light were relatively unknown, assumed to be those affected in low R:FR light induced SAS (see review by Wang and Folta, 2013). However new High Performance Liquid Chromatography work by Su *et al.*, (2014) has revealed that green ($530\pm 5\text{nm}$ and $585\pm 5\text{nm}$)-light induced hypocotyl elongation in *Cucumis*

sativus due to high gibberellic acid and low abscisic acid concentrations in the hypocotyl.

During SAS, the meristematic change from the vegetative to the reproductive phase causing flowering is greatly accelerated (Tao *et al.*, 2008) – a response that likely evolved to give the plant the best chance at propagation before lowered levels of photosynthesis result in decreased metabolism to such an extent that causes plant death. When exposure to red light falls and far-red light rises due to shading, this causes less PHYB and more PHYA to be in the light activated biologically active Pfr form (Quail, 1998). Cerdán and Chory (2003) have proposed that decreased PHYB activation leads to the upregulation of nuclear protein PHYTOCHROME AND FLOWERING TIME 1 (PFT1) which, independent of photoperiod, causes gene expression at FLOWERING LOCUS T (FT) which activates flowering. This is concurrent with the findings of Endo *et al* (2005), who found that in a high R:FR ratio, mesophyll PHYB suppresses the expression of FT in the vascular bundles of the cotyledons via inter-tissue signalling. This response to far-red light can be especially useful to growers who want to rapidly bring about and/or increase flowering in long day plants. The highly energy inefficient incandescent bulb, which provides a high proportion of far-red light, has often been used to promote flowering in the cut flower industry, but now incandescent bulbs are being phased out of UK production (Runkle *et al.*, 2012). Far-red wavelength LEDs could be a suitable replacement.

1.21 Adaptation of morphology and protection and repair of the photosynthetic apparatus under high light

Where far-red light is often the shade signal for plants, UV-A/blue light is often the signal used by the plant to detect high light intensity. In nature when leaves/ whole plants are not shaded by neighbouring vegetation or other parts of the canopy, blue light is abundant. However when in competition with surrounding plants for light, the plant will be exposed to decreasing amounts of blue light as shading increases due to the absorption profile through leaves and canopies (see section 1.8 and chapter 3). This makes blue light an excellent indicator of whether the plant is exposed to full sunlight or not. UV-A/ blue light therefore has a role in many adaptive and photoprotective mechanisms.

Blue light appears to cause the opposite reaction to SAS. For example, leaves exposed to high levels of blue light have also been demonstrated to develop a thicker, 'sun-leaf' morphology with higher photosynthetic capacity, and those exposed to lower levels become thinner, flatter and broader 'shade-leaves' (Kim *et al.*, 2005; Cookson and Granier, 2006; Hogewoning *et al.*, 2010; Hoffmann *et al.*, 2015). Increased blue light exposure has been shown to decrease stem and internode length by inducing cryptochrome mediated inhibition of cell extension in the short term and cell division in the long term (Dougher and Bugbee, 2004) in poinsettias (Islam *et al.*, 2012), soybean, lettuce (Dougher and Bugbee, 2001) and white clover (Gautier *et al.*, 1998). It is often beneficial to have shorter, more compact plants. In the cut flower industry this is usually for aesthetic reasons (Islam *et al.*, 2012) but in the agronomic/ edible horticulture industry a shorter plant prevents lodging and allows more

assimilates to be channelled into leaves, fruits and/or seeds, increasing yield. Plant growth retardants which inhibit the phytohormone gibberellin (GA) are often used to achieve reduced internode length (Rademacher, 2000). If the blue light shortening effect could be built into a light recipe, this would save the grower time and money.

The bending of the hypocotyl towards a blue light source (phototropism) was reported by Charles Darwin over a century ago (Darwin, 1881). It has since become apparent that short term, often reversible changes in response to UV-A/ blue light such as this adaptation response are most often mediated by the phototropin photoreceptors (Kang *et al.*, 2008). UV-A/blue light via the phototropins controls chloroplast migration – movement of chloroplasts within the leaf in response to fluctuating light intensities (Łabuz *et al.*, 2012; Banaś *et al.*, 2012). For example, in *A. thaliana*, mediated by PHOT2, high levels of UV-A/blue light trigger the chloroplast avoidance response, whereby chloroplasts are angled away from the incident light in order to prevent photodamage (Łabuz *et al.*, 2012).

Cryptochromes have a vital role in the PSII repair cycle. Coded by the genes PsbA and PsbD respectively, D1 and D2 are the chlorophyll binding proteins which make up the core of the PSII reaction centre. They therefore have a crucial role in photosynthesis. However, under high light intensities the PSII proteins are particularly susceptible to photodamage (Thum *et al.*, 2001).

The absorption of photonic energy by the photosynthetic pigments of PSII is the first crucial step in the light dependent reactions of photosynthesis (Zhu *et*

al., 2008). However, this vital process causes continuous damage to the photosynthetic apparatus. Rate of damage and therefore inactivation of the proteins of PSII is directly proportional to photosynthetic photon flux density (PPFD) (Chow *et al.*, 1990; Tyystjärvi and Aro, 1996). This is why photoinhibition – a decreased efficiency of photosynthesis caused by photodamage to PSII (Nishiyama *et al.*, 2006) – most often occurs at high light intensities.

Photodamage first occurs to the oxygen-evolving complex due to light absorbed by the manganese at its core, and subsequently to other parts of PSII due to light absorbed by the photosynthetic pigments (Nishiyama *et al.*, 2006). Damage, in particular to the chlorophyll binding proteins D1 and D2 which make up the core of PSII (Thum *et al.*, 2001), is caused by a build-up of reactive oxygen species (ROS) that are the by-product of several stages of the light dependent reactions (Müller *et al.*, 2001; Nishiyama *et al.*, 2006). In order to continue photosynthesising effectively and maintain growth and metabolism under these high irradiances, chloroplasts use their own unique quality control system, involving specific proteases, to break down D1 and D2, which are then synthesised *de novo* in the process known as the PSII repair cycle (Kato and Sakamoto, 2009). When rate of photodamage exceeds rate of repair, photoinhibition occurs (Mellis, 1999).

PsbD transcription in the PSII repair cycle is known to be blue-light dependent. At high irradiances of UV-A /blue light, it is thought that the unique plastid σ factor AtSig5 activates the transcription factor *PsbD* BLRP which induces transcription of PsbD and production of the D2 protein (Tsunoyama *et al.*,

2004). According to Thum *et al* (2001), AtSig5 is the mediator in the signalling pathway that originates from activated CRY1, CRY2 and PHYA.

Green light has been shown to cause a decrease in transcript levels of *psaA*, *rbcl* and *psbD*, which all encode proteins that have roles in maintaining the photosynthetic apparatus (Dhingra *et al.*, 2006). Perhaps down-regulation of the genes that code for proteins which support the photosynthetic apparatus is part of green-light induced low light acclimation, whereby plants do not waste resources on building reparative proteins when not exposed to high light levels.

Many studies have shown that species such as spinach, lettuce, radish and strawberries all produce higher yields when exposed to blue as well as red light and that the amount of blue light needed for healthy growth and development appears to differ between species, ranging from 7 – 30% of total irradiance (Yorio *et al.*, 2001; Kim *et al.*, 2004; Samuolienė *et al.*, 2010). It has been shown that at least 7% blue light in a red light background is needed to prevent photosynthetic dysfunction in cucumber leaves and that a further increase in blue light up to a red:blue ratio of 1:1 can increase maximum photosynthetic capacity/ carbon assimilation rate (A_{max}) and other parameters associated with acclimation to high light proportionally (Hogewoning *et al.*, 2010).

1.22 Conclusions, aims and hypotheses

In conclusion, LED lighting systems look to be a more reliable and energy efficient alternative to lighting currently used in research and horticulture. However, before replacing conventional broad-waveband white lighting, LED

light recipes must be formulated to ensure that new lighting systems produce plants of the same or higher quality and yield. As discussed above, the ability of individual portions of the light spectrum to cause developmental and physiological changes during plant development has been demonstrated in experimental settings. Once a basic light recipe containing the right combinations of wavelengths in the right proportions for healthy plant growth and development has been formulated, the plastic nature of the plant developmental pattern means that further optimisation of light recipes could lead to changes in plant physiology linked with increased resource use efficiency, quality and yield of commercially grown horticultural crops. Highly energy efficient LED lighting and control system technology could give us the degree of control we need to make this possible.

The aim of the following experimental work was to develop LED light recipes suitable for use as a replacement for conventional broad-waveband white light sources in a research or commercial horticultural setting through:

- a) Analysis of the natural sunlight spectra under which plants have evolved in order to determine what proportions of different wavelengths induce certain acclimation responses which may be beneficial in controlled environments.
- b) Filling gaps in the current knowledge about how light signals from photons in multiple wavebands (with particular attention to green light) work in combination to induce desirable traits in plant physiology, such as those that increase resource use efficiency, biomass accumulation

and ultimately fresh-weight yield, in order to define an ideal light spectrum for the growth of common horticultural crops.

- c) Comparison of the energy/ cost efficiency of conventional horticultural lighting and LED lighting in the glasshouse environment in terms of energy input per biomass output, or 'crop per watt', and linking differences in fresh-weight yield directly to physiological changes brought about by the differences in supplementary light spectral quality.

Initial hypotheses:

- a) Due to the plant requirement for signals from multiple photoreceptors for various aspects of plant development, monochromatic LED light as the sole source of irradiance in controlled environment growth rooms will be unsuitable for plant growth
- b) Supplementation of the natural sunlight spectrum with individual wavebands of light may however be beneficial in a glasshouse environment, where no wavebands are excluded, but the change in ratio of different wavelengths may bring about beneficial effects.
- c) Lighting which produces the light quality which best allows the plant to acclimate to light quantity (i.e. PAR level) in the growth environment will result in crops with the highest fresh-weight yield. Acclimation of morphology, the photosynthetic apparatus and photosynthetic capacity is likely to be directly influenced by light quality.

2 Materials and Methods

The materials and methods used in the following three experimental chapters are presented here in full.

2.1 Chapter 3 - Spectral variation in natural light environments

2.1.1 Growth environment and plant material

Bean (*Vicia faba* cv. Wizard) and oat (*Avena sativa* cv. Humbolt) plants were sown in the field at the University of Nottingham Sutton Bonington Campus, Leicestershire, UK on 11/09/2014 at seed rates of 420 and 105 kg/ha and 50 and 250 seeds/ m² respectively. Plot dimensions were 12x1.625m with an area of 19.5m². The plots were treated with various herbicides and fungicides pre and post emergence. For bean this was ‘full rate Simazine’ pre-emergence and ‘Laser’ at 1.0 l/ha post-emergence. For oat it was ‘Hurricane’ at 0.2 l/ha pre-emergence and ‘Lexus’ at 100g/ha post-emergence. Fertiliser ‘Human Extra’ was applied once on 18/11/14 at 1 l/ha. The measurements outlined in section 2.1.3 were taken on the 15th of June in the following year between 11am and 2pm (for maximum natural sunlight), whilst bean was at anthesis and oat was at Zadoks stage GS80-89 or dough development grain fill period (Zadoks J.C., Chang T.T., 1974)

Rice (*Oryza sativa* cv. Morobereken) was grown in the FutureCrop Glasshouse facilities in south facing glasshouse also at the University of Nottingham Sutton Bonington Campus (52°49’59” N, 1°14’50” W, UK). The FutureCrop glasshouse contains large, deep soil tanks which allow for the growth of entire crop stands in a controlled environment. Glasshouse conditions were kept the same throughout the experiment. Temperature was maintained at 28 ± 3°C and

relative humidity (RH) between 50 and 60%. in late March 2015 (seven days after root establishment), seedlings were transplanted from module trays of Levington Module compost into five 1x1 m² plots of sandy loam soil (1.25 m deep) acquired from local fields, with 10cm spacing between plants (8x8 plants in each). Drip irrigation supplied water twice daily for 15 minutes. A 12 hour photoperiod was maintained with blackout blinds and high-pressure sodium (Son T-Agro, Philips) lamps provided additional lighting when PAR fell below 300μmol m⁻²s⁻¹. The measurements outlined in section 2.1.3 were taken at full canopy development during the vegetative phase, approximately 80 days after transplanting, on the 19th June 2015 between 11am and 2pm. For this purpose all supplementary lighting was switched off so that only natural sunlight spectra would be measured.

2.1.2 Equipment

The LI-6400XT portable photosynthesis system (LI-COR, Nebraska) is an infrared gas analyser (IRGA) which measures carbon assimilation rate and stomatal conductance based on gas (H₂O and O₂) exchange across the measured area of leaf surface. The sun and sky leaf chamber head with clear plastic windows (Fig2.1) allows measurement of photosynthesis under ambient light.



Figure 2.1: The LI-6400XT portable photosynthesis machine measuring head with sun and sky attachment.

The FieldSpec HandHeld 2 spectroradiometer (ASD, Boulder Colorado) is a handheld device which contains a photodiode array which can detect the energy of incoming wavelengths between 325 and 1075nm to a 1nm accuracy. The bare fibre sensor has a viewing angle of 25° but a cosine diffuser attachment (Remote cosine receptor – RCR) widens this to a dome, allowing the measurement of light coming in from all angles around the top of the attachment. The device can be used either connected to a laptop with the RS3 controlling software (Fig. 2.2a), or in ‘Stand alone’ mode (Fig. 2.2b) and the data later downloaded through the HH2 Sync interface software.



Figure 2.2: The FieldSpec HH 2 spectroradiometer connected to the laptop via USB (a) and in standalone mode with RCR attachment (b).

2.1.3 Data collection

The FieldSpec HandHeld 2 spectroradiometer with cosine diffuser attachment was used alongside the LI-6400XT infra-red gas analyser with sun and sky chamber head. This allowed simultaneous measurements of the natural light spectrum and photosynthesis/ stomatal conductance at different levels of *Vicia faba*, *Avena sativa* (field) and *O. sativa* (glasshouse) canopies. First, the LI-6400XT was set to provide a flow rate of $600\mu\text{mol s}^{-1}$ and $400\mu\text{mol} / \text{mol of CO}_2$. The spectroradiometer was then powered on in stand-alone mode and left for 30 minutes to ensure the different photodiodes were all at the same temperature. A white reference measurement was then taken by angling the bare fibre (without RCR attachment) towards a Spectralon® white panel held approximately 5cm away, under full natural sunlight. The RCR was attached to the bare fibre sensor, and the spectroradiometer set to measure irradiance.

Integration time, i.e. how long the sensor was exposed to light, was optimised to light intensity 5cm above the canopy.

After taking a spectroradiometer spectrum measurement with the RCR sensor 5cm above the canopy, simultaneous spectrum + gas analysis measurements were taken at the top (10cm from the top of the canopy), middle (half-way down the canopy) and base (10cm from soil level) of the canopies. The clear plastic window of the LI-6400XT measuring head, attached to a leaf in the canopy (adaxial side up), was held level alongside the RCR attachment of the spectroradiometer. After waiting for the chamber CO₂ to equilibrate, a spectrum measurement with the spectroradiometer was taken simultaneously with a gas analysis measurement of the leaf. Measurements of top, middle and base were carried out successively and were repeated 15 times in *V. faba* and *A. sativa* (by/on plants at least 1m apart within the 19.5m² plots) and 10 times in *O. sativa* (by/on two plants in each of the 5 plant stands) for each canopy level. For the intra-canopy measurements, it was ensured that plants were surrounded by other vegetation on all sides.

If the leaf did not fill the sun and sky measuring chamber of the LI-6400XT, an aerial photograph was taken of the chamber for later analysis (section 2.1.4.2) in order to adjust measured values of photosynthesis and stomatal conductance to per unit leaf area.

2.1.4 Data analysis

Data from the LI-6400XT was downloaded via serial port to USB and saved as .csv files which were later analysed in Microsoft Excel. Data from the FieldSpec

HH 2 spectroradiometer was downloaded through the HH2 Sync interface software and saved as .asd files which were opened with the ViewSpecPro post processing software (ASD, Boulder Colorado). Graphs of irradiance plotted against wavelength were opened for each individual file and the raw data points exported to Microsoft Excel for further analysis, including the conversion of watts per meter squared (Wm^{-2}) to photon flux density.

2.1.4.1 Conversion of irradiance watts m^{-2} data to photon flux density

All irradiance measurements taken with the Fieldspec HH2 spectroradiometer give output in Wm^{-2} . To convert these to photon flux density at each wavelength (325-1075nm) a Microsoft Excel spreadsheet was set up (Fig. 2.3) with the following equation:

$$\mu\text{E} = \{[(\text{Wm}^{-2} / ((C/\lambda) * h) / L)] / 10000\}$$

Where:

$$\mu\text{E} = \text{quantum flux} / \text{photon flux} (\mu\text{mol m}^{-2} \text{s}^{-1})$$

$$C = \text{the speed of light } (2.988 * 10^8 \text{ m s}^{-1})$$

$$\lambda = \text{wavelength}$$

$$h = \text{Planck's constant } (6.63 * 10^{-34})$$

$$L = \text{Avogadro's constant } (6.022 * 10^{23})$$

	A	B	C	D	E	F	G	H	I	J	K
10		CHANGE EACH TIME									
11	Wavelength	Irradiance	Frequency	Speed of light	Planck's constant	avogadro's constant	C/wavelength	Energy/photon	Number of photons	Photon flux per mole	Photon flux per micromole
12	λ	(Watts m ⁻²)	f (time in seconds)	C (m s ⁻¹)	h (Js)	mol ⁻¹		Ep	Np	mol ⁻¹ m ⁻² s ⁻¹	$\mu\text{mol}^{-1} \text{m}^{-2} \text{s}^{-1}$
13	325	1	1	298800000	6.63E-32	6.022E+26	919384.6154	6.09552E-26	1.64055E+25	2.72426E-06	2.724259
14	326	1	1	298800000	6.63E-32	6.022E+26	916564.4172	6.07682E-26	1.6456E+25	2.73264E-06	2.732641
15	327	1	1	298800000	6.63E-32	6.022E+26	913761.4679	6.05824E-26	1.65064E+25	2.74102E-06	2.741024
16	328	1	1	298800000	6.63E-32	6.022E+26	910975.6098	6.03977E-26	1.65569E+25	2.74941E-06	2.749406
17	329	1	1	298800000	6.63E-32	6.022E+26	908206.6869	6.02141E-26	1.66074E+25	2.75779E-06	2.757788
18	330	1	1	298800000	6.63E-32	6.022E+26	905454.5455	6.00316E-26	1.66579E+25	2.76617E-06	2.766171
19	331	1	1	298800000	6.63E-32	6.022E+26	902719.0332	5.98503E-26	1.67084E+25	2.77455E-06	2.774553
20	332	1	1	298800000	6.63E-32	6.022E+26	900000	5.967E-26	1.67588E+25	2.78294E-06	2.78293
21	333	1	1	298800000	6.63E-32	6.022E+26	897297.2973	5.94908E-26	1.68093E+25	2.79132E-06	2.791318
22	334	1	1	298800000	6.63E-32	6.022E+26	894610.7784	5.93127E-26	1.68598E+25	2.7997E-06	2.799700
23	335	1	1	298800000	6.63E-32	6.022E+26	891940.2985	5.91356E-26	1.69103E+25	2.80808E-06	2.808082
24	336	1	1	298800000	6.63E-32	6.022E+26	889285.7143	5.89596E-26	1.69608E+25	2.81647E-06	2.816465
25	337	1	1	298800000	6.63E-32	6.022E+26	886646.8843	5.87847E-26	1.70112E+25	2.82485E-06	2.824847
26	338	1	1	298800000	6.63E-32	6.022E+26	884023.6686	5.86108E-26	1.70617E+25	2.83323E-06	2.833229
27	339	1	1	298800000	6.63E-32	6.022E+26	881415.9292	5.84379E-26	1.71122E+25	2.84161E-06	2.84161
28	340	1	1	298800000	6.63E-32	6.022E+26	878823.5294	5.8266E-26	1.71627E+25	2.84999E-06	2.849994
29	341	1	1	298800000	6.63E-32	6.022E+26	876246.3343	5.80951E-26	1.72131E+25	2.85838E-06	2.858376
30	342	1	1	298800000	6.63E-32	6.022E+26	873684.2105	5.79253E-26	1.72636E+25	2.86676E-06	2.866759
31	343	1	1	298800000	6.63E-32	6.022E+26	871137.0262	5.77564E-26	1.73141E+25	2.87514E-06	2.875141
32	344	1	1	298800000	6.63E-32	6.022E+26	868604.6512	5.75885E-26	1.73646E+25	2.88352E-06	2.883523
33	345	1	1	298800000	6.63E-32	6.022E+26	866086.9565	5.74216E-26	1.74151E+25	2.89191E-06	2.891906

Figure 2.3: Spreadsheet for the conversion of watts per meter squared at 1nm intervals between 325-1075 to photon flux density using the equation as described above in section 2.4.

2.1.4.2 Determination of leaf area with ImageJ

Images of leaf area were analysed with ImageJ (a public domain Java-based imaging processing program developed at the National Institutes of Health, USA). The straight-line tool was used to establish scale. A colour threshold, in order to only measure green pixels and therefore determine leaf area, was set by changing the hue to include only the green area and brightness and saturation adjusted until only the leaf samples were selected.

2.2 Chapter 4 - A comparison of growth, development and physiology of *Coriandrum sativum* grown under different combinations of blue, green, red and far-red light in controlled environment growth rooms

2.2.1 Growth environment

In a controlled environment growth room six individual cubicles (height -58 cm; length – 76 cm; depth – 43 cm) were constructed from wooden shelving and opaque-white plastic reflective material. Cubicles allowed no exterior light to enter the growth area and were accessible via the front by lifting a small area of the plastic. LED lamps providing six different light recipes (as measured with the FieldSpec HH 2 spectroradiometer – Fig 2.6; Tables 2.1, 2.2) were positioned above the centre of each cubicle. Monochromatic red (with peak output at 665 nm) and blue (peak output 455 nm) light recipes were provided by 30 watt LED floodlamps (Greengage Lighting Ltd., U.K.). These each consisted of a heatsink, a one board array of 16 2 mm wide LEDs in a 4x4 configuration with 6 mm centre to centre spacing and a metal reflector, enclosed at the front with glass (Fig. 2.4).



Figure 2.4: The 30 watt LED floodlamps from above, below and in the growth area.

The red floodlamp was positioned 34 cm above 9 cm plant pot height and provided an even $110 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR) over an area of 38x24cm, as measured with the LI-250A PAR meter (LI-COR, Nebraska). The blue light provided the same level of PAR across an area of 37x22 cm when positioned 30 cm above pot height.

To provide four light recipes containing multiple wavebands of light, lamps each containing four channels (peaks of LEDs: Red (R) = 665nm; Blue (B) = 455nm; G = 525nm; Far-red (FR) = 745nm) of fixed wavelength LEDs with adjustable intensity output were designed and built in collaboration with the Faculty of Engineering, University and Nottingham (Fig. 2.5). The 120 watt lamps each consisted of a fan-assisted heat sink, wireless control unit and 64 3mm LEDs with 6mm centre to centre spacing. LEDs were an equal mix of the different wavelengths and were alternated to allow complete mixing of the light.

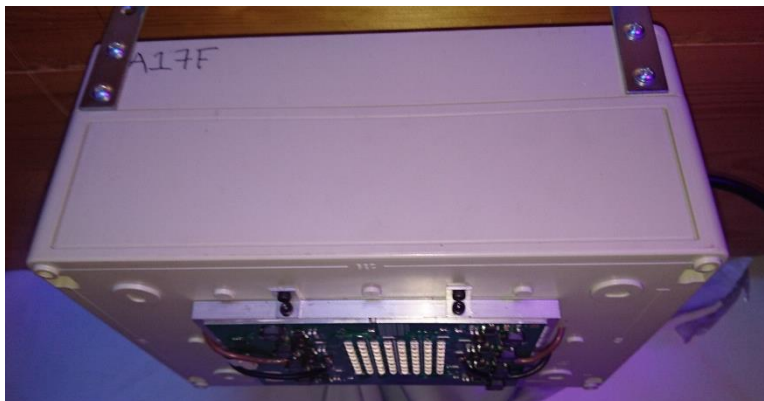


Figure 2.5: Adjustable output multi-waveband LED lamp as designed and built by members of the Faculty of Engineering, University of Nottingham, UK.

Lamps were positioned at 34 cm above pot height in the remaining four cubicles and were set to provide four different light recipes (Fig 2.6). Light recipes were measured and adjusted using the FieldSpec HH 2 spectroradiometer with RCR adaptor (operated as in section 2.1) and then intensity was adjusted to provide an even $110 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR at pot height across an area of 76x43 cm with the LI-250A light meter (LI-COR, Nebraska).

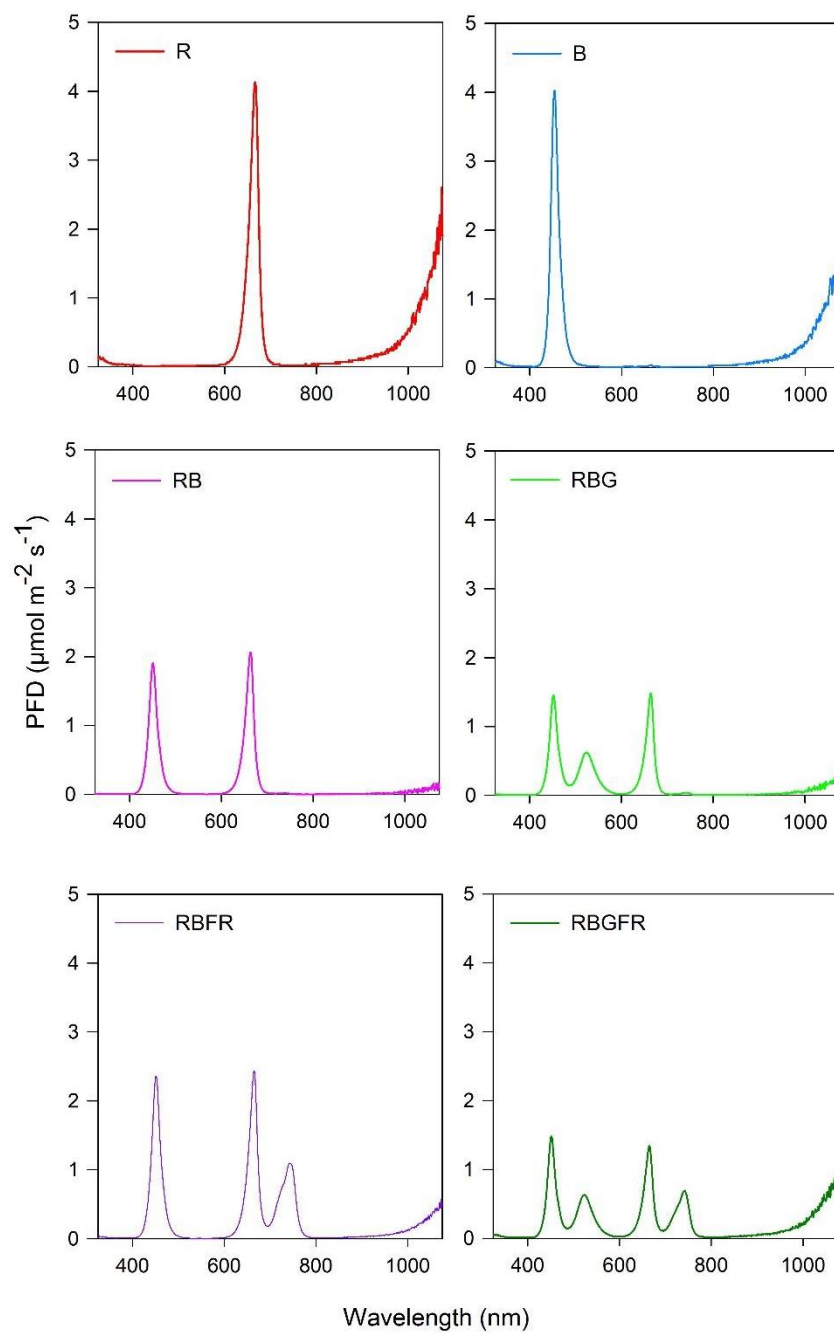


Figure 2.6: The spectra of the LED lamps as measured with the Fieldspec HH 2 spectroradiometer and converted from Wm^{-2} to photon flux density (PFD).

Table 2.1: The percentage of total photon flux density (325-750 nm) made up by each waveband (UV-A/blue 325-499 nm; green 500-599 nm; red 600-699 nm; far-red 700-750 nm) in each light recipe. Peaks of LEDs: Red (R) = 665nm; Blue (B) = 455nm; G = 525nm; Far-red (FR) = 745nm.

	R	B	RB	RBG	RBFR	RBGFR
% UVA/Blue	5	94	48	38	39	32
% Green	1	2	1	26	1	20
% Red	92	2	50	35	40	28
% Far-red	2	2	1	1	27	20

Table 2.2: The ratios of each waveband (UV-A/blue 325-499nm; green 500-599nm; red 600-699nm; far-red 700-750nm) in each light recipe.

	R	B	RB	RBG	RBFR	RBGFR
R:G	-	-	-	1.3:1	-	1.4:1
R:FR	-	-	-	-	1.4:1	1.4:1
UV-A/B:G	-	-	-	1.5:1	-	1.5:1
R:UV-A/B	-	-	1:1	0.9:1	1:1	0.9:1

Tinytag Ultra 2 data loggers (Gemini Data Loggers Ltd, Chichester) were used to confirm that temperature and humidity was uniform across all cubicles. Temperature day and night set points were 22°C and 16°C respectively and temperature was kept within 0.5°C between cubicles at all times. Relative humidity was maintained at 45±5% across all cubicles.

2.2.2 Plant material

In the following experimental work, coriander (either *Coriandrum sativum* cv. Standard, sourced from Haddons Seed Company, UK or *Coriandrum sativum* cv. Cruiser, sourced from Langmead Farms, UK) was grown in a soil mixture containing 3 parts Levington M3 compost to 1 part vermiculite. Soil was first dampened and then seeds sown 1cm deep. Plants were watered from the roots via watering trays as needed to keep the top of the soil moist and positions rotated under the light weekly. Photoperiod, cultivar, pot size, seeds per pot and length of period of growth are specified for each section work.

2.2.3 Data collection

2.2.3.1 Germination assays

Lamps were set to a 12-hour photoperiod. For each cubicle, cv. Standard was sown evenly 6 seeds per 1 litre pot. Cubicles were checked daily and germination (taken as when the seedling broke through the soil surface) rate was recorded. Fifteen days after sowing, germination success (% of seeds germinated under each light recipe) was recorded. The experiment was then repeated twice.

2.2.3.2 Experiment 1: Comparison of growth, development and physiology

Photoperiod was set to 12 hours. For each cubicle, cv. Standard was sown 1 seed per pot into eight 9cm pots. The day of the first germination event was

recorded as 'Day 0'. On day 7 seedlings were thinned to 1 per pot (coriander produces two plants per seed case). Spare seedlings were transplanted into empty pots to keep the number of replicates at 8.

2.2.3.2.1 Basic growth measurements

Plant height (soil surface to highest point of supported plant), true leaf number (not including cotyledons) and stem number were recorded on day 7, 14, 21 and 28 (harvest day). Photographs of an example of a plant from each light recipe were also taken on these days.

2.2.3.2.2 Measurement of stomatal number and density

A thin layer of clear, quick drying nail varnish was applied to the adaxial and abaxial leaf surfaces of the first true leaf of 3 plants under each light recipe. After approximately 60 seconds, once completely dry, clear sticky tape was pressed firmly over the varnish and the varnish impression pulled away from the leaf. The tape and impression was then fixed to a microscope slide for immediate analysis with a bright-field microscope.

The Laborlux 8 brightfield microscope (Ernst Leitz GmbH, Germany) was used at 25x zoom with a gridded graticule of 10x10 0.1mm², allowing analysis of 0.1mm² of leaf surface. Stomata inside the grid (including those that fell on the bottom or left grid edges and excluding those that fell on the top or right grid edges) were counted enabling determination of stomatal density and ratio of stomata on the abaxial to adaxial side of the leaf. Final counts were an average of three 0.1mm areas of the leaf.

2.2.3.2.3 Measurement of rapid light response curves of chlorophyll fluorescence

In order to compare relative quantum efficiency of photosystem II between leaves of plants grown under different light treatments, on day 14-15 the JuniorPAM (Walz, Effeltrich, Germany) chlorophyll fluorometer was used to measure rapid light response curves of chlorophyll fluorescence on the last fully expanded leaf of 3 plants under each light recipe. The instrument measured pulse-amplitude modulated (PAM) chlorophyll fluorescence in response to increasing levels of blue (460nm) actinic light. The control box was connected to a laptop via USB and the measuring head (which also measured and accounted for ambient light) with fibre-optic cable was attached (Fig. 2.7 left). The non-dark adapted leaf was positioned into the leaf clip with the fibre-optic 1mm from the centre of the adaxial leaf surface, avoiding any leaf veins (Fig. 2.7 right). WinControl-3 software was used to run the light curve program. Pulses of light in addition to ambient light were delivered 5 seconds apart at photon flux densities of 0, 25, 45, 65, 90, 125, 190, 285, 420, 625, 820, 1150 and 1500 $\mu\text{mol m}^{-2} \text{s}^{-2}$.

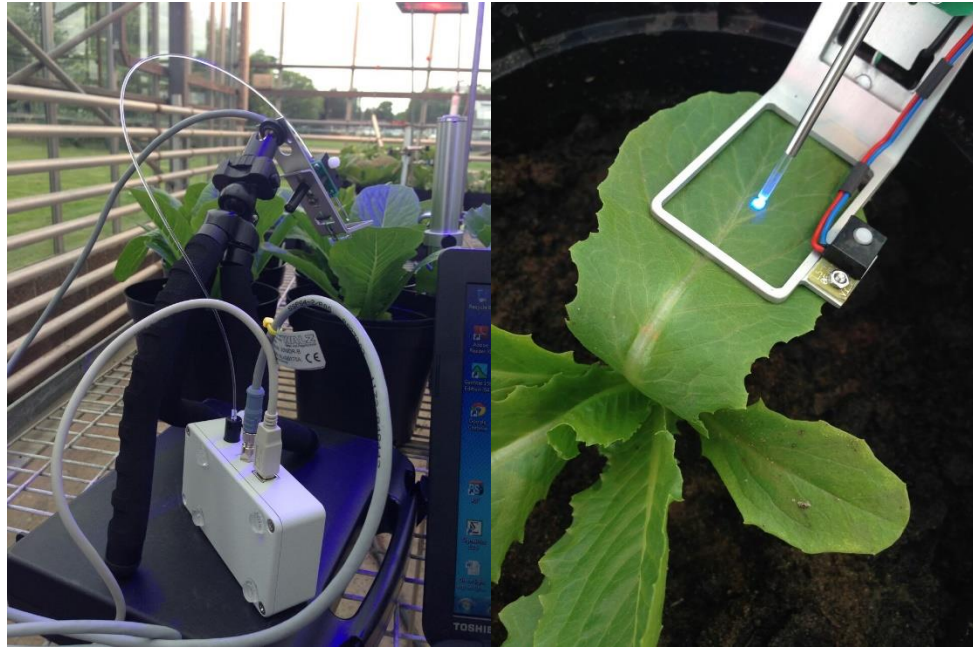


Figure 2.7: Demonstration (on lettuce) of the use of the Walz JuniorPAM chlorophyll fluorometer for measurement of rapid light curves of chlorophyll fluorescence.

Data including the parameters of ϕPSII (quantum yield of photosystem II) and ETR (electron transport rate) was exported as .csv files and further analysed in Microsoft Excel.

$$\phi\text{PSII} = \frac{F_m' - F'}{F_m'}$$

Where F_m' is the maximum fluorescence yield after the saturating pulse of light in a light adapted leaf and F is the steady state fluorescence in response to the measuring light ($< 1\mu\text{mol m}^{-2} \text{s}^{-1}$) applied before the saturating pulse (Genty *et al.*, 1989; Murchie and Lawson, 2013).

$$\text{ETR} = \phi\text{PSII } I \text{ a b}$$

Where I is the PAR at leaf level, a is the distribution of photons absorbed across the photosystems I and II (here assumed to be equal, and therefore 0.5) and b

is the absorbance value (ratio of leaf-absorbed photons to incident photons) which is often assumed to be 0.84 (Murchie and Lawson, 2013).

Actual absorbance values were then determined with use of the FieldSpec HH 2 spectroradiometer and integrating sphere (LI-COR, Nebraska) and used to replace the estimate of 0.8 (as detailed below in section 2.2.3.2.4).

2.2.3.2.4 Integrating sphere leaf reflectance, transmittance and absorption measurements

An integrating sphere and white light source illuminator (model number 1800-12, LI-COR, Nebraska) was used in conjunction with the FieldSpec HH 2 spectroradiometer (Boulder, Colorado) in order to calculate the actual absorption value of the third true leaf one plant under each light recipe. The spectroradiometer was connected to the a laptop via USB, and the bare fibre sensor of the spectroradiometer was connected to the integrating sphere by a fibre-optic cable (Fig.2.8a). The spectroradiometer was then powered on and left to warm up for 30 minutes, as in section 2.1.3. RS³ spectroradiometer software (ASD, Bolder Colorado) was then used to set integration time to 2.18 seconds.

The following reference, transmittance and reflectance measurements were taken by moving the illuminator, white plug and black plug (as shown in Fig. 2.8) into the configurations below (Table 2.3). The coloured dots on these and the ports of the integrating sphere acted as a guide for ensuring the instruments are in the right configuration.

Table 2.3: Configuration of integrating sphere components for each measurement.

	Reflectance reference	Reflectance dark reading	Reflectance sample	Transmittance reference	Transmittance sample
Sample port	Sample	Nothing	Sample	Sample	Sample
Port A	Black plug	Black plug	Black plug	Black plug	Illuminator
Port B	Lamp	White plug	White plug	Illuminator	Black plug
Port C	White plug	Illuminator	Illuminator	White plug	White plug
Dot colour	White	Red	Red	White	Yellow
Measurement	R_Ir	R_Id	R_Is	T_Ir	T_IL

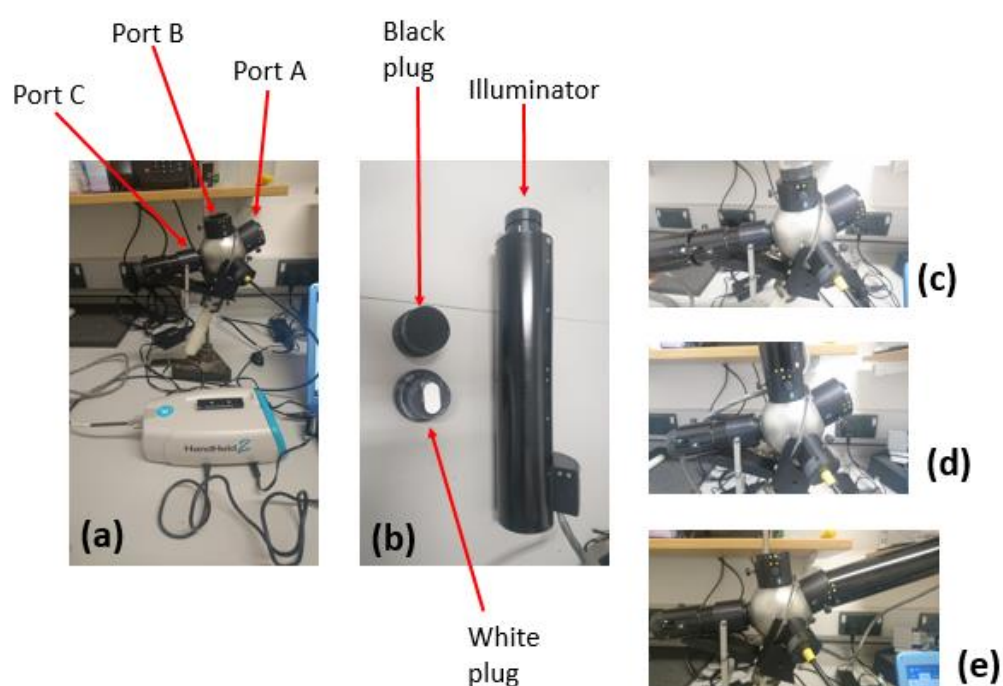


Figure 2.8: (a) The FieldSpec HH 2 spectroradiometer connected to the integrating sphere and laptop. (b) The black plug, white plug and their configuration for (c) R_Id and R_Is, (d) R_Ir and T_Ir and (e) T_IL measurements.

For the most accurate readings it was important to choose a leaf that filled as much of the leaf chamber as possible. This leaf was removed from the plant and moved into place with tweezers. To measure the adaxial side, the leaf was placed into the sample port with the adaxial side orientated towards port C during the reflectance readings, and towards port A for the transmittance

readings. To measure the abaxial side, the leaf was placed in the opposite orientations.

Files were saved in the .ASD file format and were opened with ViewSpecPro data analysis software (ASD, Boulder Colorado). The raw numbers from each measurement file were exported into a Microsoft Excel spreadsheet that was designed to perform the calculations for the equations below.

$$\text{Reflectance} = \frac{(I_s - I_d)R_r}{(I_r - I_d)}$$

(Assuming that the reflectance reference is 1 for a newly pressed barium sulphate reference on the white plug.)

$$\text{Transmittance} = \frac{I_L R_r}{I_r}$$

$$\text{Absorption} = 1 - (\text{Reflectance} + \text{Transmission})$$

Total absorption in the region of PAR could then be obtained. Percentage reflectance, transmittance and absorption of light in the blue (400-499nm), green (500-599nm) and red (600-699nm) could also be calculated for the adaxial and abaxial sides of each leaf sample by summing the readings in each waveband.

2.2.3.2.5 Chlorophyll assay

On day 18-20 leaf 3 of 3 plants under each light recipe were removed to determine chlorophyll content and chlorophyll a:b ratio. Fifteen millilitre falcon

tubes were labelled and filled with 5ml 80% acetone (20% pure water) before being placed on ice in a light-tight polystyrene box. Whole leaf samples were stripped from the stem and then laid flat on a blank white surface after which an aerial photo was taken with a ruler for scale. Leaf area for each sample was then determined in ImageJ, as in section 2.1.4.2. Leaves were then placed directly into the falcon tubes. In the fume hood, samples were ground in the 80% acetone with a pestle and mortar until only small particles remained and then returned to the falcon tube and placed in the ice box. All tubes were then transferred to the MSE Centaur 2 centrifuge and spun for 5 minutes at 3000rpm. The supernatant of each sample was transferred to a 1cm cuvette and the Varian Cary spectrophotometer (Agilent Technologies, Santa Clara, California) used to measure absorption at wavelengths of 646.6 and 663.6 for chlorophyll b and a respectively and 750nm to allow for a turbidity correction. Values were then entered into a Microsoft Excel spreadsheet containing the following equations (as in Porra et al. 1989):

$$\text{Chl a } (\mu\text{g}/\text{cm}^2) = \{[12.25*(A_{663.6}-A_{750})] - [2.55*(A_{646.6}-A_{750})] *V\}/S$$

$$\text{Chl b } (\mu\text{g}/\text{cm}^2) = \{[20.31*(A_{646.6}-A_{750})] - [4.91*(A_{663.6}-A_{750})] *V\}/S$$

$$\text{Chl a+b } (\mu\text{g}/\text{cm}^2) = \{[17.768*(A_{663.6}-A_{750})] + [7.34*(A_{645}-A_{750})] *V\}/S$$

Where A = absorption value, S = Sample area (cm²) and V = volume of the extract (ml).

2.2.3.2.6 Infra-red gas analysis

Between days 21 and 28, gas exchange and chlorophyll fluorescence parameters of the last fully expanded leaves of 3-4 plants under each light recipe were measured with the GFS-3000 photosynthesis system (Walz,

Effeltrich, Germany). The machine was operating with narrow rectangular leaf adapter 3010-1, flow rate of $800\mu\text{mol s}^{-1}$, cuvette temperature of 21°C and relative humidity of $50\pm 1\%$. Light was provided by a combination of in-built red and blue LEDs. For the light-response curves, carbon dioxide level was set to 400mmol/ mol . Leaves were placed adaxial side upwards in the leaf cuvette and exposed to $400\mu\text{mol m}^{-2} \text{s}^{-1}$ for three minutes before beginning illumination with a series of PAR values. PAR set points were 0, 50, 150, 300, 400, 600, 800, 1000, 1200, 1500, 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 90% red light and 10% blue light with 120 second intervals. For the carbon dioxide response curves, PAR was set to a constant $1000\mu\text{mol m}^{-2} \text{s}^{-1}$ and the CO_2 set points were 400, 100, 50, 0, 200, 400, 400, 600, 800, and 1000ppm with 180 second intervals.

Leaf area within the cuvette was determined in ImageJ as in section 2.1.4.2 but with one modification. The leaf in the aerial photo was orientated in the way it would be placed into the cuvette and the rectangle selection tool used to select only the area of leaf that would be inside. (Fig. 2.9).

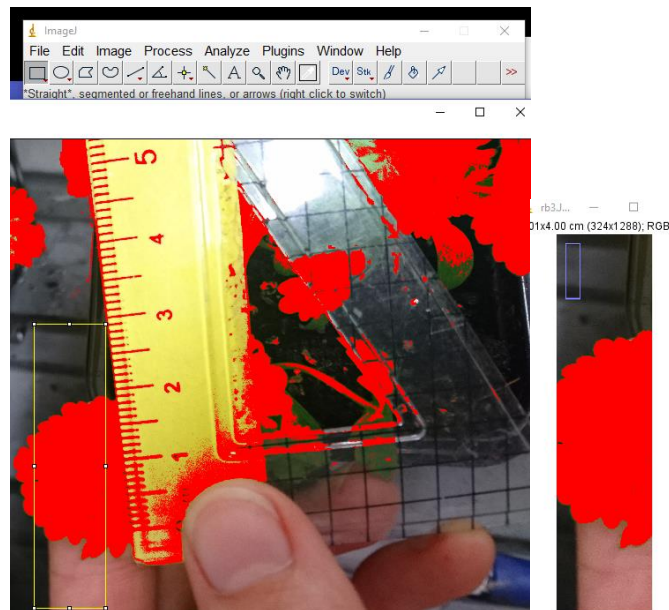


Figure 2.9: Selection and cropped leaf image in ImageJ for determination of leaf area inside GFS-3000 cuvette.

GFS-3000 data was exported as .csv files which were further analysed in Microsoft Excel. Dark respiration (RD), light compensation point (LCP) and maximum rate of carbon assimilation (A_{max}) were calculated by entering the photosynthetic light response curve values into the model described by (Prioul and Chartier, 1977) with use of a Microsoft Excel spreadsheet produced by Lobo *et al.*, (2013). V_{cmax} and J_{max} were calculated by entering the values from the CO_2 response curves into the two-segment PS-FIT model, which used the equations of Farquhar *et al.*, 1980 and was produced by Bernacchi (2013), also in Microsoft Excel.

2.2.3.2.7 Measurement of in situ photosynthesis

Spot measurements of in situ photosynthesis were taken on the last fully expanded leaf of 3 plants under each light recipe on day 21, using the Licor-LI6400XT (LI-COR, Nebraska) with sun and sky clear plastic window. First, the

LI-6400XT was set to provide a flow rate of $600\mu\text{mol s}^{-1}$ with a cuvette CO_2 concentration of $400\mu\text{mol / mol}$ and temperature of 21°C . The attached leaf of the selected plant was then clamped into the leaf chamber, adaxial side upwards, towards the light. If leaves did not fill the measurement area in the chamber, the leaf area measurement protocol described in section 2.1.4.2 was used to work out the area of the leaf inside the chamber. Determination of the correct area enabled the re-calculation of photosynthesis on a per unit leaf area (cm^2) basis. The clear plastic window was positioned so that the leaf received $150\mu\text{mol m}^{-2} \text{s}^{-1}$ of the ambient growth light. After waiting for chamber conditions to re-equilibrate, three measurements of carbon assimilation rate were logged at 60 second intervals.

2.2.3.2.8 Harvesting of plant material

On day 28 above ground plant material was harvested. For determination of total plant leaf area of each plant, leaves were stripped from the stem and laid on a blank white surface. An aerial photo was taken with a ruler for scale. Leaf area was then calculated in ImageJ, as in section 2.1.4.2. Leaf fresh weight and stem fresh weight of individual plants were recorded. All plant material was then dried in paper bags in an oven at 80°C for 7 days before dry leaf and stem weights were recorded. The entire experiment was then repeated twice.

2.2.3.3 Experiment 2 - Comparison of biomass partitioning and root development

To further investigate the effects of combinations of red, blue and green light on the physiology, particularly with regards to biomass partitioning, cv. Cruiser was grown under the same R, B, RB and RBG light recipes and conditions as used above, but with a photoperiod of 16 hours. For each cubicle seeds were

sown evenly 6 seeds per pot 1 litre pot. 21 days after sowing, plant height was recorded before plants were washed out of the soil. Each pot was squeezed lightly to break up the soil and then submerged entirely in a tub of room temperature water. The water was gently swirled to isolate individual plants and these were transferred one by one to another tub of clean water. All soil was carefully removed from the shoots and roots before plants were placed next to a ruler for scale and photographed. The roots were then removed with scissors and transferred to shallow, clear plastic trays of water which were placed in a flatbed scanner, with a ruler for scale. The trays were covered with a white sheet of card and then the scanner lid closed. Scans were analysed with WinRHIZO (Regent Instruments Inc., Canada) root analysis software, as in Arsenault *et al.*, (1995). Leaf, stem and root fresh weights were recorded and plants placed in individual paper bags in an 80°C oven for one week before overall dry weight was recorded. The experiment was then repeated twice.

2.2.3.4 Experiment 3 - Visualisation of leaf vein networks

To allow visualisation of the vein networks of the fully expanded first true leaf from a plant in were grown again as in section 2.2.3.3. Whole leaves from each light treatment were placed in labelled glass petri-dishes, covered with filter paper and then a thin layer of 5% NaOH. After being left overnight in a 40°C oven the filter paper was removed with tweezers and the 5% NaOH using a pasteur pipette, taking care not to touch the fragile leaves. Leaves were washed 3 times in distilled water and then a 6% bleach solution was added. The bleach was removed once leaves had become entirely see-through with the veins clearly visible. Leaves were again washed three times in distilled water,

and then left for 30 minutes each in a series of 40, 60 and 80% ethanol. The now less fragile leaves were now stained for 3 hours with a mix of 5% safranin and 95% ethanol. The stain was then mostly removed with 100% ethanol, leaving the veins of the leaves a dark pink colour and the leaves themselves mostly clear.

The leaves were floated in 95% ethanol in clear plastic petri-dishes and the petri-dishes placed in a flat-bed scanner next to a ruler for scale. They were then covered with a sheet of white card and then scanner lid closed before running a high resolution 1200dpi scan on each light treatment set and then individual leaves to produce high quality images of the vein networks.

2.2.3.5 Experiment 4 - Comparison of absorption spectrum and chlorophyll content

Photoperiod was set to 16 hours. Under all six recipes, for each cubicle seeds of cv. Cruiser were sown evenly 6 seeds per 1 litre pot. Twenty-one days after sowing, to compare absorption of light by leaves grown under the different spectra in more detail, the FieldSpec HH 2 spectroradiometer (ASD, Boulder Colorado) along with an integrating sphere with broad-wavelength white light illuminator (LI-COR, Nebraska) was used to measure reflectance and transmission values of the third true leaf (as in section 2.2.3.2.4) of 4 plants under each light recipe. Leaves were then immediately used for chlorophyll assay (as in section 2.2.3.2.5).

2.2.3.6 Supplementary experiment - Investigation into the source of differences in growth between cultivars under the B light recipe

Due to the differences in average leaf area and fresh-weight yield at harvest of cv. Standard and cv. Cruiser (as compared to plants grown under the other light recipes) when grown under the monochromatic blue light recipe, extra physiological measurements were carried out to find the cause. Plants were grown again in their original conditions and photosynthetic light response curves were measured on the third true leaves of cv. Cruiser 24 days after germination with the LI-6400XT (LI-COR, Nebraska) infra-red gas analyser (see below) whilst roots of cv. Standard were imaged 21 days after germination (as in section 2.2.3.3).

The LI-6400XT was set to operate at a flow rate of $600\mu\text{mol s}^{-1}$, with cuvette carbon dioxide concentration at $400\mu\text{mol/mol}$ and temperature at 21°C . Leaves were placed into the chamber adaxial side upwards. The leaf was then illuminated with $400\mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR provided by inbuilt red and blue LEDs for 180 seconds before a sequence of PAR levels was applied with 180 seconds at each step: 0, 100, 300, 600, 1200, 1500 and $2000\mu\text{mol m}^{-2} \text{s}^{-1}$.

2.3 Chapter 5

2.3.1 Growth environment

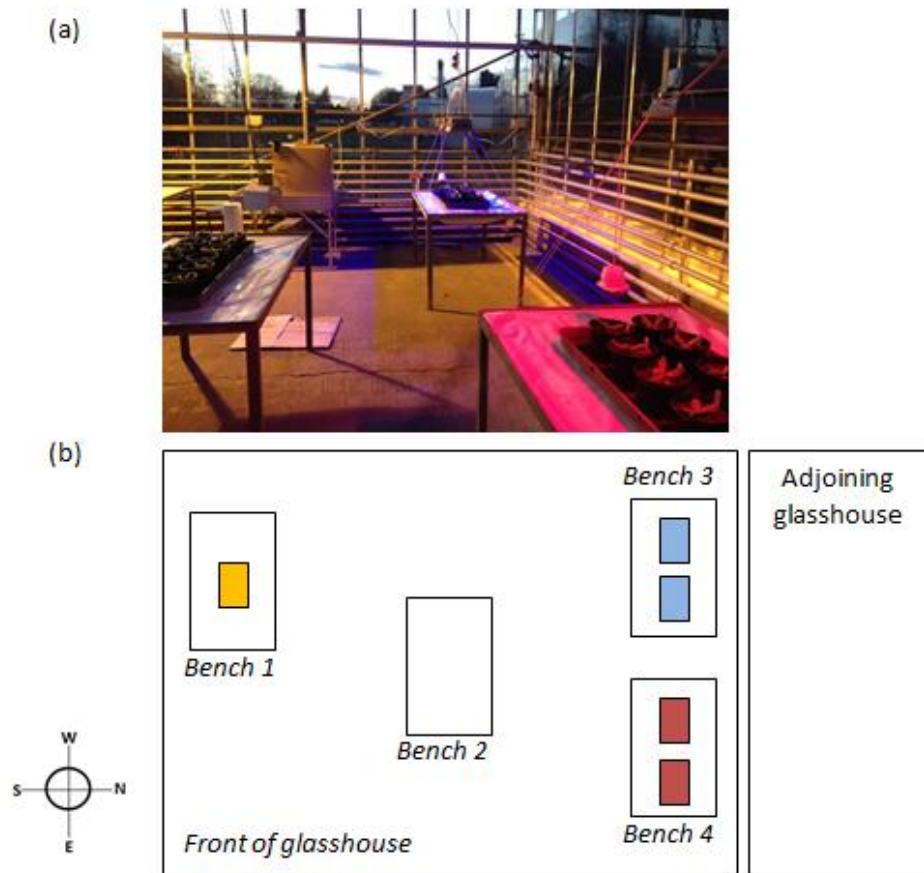


Figure 2.10: Photograph (a) and schematic (b) of the glasshouse set-up. Benches were arranged in a south-facing glasshouse in Leicestershire, UK. One 400 watt HPS lamp was suspended 167 cm above bench 1, two 30 watt blue LED lamps suspended 60 cm above bench 3 and two 30 watt red LED lamps suspended 66 cm above bench 4. Bench 2 was left as a sunlight only control.

Metal grid-top benches (76 cm high) covered with white reflective plastic were arranged in a south facing glasshouse in Leicestershire, UK (Fig 2.10). One 400 watt HPS lamp (Son T-Agro, Philips) was suspended 167 cm above bench 1, two 30 watt blue LED lamps (Greengage Lighting Ltd, U.K., - peak wavelength output 455 nm) suspended 60 cm above bench 3 and two 30 watt red LED lamps

(Greengage Lighting Ltd, U.K., - peak wavelength output 665 nm) suspended 66cm above bench 4. Bench 2 was left as a sunlight only control. After sunset, the lamps were adjusted to give the most even amount of light across their respective benches. The tables were split into a grid pattern and PAR and spectra were measured at each section with the LI-250A PAR meter (LI-COR, Nebraska) and Fieldspec HH 2 spectroradiometer (ASD, Boulder Colorado) respectively. This was to ensure that the same amount of supplementary light was being given to each treatment (none to the sunlight control), and that there was no 'contamination' or spill-over between light treatments.

When positioned 156cm above 1 litre pot height (10.3cm), one 400W HPS lamp provided an even $50 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR over 108 by 159cm (total 17172 cm²), quickly dropping off in intensity outside of this area. Placed 59 cm (centre to centre) apart and 55.5 cm above pot height, 2 30W red LED lamps provided the same intensity of PAR over 117x66 cm (total 7722 cm²). Two 30W blue LED lamps positioned 49 cm above pot height and 59 cm apart also provided $50 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR over 117x66 cm of bench. Analysis of the spectra as measured by the spectroradiometer after sunset confirmed that there was no light contamination between benches of wavelengths from other treatments or lights in the adjoining glasshouse. The control bench received $<1 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR from artificial lighting. Spectra were also measured on one bright day (clear skies) and one cloudy day. Final measurements of spectra can be seen in Figure 2.11.

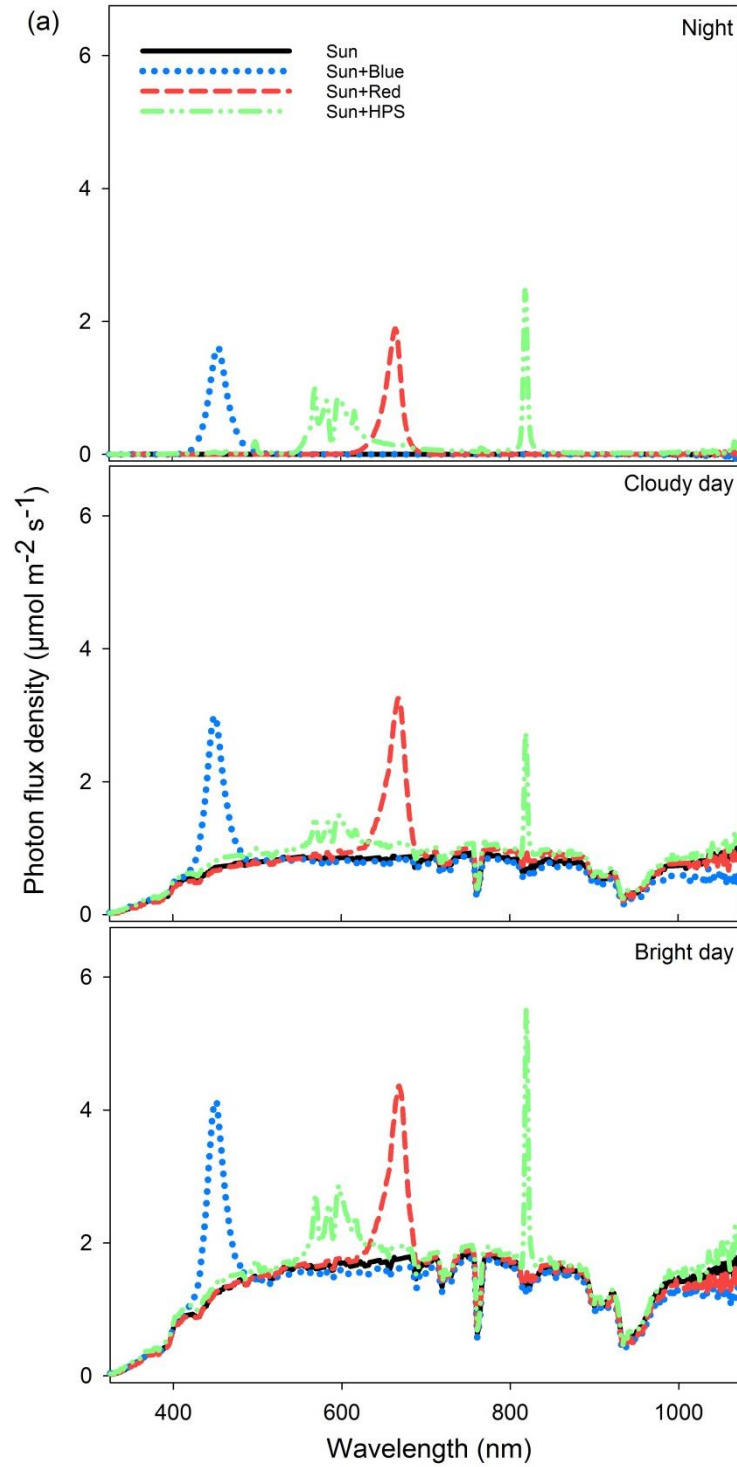


Figure 2.11a: Spectra of the supplementary lights as measured in the hours of darkness (night), on a dull day at midday (cloudy day) and on a bright day at midday (bright day) with the ASD Fieldspec HH 2 spectroradiometer at plant height directly under the lamps (no lamps for sun control).

(b)

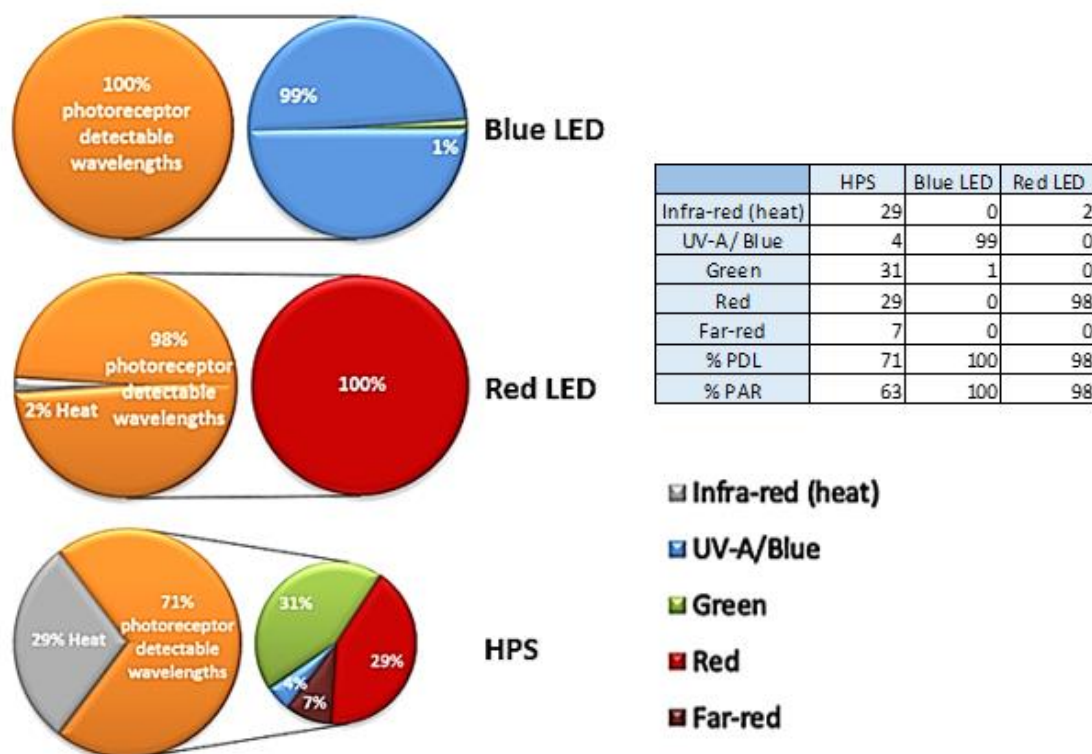


Figure 2.11b: Pie charts and accompanying table show the composition of the light emitted by the HPS, blue LED and red LED supplementary light. Size of pie charts on the right in each set is proportional to the percentage of photoreceptor detectable wavelengths – i.e. wavelengths of photons absorbed by the leaf which are between 325 and 750nm which can also be defined as plant detectable light (PDL) - received by the plant when under that light treatment. LED wavelengths provided: Red – 605-705nm with a peak at 665nm; Blue – 411-516nm with a peak at 454nm. Wavebands are taken as: UV-A/Blue = 325-399nm; Green = 400-499nm; Red = 500 -599nm; Far-red = 700-750nm; Infra-red (heat) = 751-1075nm

All lamps were set to provide 16 hours of supplementary light per day (5am – 9pm), to match sunrise and sunset times. TinyTag Ultra 2 data loggers (Gemini Data Loggers Ltd, Chichester) were then positioned at pot height at each of the four benches to log temperature throughout the experiment. Temperature in

the glasshouse was controlled automatically only via roof vents for cooling. During run 1 average day time (5am – 9pm) and night temperatures were 27°C and 15°C respectively. During run 2 they were 30°C and 20°C. There were no significant differences between average temperatures at plant height in any of the light treatments during either run of the experiment.

2.3.2 Plant material

Two spring/summer runs of the experiment were carried out: May/June 2014; June/July 2014. For each experiment, 12 cell seed trays were filled with John Innes number 3 compost, placed in watering trays and the soil dampened. Half were sown with Cos (*Lactuca sativa* L. cv. Lobjoits Green) and half with Iceberg (*Lactuca sativa* L. cv. Match) summer-lettuce cultivars. Seeds were placed on the surface of the damp soil, two per cell, and covered with a thin layer of compost. They were then placed in a potting shed to germinate to avoid thermal dormancy from the summer heat in the glasshouse. Once germinated the plants were moved to the glasshouse and one tray of each cultivar placed under each of the four light treatments. Ten days later 4 (run 1) or 8 (run 2) healthy plants of each variety per light treatment were transferred to 1 litre pots (height 10.3cm and diameter 12.9cm) of damp John Innes number 3 compost for the remainder of the experiment. Pots were spaced evenly in their respective growth area and rotated regularly during growth, ensuring the most even absorption of light.

2.3.3 Data collection

2.3.3.1 Basic growth measurements

Leaf number was recorded from germination until head formation. Green area over time was measured throughout the experiment by taking aerial images of

each plant inside a photographic tent (to reduce shadow), with a ruler for scale, and analysed in the ImageJ computer program (as in section 2.1.4.2).

2.3.3.2 Diurnal measurements of PAR and chlorophyll fluorescence

MONITORING-PAM Chlorophyll Fluorometers (Walz, Effeltrich, Germany) were used to track diurnal changes in photosynthetically active radiation at leaf level as well as monitor the quantum efficiency of photosystem II (Φ_{PSII}), electron transport rate through photosystem II (ETR) and non-photochemical quenching (NPQ) of plants of each cultivar under each light treatment. Multiple measuring heads were connected to a main control unit which stored data. Fluorometers were clamped to a healthy, fully light exposed leaf of one plant from each cultivar in each light treatment (Fig. 2.12) to deliver a saturating pulse of blue (with a peak at 460nm) light ($>8500\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) every 15 minutes. In the first run of the experiment, four fluorometer heads were used on days 37-42, allowing a comparison of plants of one cultivar under each light treatment. The fluorometers were repositioned each day to additively measure three plants under each light treatment over three consecutive days. They were then moved to the other cultivar to take the same measurements over the next three days. In run 2 of the experiment on days 41-43, 8 fluorescence heads were used, enabling simultaneous monitoring of 1 plant of each cultivar under each light treatment and allowing a comparison between cultivars as well as light treatments.



Figure 2.12: Walz MONITORING-PAM Chlorophyll fluorometers for the continuous measurement of ϕPSII , ETR and NPQ of lettuce leaves.

ϕPSII and ETR were calculated as in section 2.2.3.2.3.

$$\text{NPQ} = \frac{F_m - F_m'}{F_m'}$$

Where F_m is the maximum fluorescence yield after the saturating pulse of light in a dark -adapted leaf and F_m' is the same measurement taken in the light. F_m was taken to be the one highest maximum yield of chlorophyll fluorescence recorded by the MONITORING-PAMs after sunset, whilst F_m' was taken as the maximum yield of chlorophyll fluorescence taken after each saturating pulse during the day.

Twenty-one days after sowing, the Walz JuniorPAM chlorophyll fluorometer (Walz, Effeltrich, Germany) was used to measure rapid light curves of chlorophyll fluorescence (as in section 2.2.3.2.3) on the last fully formed outer leaf of three plants of each cultivar under each light treatment.

To allow for correction of ETR values absorbance of wavelengths 325-1075nm of light by four leaves from each cultivar under each light treatment was compared by removing one healthy, light exposed leaf and measuring with the FieldSpec HH 2 spectroradiometer (ASD, Boulder Colorado) in conjunction with an integrating sphere (LI-COR, Nebraska) as in section 2.2.3.2.4. Absorbances at 455 and 460nm (the wavelengths of the Walz MONITORING-PAM and Walz JuniorPAM measuring beams respectively) were determined and used to replace the standard 0.84 absorbance value in the ETR equation (Table 2.4).

Table 2.4: Absorbance at 455nm and 460nm of the adaxial sides of leaves of two cultivars of lettuce, cv. Match and cv. Lobjoits Green (L.G.) as measured with the Fieldspec HH 2 spectroradiometer (ASD, Boulder Colorado) in conjunction with an integrating sphere (LI-COR, Nebraska).

Light treatment	Match (455nm)	L.G. (455nm)	Match (460nm)	L.G. (460nm)
Sun	0.899488	0.910288	0.888455	0.912004
Sun + Blue	0.902273	0.902416	0.895032	0.913462
Sun + Red	0.908703	0.925994	0.897455	0.922149
Sun + HPS	0.886773	0.913218	0.882371	0.909487

2.3.3.3 *Harvesting of plant material*

The lettuce was harvested 45 days after sowing by cutting plants off at the base, just above the soil. The LI-3100C Leaf Area Meter (LI-COR, Nebraska) was used to enable rapid calculation of total leaf area of each plant. The LI-300C is a conveyor-belt style benchtop instrument which, calibrated using a metal disc of known area, digitises the area of leaves. Individual leaves were fed between

press-rollers which flattened and trapped them between two transparent belts before they were moved over the scanner. The leaves were then ejected and the total leaf area in mm² was displayed shown. Fresh weights were then recorded before all material from each plant was transferred to labelled paper bags and dried in an 80°C oven for 1 week before weighing again for recording of dry weights.

2.4 Statistical analysis

All statistical analysis was carried out in SPSS. Analysis of variance (ANOVA) at a confidence interval of 95% with post-hoc pairwise comparisons (with either Fishers LSD or Bonferroni correction for multiple comparisons, as indicated) was used to detect significant differences.

3 Spectral variation in natural light environments

3.1 Introduction:

When designing the ideal 'light recipe' for commercial plant growth in artificial light (i.e. combination of wavelengths required for optimal plant growth and development) it is first worth considering the light quality in which plants have evolved. Plants in their light natural environment not only experience fluctuations in light intensity but also in light spectral quality. This is due to both diurnal variation in sunlight intensity, total transmission of photons and variation in transmission of photons of different wavelengths through the plant canopy structure (Kasperbauer, 1971; Vandenbussche *et al.*, 2005; Burgess *et al.*, 2015). As discussed in chapter 1. 3, plants are able to sense light from the UV portions of the spectrum (280nm) all the way through to the far-red (750nm) and adjust their growth and development accordingly (Carvalho *et al.*, 2010) in order to best survive and reproduce in their light environment.

Due to the high sensitivity of plants to their light environment (as reviewed extensively in chapter 1), the effects that individual wavelengths present within sunlight have on photoacclimation of plant physiology and photosynthesis in the field and glasshouse environment are likely to be varied and complex and it is important to understand this if successful (commercial) growth under artificial lighting conditions is to be achieved. Choosing the correct proportions of wavelengths for growth 'recipes' in controlled light environments is likely to be made easier by understanding how the light spectrum changes through

canopies in a natural light environment and how plants adapt their physiology under these light qualities.

In chapter 1.8, the way the light spectrum changes through the vertical profile of the leaf was discussed. To briefly review; due to the structure of and absorption properties of the pigments in the leaf, different proportions of each wavelength reach the chloroplasts at different depths within the leaf profile to drive photosynthesis. Blue photons and to some degree red photons are more severely depleted higher in the leaf profile due to the preference of their absorption by chlorophyll and other leaf pigments, whilst green photons are able to penetrate further into the leaf profile due to 'sieve' and 'scattering' effects (see chapter 1.8 for more detail).

Leaves do not exist in isolation. In nature and field conditions, they are usually present within a structurally complex canopy. The light spectrum through the canopy profile likely changes in much the same way as through the leaf (Kasperbauer, 1971; Vandenbussche *et al.*, 2005), but on a larger scale, where photons are absorbed, reflected and scattered not only between chloroplasts but whole leaves. This causes spectral gradients through the canopy profile which provide positional signals enabling photoacclimation of the whole plant or individual leaves to the canopy light environment (Vandenbussche *et al.*, 2005; Franklin, 2008; Zhang and Folta, 2012; Wang and Folta, 2013).

Kasperbauer (1971) measured the spectral distribution at different levels of a tobacco canopy and highlighted the declining red to far red (R:FR) ratio. Over 20% of far-red light whilst only $\leq 2.1\%$ of red light reached the base of the

canopy. The tendency toward rapid depletion of red light and an increase in far-red light as a proportion of total plant detectable light (TPDL) and subsequent photomorphogenic effects have been well documented (see chapter 1.20; Franklin and Whitelam, 2005). The proportion of far-red light through the canopy profile provides a strong positional signal to the plant. Mediated by the red/ far-red light absorbing phytochrome photoreceptors, plants sense the decrease in R:FR ratio which brings about a suite of effects collectively termed shade avoidance syndrome (SAS). This includes induction of petiole elongation, upward leaf orientation and decreased leaf area expansion as leaves try to grow upwards out of unfavourable low photosynthetically active radiation (PAR – see chapter 1.4) conditions and into the light (for more detail see chapter 1.22 and for comprehensive reviews see Smith, 2000; Franklin and Whitelam, 2005; Vandenbussche *et al.*, 2005).

Interestingly, the data from the study by Kasperbauer (1971) also showed that green light at $\lambda 543\text{nm}$ and $\lambda 576\text{nm}$ was transmitted through the canopy more readily than blue and red light, although not as readily as far-red light. Just $\leq 0.5\%$ of blue and $\leq 2.1\%$ red light from above the canopy penetrated through to the bottom layers, whereas up to 6.5% of green light (which drives photosynthesis via photosystem I and photosystem II – see chapter 1.9) The implications of green light therefore appearing to make up a higher proportion of TPDL and PAR as canopy depth increased were however not discussed.

As discussed in chapter 1.8, contrary to popular perception, green light is absorbed largely by chlorophyll b and has an important role in driving

photosynthesis deep within the leaf profile via both photosystem I (PSI) and photosystem II (PSII) (Terashima *et al.*, 2009). As far-red light is only able to drive photosynthesis through PSI, green light may also have a crucial role in driving photosynthesis deep within the canopy, where other wavelengths of PAR (blue and red light) have been more severely depleted. Green light has also been shown to activate some of the same responses that would be seen in phytochrome (PHY) mediated far-red light induced SAS (Chapter 1.20; Zhang *et al.*, 2011). This suggests that green light may act as an additional positional signal to far-red light in order to enable the plant to best acclimate to the light intensity to which it is exposed.

As discussed in chapter 1.19, the relative proportions of blue and green light have been shown to influence stomatal aperture (Frechilla *et al.*, 2000; Talbott *et al.*, 2002, 2006; Bouly *et al.*, 2007). The guard cells respond to a complex hierarchy of signals which are both intracellular and external. Light is one of the key determinants of these responses, along with [CO₂] and humidity, and has been shown to be dynamic in canopy conditions, with fluctuations in intensity occurring on a scale of hours to seconds and which vary in magnitude (Bartley and Scolnik, 1995; Morison, 1998; Pearcy and Way, 2012; Way and Pearcy, 2012; Chen *et al.*, 2012; Bauer *et al.*, 2013; Burgess *et al.*, 2015).

Regulation of stomatal aperture via manipulation of guard cell turgidity is the principle means of regulating the uptake of CO₂ for photosynthesis and the release of H₂O for maintaining transpiration. The plant's ability to precisely regulate guard cell turgidity over minutes or seconds, causing changes in

pressure which dictate the exposure of the stomatal pore, is crucial for optimisation of the balance of carbon dioxide uptake versus water loss (for a detailed review see Lawson and Blatt, 2014). Therefore, the signal to the leaf through changes in relative proportions of blue and green light through the canopy profile may have a role in increasing canopy water use efficiency.

It could be hypothesised that stomatal closure with increasing canopy depth at the lowest level of PAR is directly linked to the decreasing blue to green (B:G) light gradient. If the same degree of canopy stomatal closure occurred concurrently with the steep decrease in the level of red or the R:FR ratio, closure would be complete well before light levels dropped to the light compensation point below which photosynthesis cannot take place. It would be useful to ascertain whether the phenomenon of an increasing proportion of green light through the vertical canopy profile is common to all plant canopies in natural sun spectrum light environments, how strong the subsequent gradients in light ratios (particularly B:G light ratio) are and if they would be able to exert the same physiological effects as those discussed above.

3.2 Methodology and hypotheses

To investigate the phenomenon of spectral distribution changing in relation to canopy position, and to determine the proportions of wavelengths that plants/leaves are exposed to under full sunlight and various levels of shade, natural spectra (with no additional artificial lighting) were measured at three levels of the canopy profile in bean (*Vicia faba* cv. Wizard) and oat (*Avena sativa* cv. Humbolt) canopies in the field and a rice (*Oryza sativa* cv. Morobereken) canopy in the glasshouse. Measurements were taken with the FieldSpec HH 2 spectroradiometer which has a range of 325-1075nm and therefore UV-B light was not considered, however levels of UV-A (325-399), blue (400-499), green (500-599), red (600-699) and far-red (700-750nm) waveband light were measured and analysed. The effects of light quantity and quality on leaf gas exchange were investigated by taking infra-red gas analysis measurements simultaneously with the light spectrum measurements. For full details of methodology please refer to chapter 2.1.

The following hypotheses were proposed:

- 1) The proportion of total plant detectable light (TPDL) made up of both far-red and green light will increase with depth into the vertical canopy profile. However preferential decay of the R:FR light ratio over the B:G light ratio will result in a marked difference in the distribution between the two, causing an extreme R:FR gradient and a more shallow B:G gradient.

- 2) Leaf photosynthesis and stomatal conductance will fall in relation to levels of available photosynthetically active radiation (PAR) and stomatal conductance will correlate directly to the B:G light gradient.

3.3 Results

3.1.1 Sunlight spectrum above the canopy in the field and glasshouse

UV-A light made up the smallest proportion of the spectrum both in the field and glasshouse (Fig. 3.1b). Photosynthetically active radiation wavebands (blue, green and red) accounted for between 22.5 and 27.8% each of TPD (roughly equal proportions) in the sunlight spectrum above the canopy in the field and glasshouse. There were however some significant differences. In the field, blue light made up a significantly smaller proportion of TPD than green ($P<0.001$) and red ($P=0.003$) light and made up a statistically similar proportion to far-red light. However, UV-A and blue light combined made up 29% of TPD, a statistically similar proportion to green and red light. In the glasshouse blue, green and red light made up statistically similar proportions of TPD, however UV-A and blue light combined made up a higher proportion of light than green ($P=0.006$) or red ($P=0.001$) light. A significantly higher proportion of the spectrum in the glasshouse was made of blue ($P=0.016$) wavelengths than in the field. Far-red light was lower than red light in the field ($P<0.001$) and glasshouse ($P<0.001$).

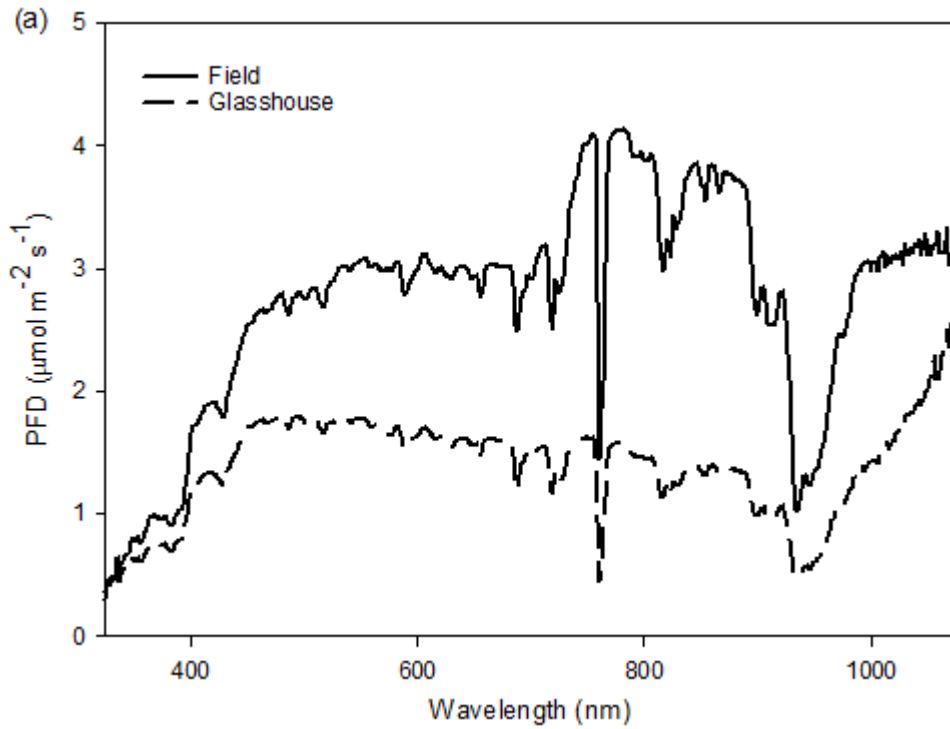


Figure 3.1a: The solar spectrum as measured as photon flux density (PFD) on a clear, bright summer day in the field and the glasshouse with the FieldSpec HH 2 spectroradiometer (ASD). Readings averaged from 5 measurements taken between 11am and 1pm. Average total photosynthetically active radiation (PAR - λ 400-700nm) in the field was $824 \mu\text{mol m}^{-2} \text{s}^{-1}$ and average total plant detectable light (TPDL - λ 325-750nm) was $1049 \mu\text{mol m}^{-2} \text{s}^{-1}$. In the glasshouse, average total PAR was $434 \mu\text{mol m}^{-2} \text{s}^{-1}$ and average TPDL was $544 \mu\text{mol m}^{-2} \text{s}^{-1}$.

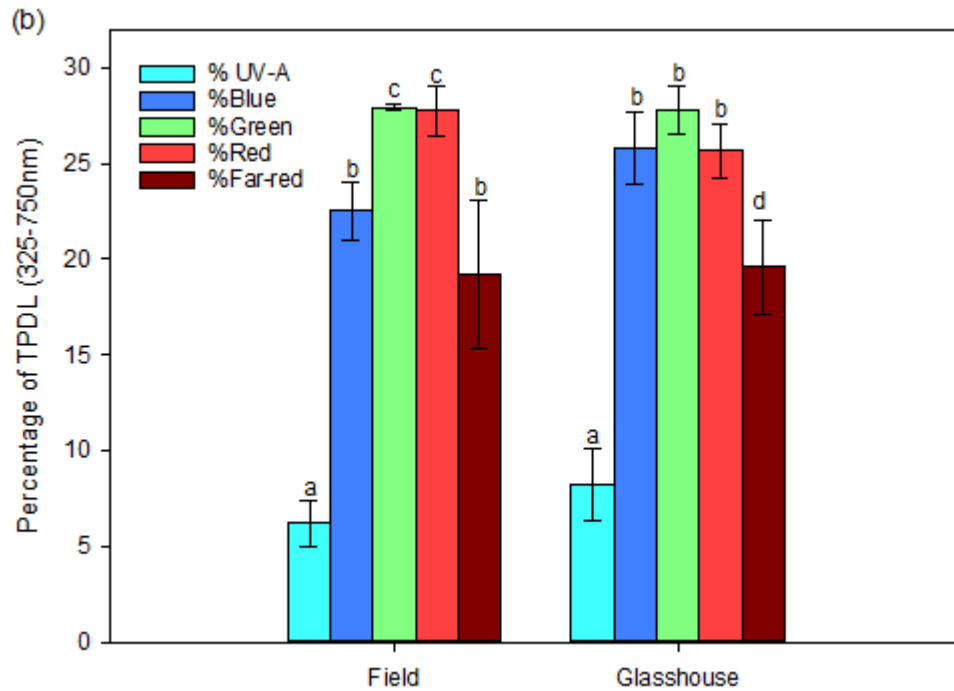


Figure 3.1b The spectra in Fig 3.1a broken down into the five wavebands as a percentage of TPD L: UV-A = λ 325-399nm; Blue = λ 400-499nm; Green = λ 500-599nm; Red = λ 600-699nm; Far-red = λ 700-750nm. N=5. Error bars represent standard deviation from the mean. Letters indicate significant differences within field and glasshouse ($P<0.05$).

Table 3.1: Light ratios in full sunlight in the field and glasshouse. UV-A = λ 325-399nm; Blue = λ 400-499nm; Green = λ 500-599nm; Red = λ 600-699nm; Far-red = λ 700-750nm. Measurements were taken on a clear, bright summer day with the FieldSpec HH 2 spectroradiometer (ASD). Readings averaged from 5 measurements taken between 11am and 1pm. Standard error of means (SEM) are also presented.

	Field	SEM	Glasshouse	SEM
R:FR	1.60	0.2	1.33	0.09
R:G	0.99	0.03	0.91	0.02
B:G	0.81	0.06	0.92	0.03
R:B	0.99	0.03	0.91	0.02
UV-A/B:G	1.03	0.05	1.21	0.06
R:UV-A/B	0.97	0.06	0.75	0.06

3.1.2 Changes in level of plant detectable light and spectra within crop canopies

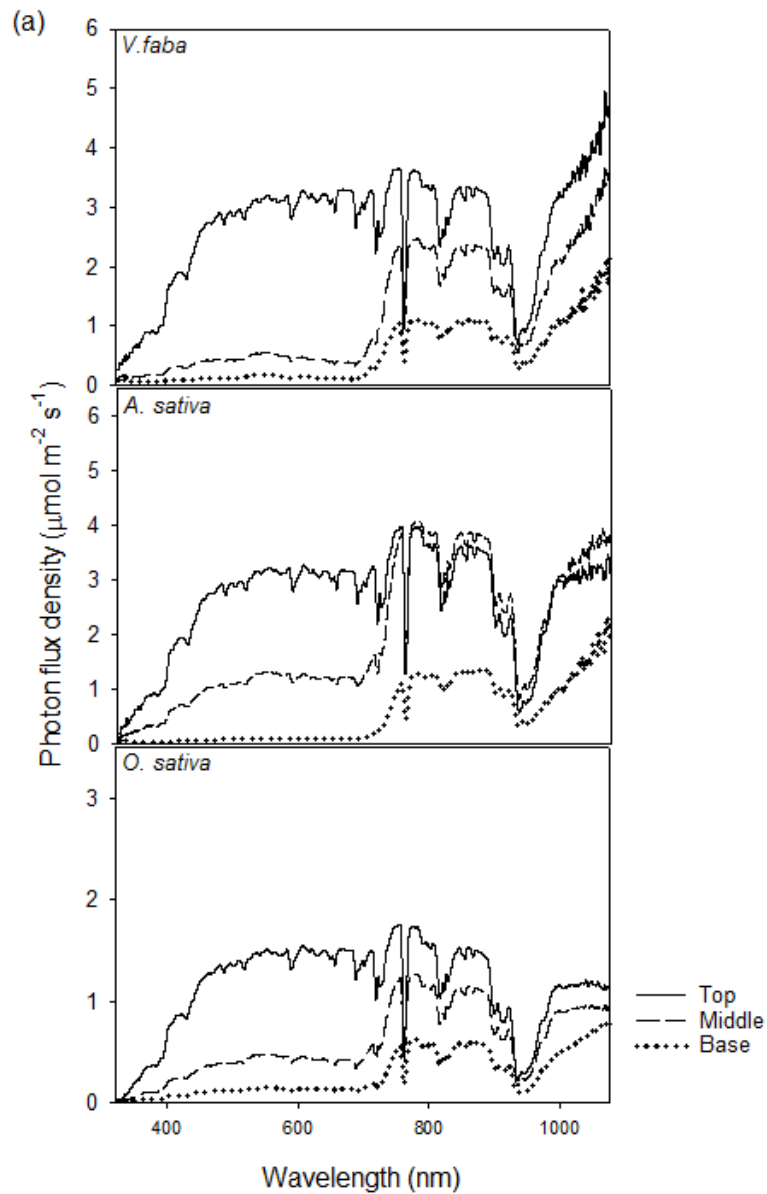


Figure 3.2: (a) Light spectra at three canopy levels in mature canopies of *V. faba*, *A. sativa* and *O. sativa* as measured with the Fieldspec HH 2 spectroradiometer (ASD). 'Top', 'middle' and 'base' refer to positions in the canopy profile 10cm from the tallest point, at the middle point and 10cm from the ground respectively. $N=15$ for *V. faba* and *A. sativa* and $n=10$ for *O. sativa*. UV-A = 325-399nm; Blue = λ 400-499nm; Green = λ 500-599nm; Red = λ 600-699nm; Far-red = λ 700-750nm.

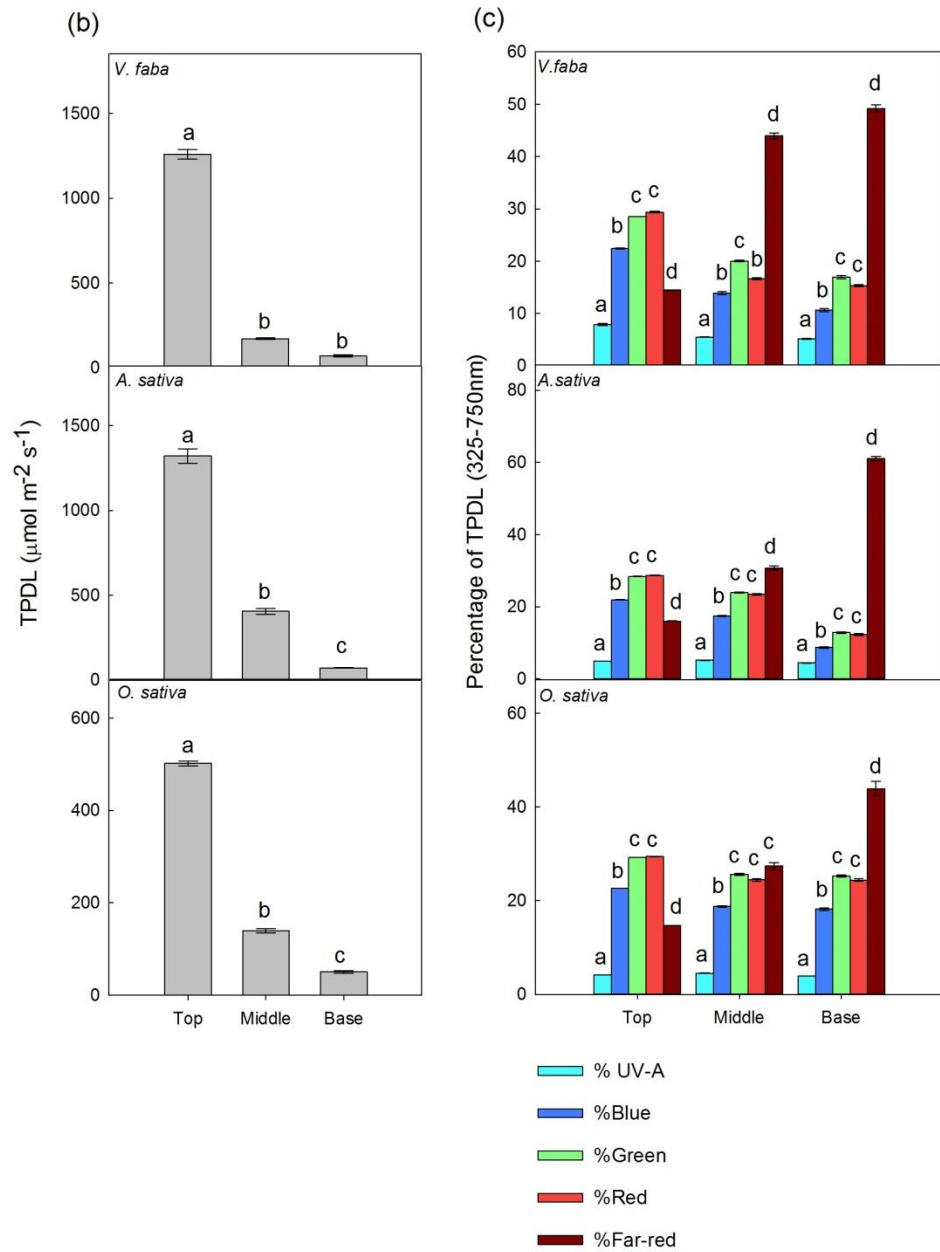


Figure 3.2: (b) The level of total plant detectable light (TPDL), defined as wavelengths of 325-750nm and (c) the percentage of light made up of each waveband (left) at three canopy levels in mature canopies of *V. faba*, *A. sativa* and *O. sativa* as measured with the Fieldspec HH 2 spectroradiometer (ASD). Bars show standard error of means. N=15 for *V. faba* and *A. sativa* and n=10 for *O. sativa*. Letters indicate significant differences within (percentage of TPDL) or between (total TPDL) canopy levels ($P < 0.05$).

The level of TPDL fell significantly from top to base of the canopy profile in all species ($P < 0.01$), however TPDL was more severely depleted from top to middle in *V.faba* and there was no significant difference between middle and base (Fig 3.2b). When comparing wavebands between canopy levels, proportions of TPDL made up by all wavebands except far-red fell significantly from top to base in all species (Fig 3.2c). Far-red light was the only waveband which increased as a proportion of TPDL and significantly increased from top to middle and middle to base in all species ($P < 0.001$). Blue, green and red wavebands as a proportion of TPDL in *V. faba* and *A. sativa* all fell significantly from middle to base, but UV-A light did not decrease further (Fig 2.3c). There were no significant differences in proportions of TPDL made up of UV-A, blue, green or red wavebands between the middle and base of the canopy in *O. sativa*.

When comparing proportions of TPDL made up of each waveband within canopy levels (Fig 3.2c), in *V. faba* there were significant differences between all wavebands ($P < 0.001$) except green and red, which made up a statistically similar amount. Photons of UV-A light made up the smallest proportion of TPDL, contributing 5.15% (SEM 0.25), followed by far-red (14.48% - SEM 0.16), blue (22.43 – SEM 0.39), green (28.54% - SEM 0.09) and finally red wavelength photons (29.39% - SEM 0.05). Half way down the canopy profile, the proportion TPDL made up by red (16.64% - SEM 0.65) and blue (13.92% – SEM 1.06) light became statistically similar, whilst the proportion made up by green light (20.01% – SEM 0.62) became significantly higher than that made up of red

($P=0.045$). The proportion of far-red light rose to 43.96% (SEM 2.2), an over three-fold increase when compared to that at the top of the canopy. At the base of the canopy the proportion of TPD made up by blue light (12.82% - SEM 0.93) became significantly smaller than both green ($P=0.001$) and red ($P=0.009$) light, which once again made up a statistically similar portion of TPD accounting for 19.32% (SEM 0.74) and 17.02% (SEM 0.42).

At the top of the *A. sativa* canopy, again there were significant differences between amount of TPD made up of all waveband photons ($P<0.001$) except green and red. UV-A light again made up the smallest portion of TPD accounting for just 5.94% (SEM 0.13) followed by far-red (16.04% – SEM 0.25), blue (21.94% – SEM 0.21), green (28.4% - SEM 0.06) and red (28.68% - SEM 0.32) photons. In the middle of the canopy profile, the proportion of TPD made up of red (23.45% - SEM 0.8) and green (23.95% - SEM 0.7) light this time remained statistically similar, and the proportion of blue light (17.46% - SEM 0.79) remained significantly lower than that of red ($P=0.001$) and green ($P<0.001$). UV-A light once again made up the smallest portion of the spectrum (4.45% - SEM 0.24) whilst far-red light once again became the largest contributor, accounting for 30.67% (SEM 2.4), an almost two-fold increase when compared to the top of the canopy.

At the top of the canopy of *O. sativa*, once again there were significant differences between amount of TPD made up of all waveband photons ($P<0.001$) except green and red. UV-A light again made up the smallest portion of TPD accounting for 4.12% (SEM 0.21) followed by far-red (14.69% – SEM

0.05), blue (22.64% – SEM 0.05), green (29.25% - SEM 0.02) and red (29.41% - SEM 0.07) photons. In the middle of the canopy profile, far-red light increased to 27.37% (SEM 2.28) of TPD, an almost two-fold increase when compared to the top of the canopy and now making up a statistically similar proportion of TPD to green (25.63% - SEM 0.69) and red (24.36% - SEM 0.91) light. Proportions of the spectrum made up of blue (18.73% -SEM 0.66) and UV-A (3.92% - SEM 0.08) remained significantly lower. At the base of the canopy, green light (25.25% - SEM 0.68) made up a significantly higher proportion of the spectrum than blue (18.17% - SEM 0.79) light ($P=0.032$), but statistically similar to red light (24.43% - SEM 0.93). Red light also made up a larger proportion than blue light, although this was not quite statistically significant ($P=0.056$). UV-A light (4.54% - SEM 0.28) remained the smallest contributor to the spectrum, whilst far-red (43.96% - SEM 4.85) was once again the largest.

The differences in rates of depletion of TPD between canopies are likely due to the differences in canopy structure. The broad and flat leaves of the *V. faba* canopy caused the most rapid depletion of TPD down the vertical canopy profile, with the most significant drop being between the top and middle whilst the two cereals with their thinner, more erect leaf structures had significant decreases in light levels from both top to middle and top to base of the canopy. The light quality likely remained the same between the middle and base of the canopy in *O. sativa* due to its very high leaf angle allowing better transmission of all light through the canopy profile.

3.3.1 Changes in light ratios through the canopy profile

The variation in the level of transmission of different wavelengths through the vertical canopy profile created a number of light gradients as presented in Fig. 3.3.

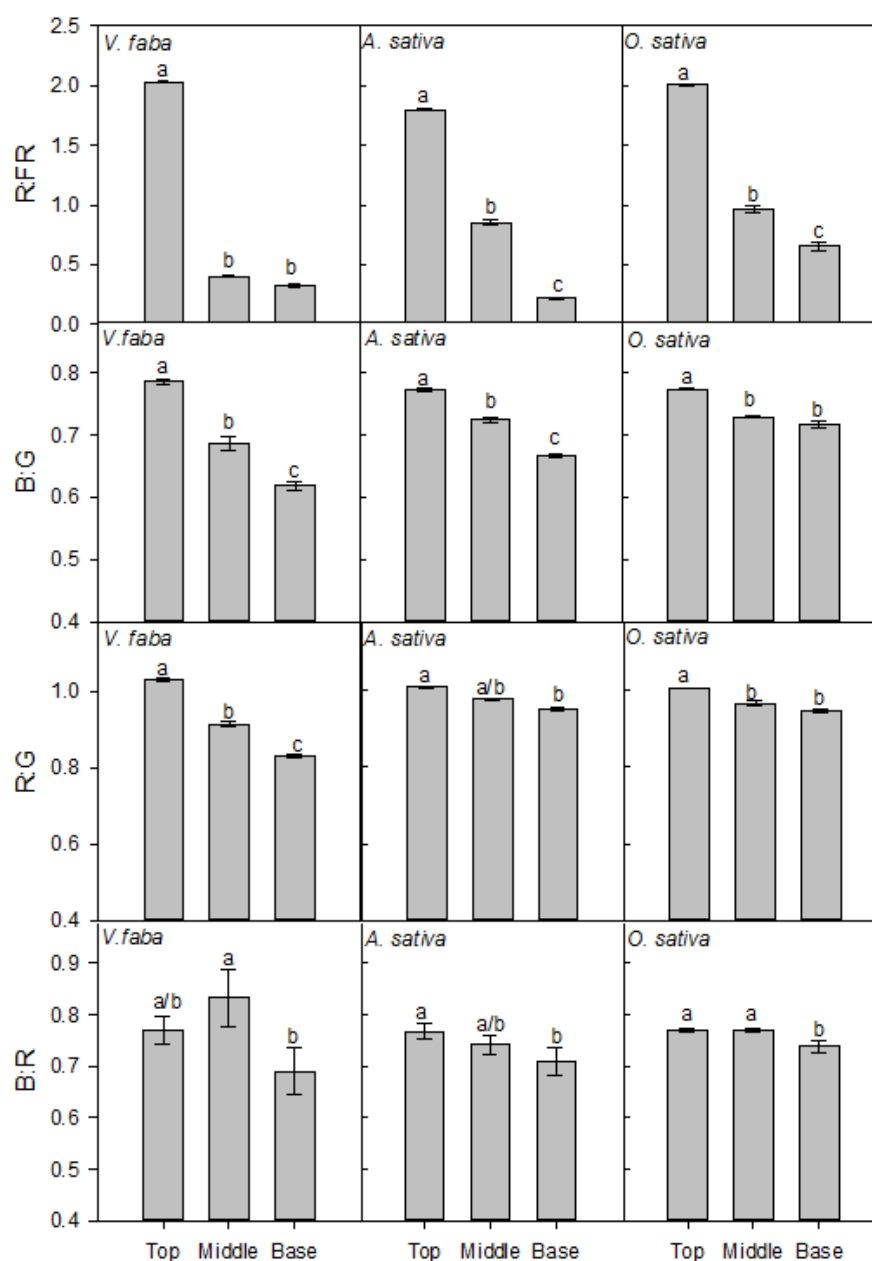


Figure 3.3: Red to far-red (R:FR), blue to green (B:G), red to green (R:G) and blue to red (B:R) light ratios through the canopy profile of mature canopies of *Vicia faba*, *Avena sativa* and *Oryza sativa* as measured with the Fieldspec HH 2 spectroradiometer (ASD). 'Top', 'middle' and 'base' refer to positions in the canopy profile 10cm from the tallest point, at the middle point and 10cm from the ground respectively. Bars show standard error of means and letters represent significant differences ($P < 0.05$). $N = 15$ for *V. faba* and *A. sativa* and $n = 10$ for *O. sativa*. Blue = $\lambda 400-499\text{nm}$; Green = $\lambda 500-599\text{nm}$; Red = $\lambda 600-699\text{nm}$; Far-red = $\lambda 700-750\text{nm}$.

As shown in Fig. 3.3, all ratios except B:R in *V. faba* declined significantly from top to base of the canopy profile ($P < 0.05$), with the sharpest decline in R:FR ratio which decreased by as much as 80% from top to middle and 84% from top to base. R:FR declined significantly from top to middle in *V. faba* ($P < 0.001$), *A. sativa* ($P < 0.001$) and *O. sativa* ($P < 0.001$) and declined from middle to base in *A. sativa* ($P < 0.001$) and *O. sativa* ($P = 0.017$). B:G ratio had a more shallow decline, decreasing by as much as 22% from top to base and middle to base. B:G ratio significantly decreased from top to base in *V. faba* ($P = 0.001$), *A. sativa* ($P < 0.001$) and *O. sativa* ($P = 0.002$) and declined from middle to base in *V. faba* ($P = 0.045$) and *A. sativa* ($P = 0.002$). R:G ratio declined as much as 19% from top to base and 9% from middle to base. R:G ratio significantly decreased from top to base in *V. faba* ($P < 0.001$), *A. sativa* ($P = 0.006$) and *O. sativa* ($P = 0.002$) and declined from middle to base in *V. faba* ($P = 0.001$) and *O. sativa* ($P = 0.011$). B:R ratio declined as little as 4% from top to base of the canopy, being only just statistically significant in *A. sativa* ($P = 0.049$) and *O. sativa* ($P = 0.034$). As shown in Fig. 3.4, Increasing B:G, R:FR and R:G light ratios all correlated with increasing PAR levels, however the B:R light ratio little correlation. When plotting B:R light ratio over TPDL rather than canopy level (Fig 3.4), there is no strong relationship between decreasing light level and a decrease in B:R ratio, suggesting that blue and red photons are depleted in more similar proportions down the canopy profile than through the vertical profile of a leaf, as described in chapter 1.8, where blue photons are depleted nearer the top of the profile than red photons (Sun *et al.*, 1998).

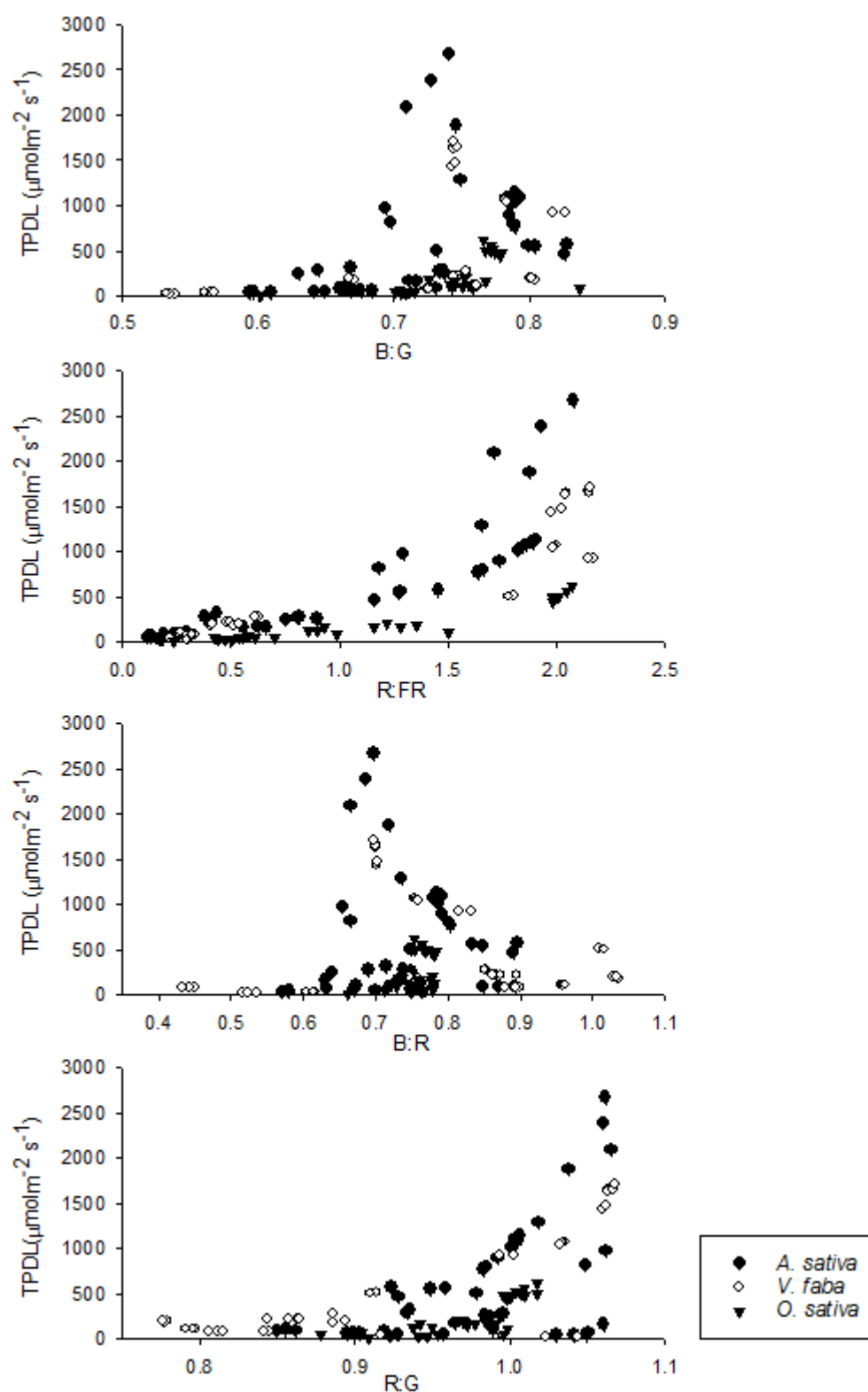


Figure 3.4: Red to far-red (R:FR), blue to green (B:G), red to green (R:G) and blue to red (B:R) light ratios plotted against total plant detectable light (TPDL), defined as wavelengths between 325 and 750nm, measured through the canopy profile of mature canopies of *Vicia faba*, *Avena sativa* and *Oryza sativa* as measured with the Fieldspec HH pro spectroradiometer (ASD). Blue = $\lambda 400-499\text{nm}$; Green = $\lambda 500-599\text{nm}$; Red = $\lambda 600-699\text{nm}$; Far-red = $\lambda 700-750\text{nm}$. N=15 for *V. faba* and *A. sativa* and n=10 for *O. sativa*.

3.3.2 Changes in PAR and leaf gas exchange

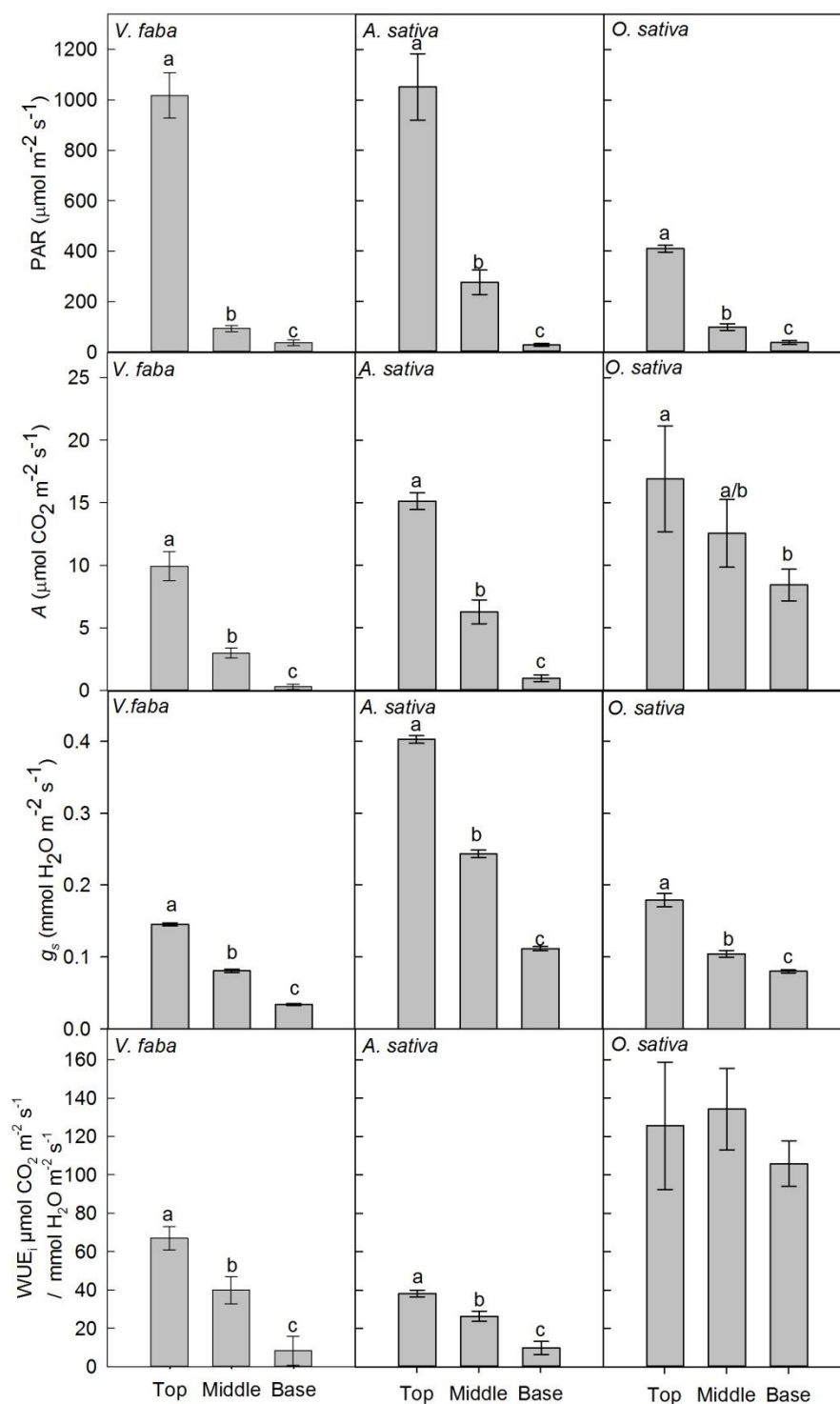


Figure 3.5: Levels of photosynthetically active radiation (PAR) defined as 400-700nm as measured with the Fieldspec HH 2 spectroradiometer (ASD) and photosynthesis (A), stomatal conductance (g_s) and intrinsic water use efficiency (WUE_i) as measured with the LI-6400XT (LI-COR, Nebraska) at three levels in the profiles of mature canopies of *V. faba*, *A. sativa* (in the field) and *O. sativa* (in the glasshouse). Top', 'middle' and 'base' refer to positions in the canopy profile 10cm from the tallest point, at the middle point and 10cm from the ground respectively. Bars show standard error of means and letters represent significant differences ($P < 0.05$). $N=15$ for *V. faba* and *A. sativa* and $n=10$ for *O. sativa*.

As hypothesized photosynthetically active radiation (Fig. 3.5) decreased significantly from the top to the base of the vertical canopy profile. As expected, photosynthesis and stomatal conductance also significantly declined with increasing canopy depth. PAR level decreased significantly from top to middle and middle to base of all three canopies (*V. faba* - $P<0.001$ and $P=0.045$; *A. sativa* – $P<0.001$, $P=0.036$; *O. sativa* – $P<0.001$, $P=0.001$). Photosynthesis decreased significantly from top to middle and middle to base of *V. faba* ($P<0.001$, $P=0.024$) and *A. sativa* ($P<0.001$, $P<0.001$) but only top to base in *O. sativa* ($P=0.003$). Stomatal conductance decreased significantly from top to middle and middle to base of all three canopies (*V. faba* - $P<0.001$ and $P<0.001$; *A. sativa* – $P<0.001$, $P<0.001$; *O. sativa* – $P=0.001$, $P=0.028$). Intrinsic water use efficiency declined significantly from top to middle and middle and middle to base in *V. faba* ($P<0.001$, $P=0.003$) and *A. sativa* ($P=0.003$, $P<0.001$) but there were no differences between canopy levels in *O. sativa*, in which WUE_i remained consistently high in comparison to the other species.

3.3.3 Determining cause of changes in stomatal conductance

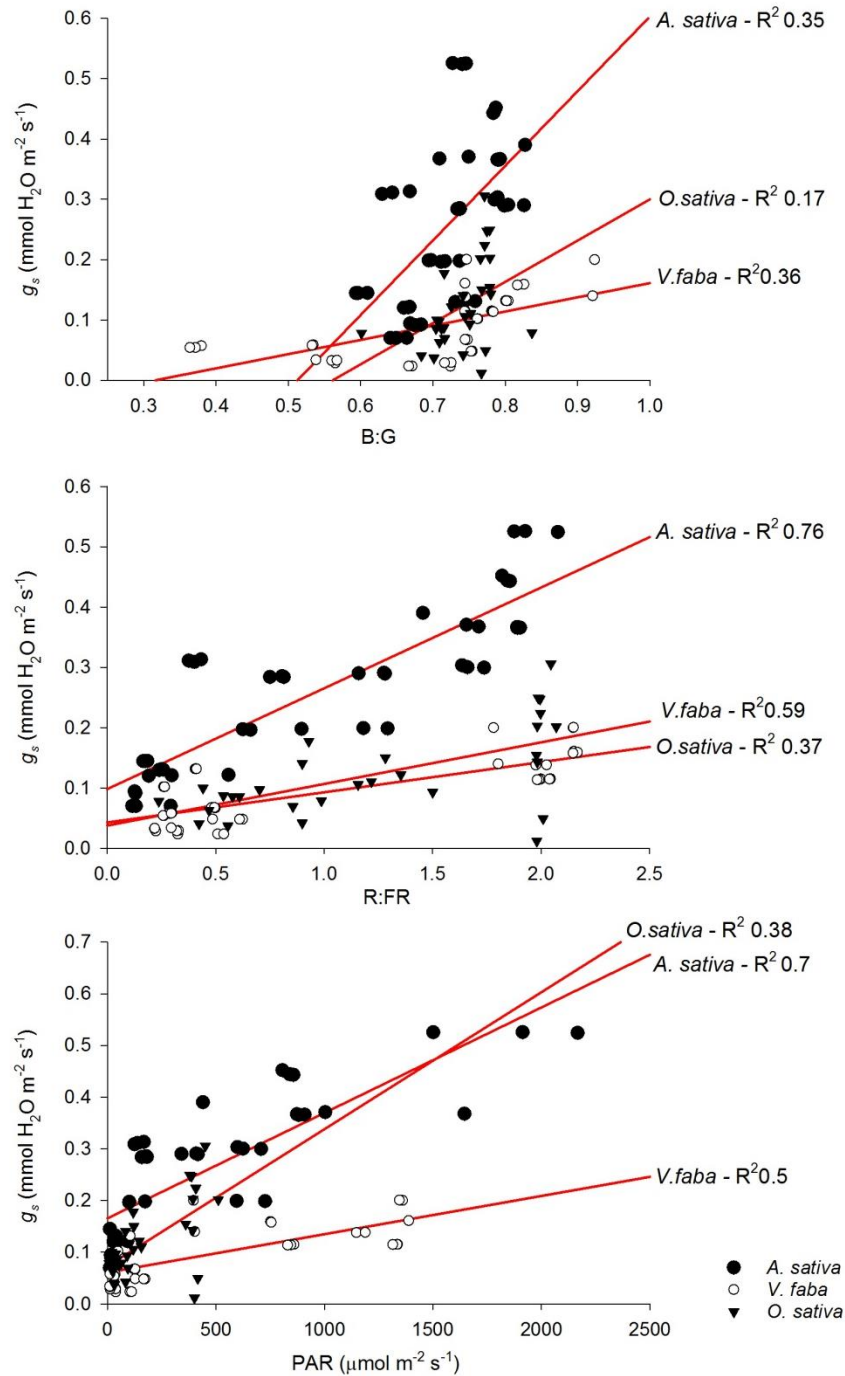


Figure 3.6: Stomatal conductance (g_s) as measured with the LI-6400XT (ASD) plotted against blue to green (B:G) light ratio red to far-red (R:FR) light ratio and overall level of photosynthetically active radiation (PAR) as measured with the Fieldspec HH 2 spectroradiometer (ASD) in mature canopies of *V.faba*, *A.sativa* and *O. sativa*. Lines of regression (R^2) are shown for each. Blue = λ 400-499nm; Green = λ 500-599nm; Red = λ 600-699nm; Far-red = λ 700-750nm. N=15 for *V. faba* and *A. sativa* and n=10 for *O. sativa*.

In Fig.3.6 stomatal conductance (g_s) was plotted against the B:G and R:FR light ratios as well as overall PAR. Trends were shown towards increased g_s with increases in all three. In *V. faba* and *A. sativa*, g_s correlated most strongly with R:FR, followed by PAR and then B:G. In *O. sativa*, g_s correlated most strongly with PAR, then R:FR and then B:G.

3.4 Discussion

As discussed in section 3.1, changing light ratios through the canopy profile provide positional signals to the plant via the photoreceptors which are crucial for the optimisation of growth and development under differing light conditions. Plants must be able to sense whether they are in high or low light intensity conditions in order to best acclimate their physiology and most efficiently capture and utilise available PAR. The significant reduction in R:FR, B:G and R:G light ratios (Fig. 3.3) indicate that, as hypothesised, TPDL decreased and the proportion of TPDL made up of both far-red and green light increased with depth into the vertical canopy profile of all three crops. As TPDL decreased, the increasing proportions of far-red and green light in relation to decreasing proportions of red and blue light – i.e. the resulting falling R:FR and B:G ratios – were of particular interest as they have both separately been associated with the induction of shade avoidance/ tolerance adaptations in leaf and whole plant physiology (see chapter 1.20; Franklin and Whitelam, 2005; Zhang *et al.*, 2011).

As discussed in chapter 1.12 and 1.20 plants use the increased proportion of far-red light to sense shade via the R:FR ratio via the phytochrome (PHY) photoreceptors. Blue and green light have a similar interaction via the cryptochrome (CRY) photoreceptors (see chapter 1.11 and 1.21) meaning that green light additively causes the reversal of blue light responses associated with high light intensity (Sellaro *et al.*, 2010), such as the increase in stomatal aperture mentioned in section 3.1.

TPDL in response to declining canopy depth fell most rapidly in *V. faba* (Fig. 3.2b), most likely due to the canopy structure of broad leaves and horizontal angles absorbing and/or reflecting most light in the upper layers. This was coupled with the sharpest decline in R:FR ratio from the top to the middle of the canopy (Fig. 3.3). Indeed, in agreement with Vandebussche *et al.*, (2005), the R:FR ratio had the steepest decline in all three canopies in comparison to B:G, B:R and R:G, (Fig.3.3). However, *A. sativa* and *O. sativa*, having a more upright canopy structure with thinner leaves, had a greater transmission of TPDL to the centre of the canopy than *V. faba* (Fig. 3.2b) and this was reflected in a more slower decline in R:FR light ratio (Fig. 3.3).

When comparing the decline in R:FR and B:G ratios down the canopy profile, all canopies experienced a relatively steady and shallow decline in B:G (Fig. 3.3). This confirmed the hypothesis that falling B:G ratio is present in multiple crop canopies, and has a profile distinct from R:FR profile, which may allow its ratio to act as an extended and sensitive source of positional information for leaves in certain regions of the canopy.

3.4.1 Green light gradient as a positional signal for increased water use efficiency

As discussed in chapter 1.19, it has been shown with epidermal peels and also in whole leaves that the closure of blue light mediated stomatal opening is only completed once the intensity of green light reaches twice that of blue light, i.e. a blue to green (B:G) light ratio of 0.5, and the reversal of the opening response is directly proportional to the change in the B:G light ratio (Frechilla *et al.*, 2000; Talbott *et al.*, 2002). Measurements at the base of the plants (Fig. 3.3) showed

that the B:G here, based on the information above, would reverse a significant amount of blue light induced stomatal opening. Indeed, simultaneous light quality and leaf-gas exchange measurements revealed trends towards increased stomatal conductance with increases in B:G ratio (Figs. 3.5; 3.6). However, in *V. faba* and *A. sativa*, g_s appeared to correlate most strongly with R:FR (Fig. 3.6). It may be that the extreme decreasing R:FR light gradient and fall in red light and overall PAR level could have masked the more subtle effects of the shallow B:G light gradient, for reasons discussed below.

As described at length in chapter 1.19, there are two distinct reactions of the stomata to light – the red light response and the blue light response (Lawson, 2009; Chen et al., 2012; Busch, 2014). The intensity of red light required for stomatal opening is far greater than that required for blue light mediated stomatal opening (Sharkey and Raschke, 1981; Gorton et al., 1993). The stomata are highly sensitive to blue light, responding to intensities of $\leq 5 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and requiring up to ten times less blue light than red light to achieve maximum aperture (Sharkey and Raschke, 1981).

At the base of the canopy, PAR and both red and blue light are severely depleted (Fig. 3.2b). However, it is common for leaves within plant canopies to exist in deep shade and still contribute to net plant photosynthesis close to the light compensation point (LCP) (Murchie and Horton 2008, Burgess et al 2015). Here, leaf conductance will not be limiting to photosynthesis but stomatal aperture should be kept low to avoid excessive water loss. Leaves at the base of canopies of *V. faba*, *A. sativa* and *O. sativa* were on average exposed to 14,

7 and 8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of blue wavelength photons respectively – slightly above the minimal amount of blue light required to induce stomatal opening. Here PAR, although severely depleted, was above the amount required to drive photosynthesis. The small amount of blue light remaining at the base of the canopy would be enough to keep the stomata open to some degree and allow gas exchange to take place.

As compared to the R:FR light ratio, the B:G light ratio remains relative stable until lower levels of PAR are observed deeper within the canopy profile (Figs. 3.3; 3.5). Only at the deepest canopy levels, where PAR begins to approach the LCP would the B:G ratio begin to induce the final portion of stomatal closure. Also, unlike to red light, stomata respond rapidly to short (<30 seconds) pulses of blue light (Zeiger and Shimazaki, 1987). Hence, in the event of canopy movement suddenly exposing the lower layers of the canopy to a higher level of PAR, stomata would be able to quickly respond to the increase in B:G light ratio in order to maximise photosynthesis under the new light conditions. Therefore the novel hypothesis is here proposed that the signal provided by the B:G ratio at the base of dense canopies, distinct from the R:FR ratio, provides fine-tuning of stomatal closure response, improving canopy water-use efficiency at low light levels.

As the B:G light ratio appears to have the strongest effect at the base of the canopy to further establish its role in the adjustment of stomatal conductance for increased water use efficiency will require further measurements at more levels in the lower part of the canopy profile. In order to further define the role

of the B:G light gradient on stomatal aperture through the vertical canopy profile it may also be beneficial to apply specific wavelengths of light to plants in combination with gas exchange measurements in a controlled light environment, where changes in R:FR ratio may be removed and PAR level at the top of the canopy kept constant.

3.4.2 Far-red light primes leaves to absorb green light for better energy distribution throughout the canopy

Penetration of green light through to the lower layers of leaves appears to work in much the same way as the detour effect within the leaf (see chapter 1.8; Vogelmann, 2008), with light being reflected and scattered between leaves rather than plant cells. As discussed above, the significant reduction in B:G and R:G light ratios (Fig. 3.3) indicate that, like far-red light, green light is also becoming more relatively abundant deeper within the canopy profile. These combined falling R:FR, B:G and R:G light ratios are particularly interesting because they appear to work together to improve both energy distribution and acclimation of photosynthesis through the canopy profile. Due to the process of photoacclimation of the photosystem stoichiometry to shade (see chapter 1.18) it is common to find a distinct gradient in the ratio of chlorophyll a:b from the top to the bottom of a plant canopy (e.g. Murchie et al 2002). The enriched far-red light environment in shaded conditions 'over-excites' PSI causing the production of more chlorophyll *b* containing PSII in order to balance the excitation of the photosystems for the most efficient photosynthesis (Walters, 2005). To best accommodate light harvesting at lower light levels, dark respiration rates and light compensation points fall, and the levels of

carotenoids which act as accessory pigments in light harvesting rise. For example, in rice lutein content rises by 13-25 % and neoxanthin by 20 % in shaded conditions (Murchie et al 2002). Similar more modest increases in these two molecules were seen across a range of plant species in FR enriched shade (Murchie and Horton, 1998). As both chlorophyll *b* and the carotenoids readily absorb light in the green portion of the spectrum (Murchie and Horton, 1998; Murchie *et al.*, 2002), it looks like far-red light mediated photoacclimation of the photosynthetic stoichiometry therefore enables the plant to take advantage of the increased proportion of green light available for photosynthesis in shaded conditions. Arguably, leaf composition and structure has evolved to both enable green light to reach leaves at the bottom of dense lateral canopies or the inner layers of plants with a folded leaf structure (such as lettuce) to provide photons that can be used to drive photosynthesis (McCree, 1972; Hogewoning *et al.*, 2010) as well as provide positional signals via the non-photosynthetic photoreceptors.

3.4.3 Mimicking light quality in natural light environments to create ideal morphologies

Growers may also choose ratios found in natural light environments known to induce certain morphologies or developmental events. For example, the ideal morphology for plants for the cut flower industry or herbs such as coriander is upright (no lodging). This morphology could be encouraged by providing them with a spectrum similar to that found at the top of a canopy. Only plants at the very top of the canopy in either the field or glasshouse are exposed to the full sunlight intensity and spectrum that reaches the crop. Leaves/ plants exposed

to the light intensities but also spectral qualities, i.e. light ratios, found at this level will therefore develop high light acclimated characteristics. For example, the high R:FR ratio of approximately 2:1 above (Fig. 3.1b) and at the top (Fig. 3.2b) of the canopy both in the field and glasshouse would cause phytochrome to mainly be in the Pfr form (see chapter 1.12) and therefore none of the shade avoidance symptoms discussed in chapter 1.20 would occur. This combined with the high level of blue light (see chapter 1.21) at the top of the canopy would ensure that rapid stem elongation does not occur, and the crops remain short and upright. In contrast, if a grower requires a crop with increased leaf surface area, or to provide flowers or fruit at a certain time, they may apply ratios of wavelengths found in shaded conditions, with increased levels of green/ far-red light to induce these traits (see chapter 1.20).

3.5 Conclusions

Growers can use the natural sunlight light ratios found in the field and glasshouse to make informed choices about which wavelengths and in what quantities/ ratios to use in light recipes for their crop. The above results combined with the information about how plants sense and use light in chapter 1 provide a strong case for the inclusion of blue, green red and far-red wavebands in any light recipe where the artificial light source will be the only source of light, especially if the crop forms a layered or canopy structure. Blue and red light are crucial for healthy development and for driving photosynthesis and promoting growth, but green light not only reaches deeper into the leaf but also into the canopy profile to drive photosynthesis where the other wavelengths are more severely depleted. The increased proportion of

far-red light through the canopy profile provides strong positional signals and better enables the absorption of this increased proportion of green light through the photoacclimation of the photosynthetic stoichiometry. In addition, the B:G light ratio could provide a relatively stable information- rich signal at deeper canopy positions which may have a positive effect on whole canopy water-use efficiency, although further work is required to fully establish its role. Removal of any of these wavebands could be detrimental to plant growth and development in the canopy environment as they interact to all have a role in photosynthesis and positional signalling.

4 A comparison of growth, development and physiology of *Coriandrum sativum* grown under different combinations of blue, green, red and far-red light in controlled environment growth rooms

4.1 Introduction:

As described in chapter 1, due to the highly plastic nature of the plant developmental program and photomorphogenesis, plants are able to acclimate their physiology to the light environment throughout growth in order to optimize capture and efficiency of use of resources. Use of controlled environment growth rooms enables precise control of all environmental variables which may affect plant physiology, such as light, temperature, water and nutrient availability. LED lamps can be used to apply specific combinations of wavelengths known as 'light recipes' (for more detail refer to chapter 1) to plants which allows researchers to characterise the effects of individual or combinations of wavelengths on plant growth and development. The knowledge gained can be used to inform growers of the best combination of wavelengths, or light recipe, for plant growth to achieve commercial success.

This study forms part of a project that was tasked with both designing a lamp for use in horticultural lighting and finding the ideal light recipe for plant growth. When designing lamps used for increasing yield and quality of horticultural crops, often referred to as 'grow lamps', LED manufacturers currently focus on including LEDs which provide different proportions of blue and red wavelengths sometimes supplemented with FR, with green light almost entirely discounted. This is likely due to two main reasons. First, green

light is often dismissed as having little effect on plant growth and development due to the focus on targeting the chlorophylls to drive photosynthesis. As shown in chapter 1 (Fig. 1.5), chlorophyll *in vitro* absorbs mainly in the blue and red portions of the spectrum, with a notable gap in the green portion.

However as discussed at length in chapters 1 and 3, the complex leaf structure, additional pigments and non-photosynthetic photoreceptors mean that green light has a whole host of under-appreciated effects on plant growth and physiology. The second reason is a problem known as the 'green gap' (Krames *et al.*, 2007; Pleasants, 2013). Up until recently, LEDs in the 'green' portion of the spectrum able to produce irradiance with the required efficiency for horticultural lighting were limited to the 520-540nm waveband and efficiency of higher wavelength green light, specifically up to 570nm was inadequate. However recent advances in technology have started to resolve this issue (Jiang *et al.*, 2015; Alhassan *et al.*, 2016). For example, LED manufacturer Lumiled now produce broader band 'mint' and 'lime' LEDs (LUMILEDS, 2016) which cover the entire 'green' portion of the spectrum. In addition, the development of new techniques such as multi-quantum well (MQW) which have enabled a 2.4 fold enhancement of emission efficiency over old green LEDs at 540nm, with the enhancement becoming even larger at the longer wavelengths (Jiang *et al.*, 2015) mean that LED emission in the green could soon be as efficient as emission in other parts of the spectrum. Now efficient green wavelength LEDs are becoming available to growers it is more commercially viable to use green light to enhance the standard red, blue, far-red lighting currently used. It was therefore concluded that green light should be included in any future lamp

design. Programmable 'smart lamps' with adjustable photoperiod, blue, green, red and far-red wavelength and intensity outputs were designed and built by the University of Nottingham Engineering department for use in the experiments (see chapter 2.2.1).

Coriandrum sativum (coriander) was chosen for this study for multiple reasons. Coriander is an herb crop cultivated practically worldwide for its flavoursome stems, leaves and seeds. The stem and leaf crop alone is often referred to as 'cilantro'. Global annual production is estimated at 300,000 – 335,000 mega tonnes (MT) and trade up to 100,000 MT with the main producer and exporter being India (FAO, 2015). Commonly, the dried seeds are used as a spice and the leaves and stems as a leafy green vegetable component of curries and other flavoured dishes. The leaves and stems can also be dried and ground for use in cookery. The essential and fatty oils contained in coriander seeds are also of interest medicinally and are used in industry (Diederichsen, 1996). The fresh leaf and stem crop is popular in the UK and is commonly cultivated and sold in small pots for sale in major supermarkets (Fig. 4.1).

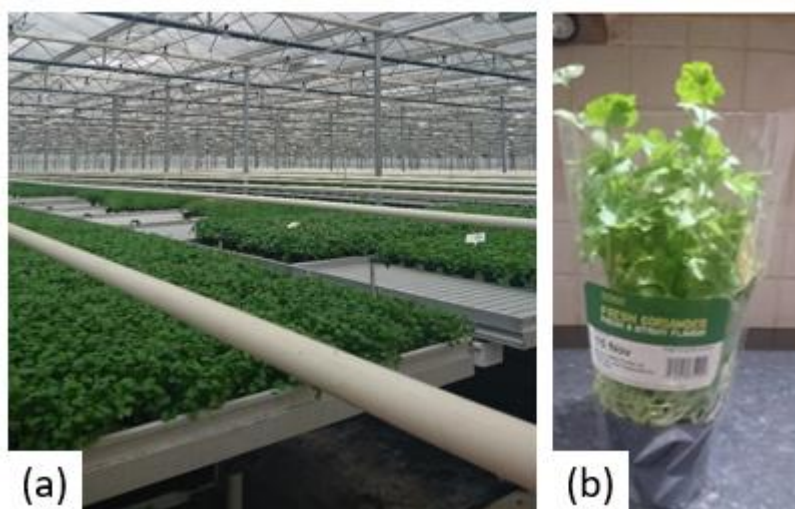


Figure 4.1: (a) Commercial glasshouse potted coriander growth, Langmead Herbs, UK. (b) Potted coriander as sold in TESCO supermarkets, UK.

The basic development of coriander is as follows:

- Seeds germinate epigeously within, depending on environmental conditions, around 7-11 days, typically producing 2 seedlings per seed case with distinctive long, thin cotyledons (Fig. 4.2a).
- The meristem then grows upward and produces the first true leaf, which is laminar, pinnatifid (or lobed) with three sections joined at the midrib, and has jagged edges (Fig. 4.2b). Coriander is glabrous and the stems and leaves are light to dark green with a slightly waxy appearance.
- This first stem continues to elongate and may produce another leaf, but a lateral branch from the base of the stem then takes over the main growth and begins to produce leaves which are formed in a rosette structure. As the stem elongates the leaf internode length increases and these leaves expand to full size.

- Eventually, especially if sown multiple seeds per pot, the stems tangle and the plants form a canopy structure.

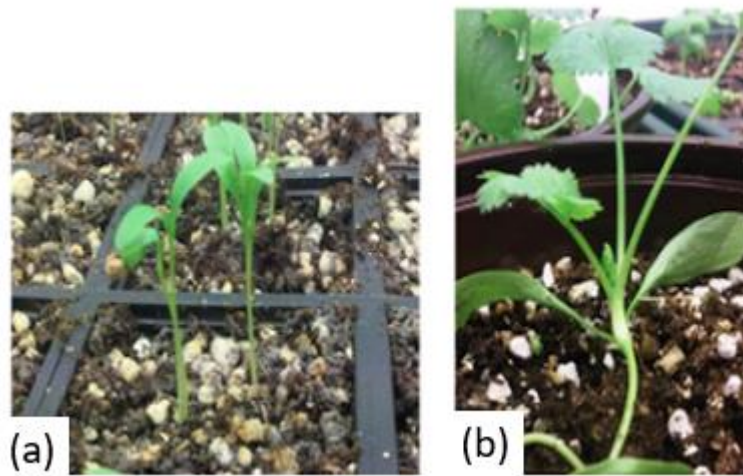


Figure 4.2: Coriander seedlings 10 days (a) and 20 days (b) after sowing in the glasshouse. Each seed case produces 2 seedlings which have a pair of cotyledons. The next leaf is the first ‘true leaf’ with the distinctive lobed coriander shape.

Depending on environmental conditions, the crop is typically ready for market 5-7 weeks from sowing at 15-20cm tall (Fig 4.1b). The popularity, compact size, rapid growth and canopy forming structure of coriander make it an ideal candidate for research into improvement of growth and development under different light recipes. Once combinations of wavelengths which produce desirable traits have been identified, growers may use them to produce crops of a higher quality and/or yield.

4.2 Aims and hypotheses

To investigate the effects of individual/ combinations of wavebands of light on the growth, development and physiology of coriander with a view to:

- a) Identifying the wavelengths which must be provided to enable specific aspects of plant growth and development such as germination, leaf expansion, stomatal development and stem and root elongation and branching.
- b) Discovering light recipes which not only increase fresh-weight yield but also specific aspects of plant quality for commercial purposes – i.e. short stature and high leaf area to prevent lodging and produce a high leaf to stem ratio.

Hypotheses:

- Light recipes containing specific combinations of wavebands rather than single specific wavebands will produce the highest yielding plants because they will stimulate specific known photoreceptor-associated processes that are crucial to healthy plant growth and development
- Photosynthetic capacity per unit leaf area can be influenced by light quality alone. Stomata, leaf pigments/photosystems, enzymes such as rubisco and components of the electron transport chain will all be affected by light quality.

- The addition of blue light to light recipes will result in more high-light acclimation traits whilst the addition of green and far-red light will result in more low-light acclimation traits.
- Addition of far-red light will cause the same, although more extreme shade effects than green light.

4.3 Methodology

Coriander (*Coriandrum sativum*) was grown under light recipes of set light intensity but varying light quality in a controlled environment growth room. For full materials and methods please refer to chapter 2.2.

4.4 Results

4.4.1 Germination assays

The RB light recipe resulted in a significantly lower germination success than the R, RBG and RBFR light recipes ($P < 0.05$). There were no other significant differences in germination success or time to first germination (Fig 4.3).

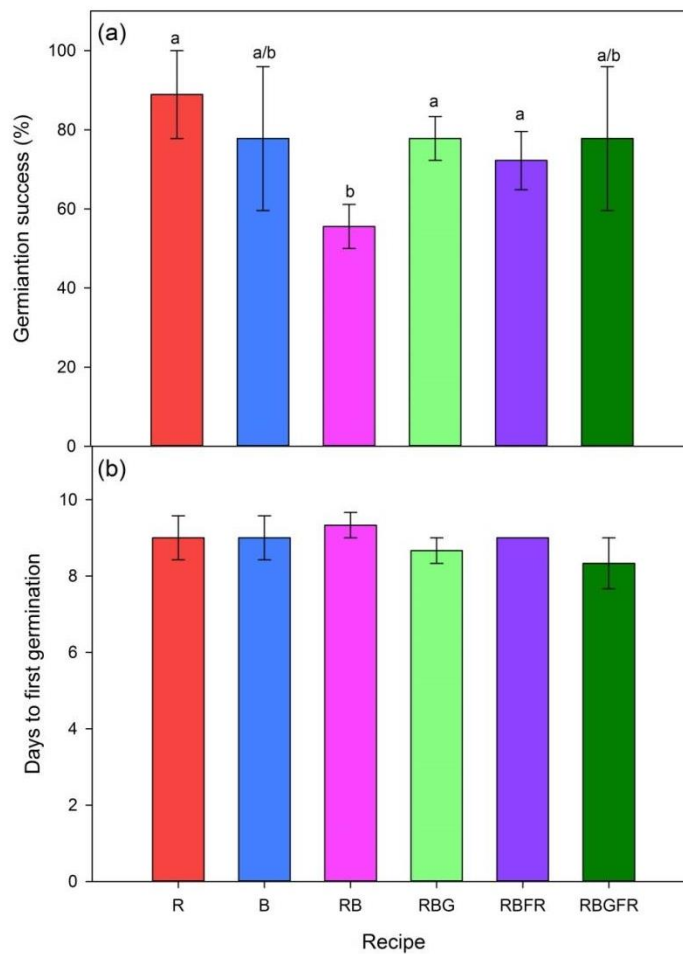
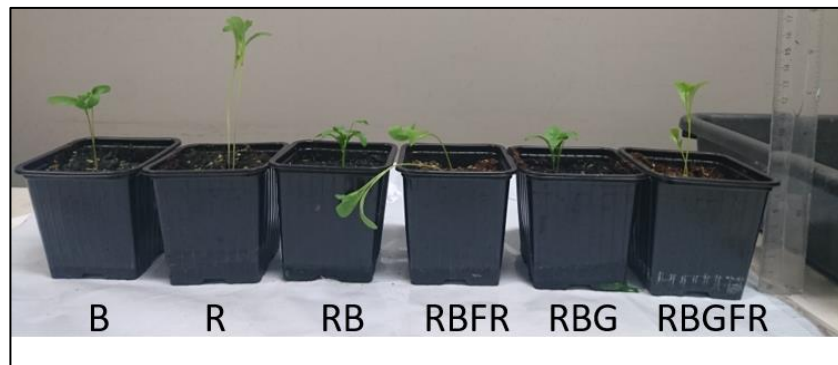


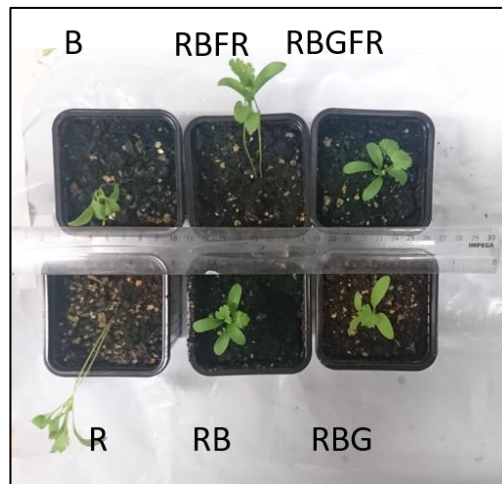
Figure 4.3: For each light recipe (see chapter 2.1.1), (a) germination success as calculated as the percentage of a possible 12 seedlings of cv. Standard to emerge from the soil within 15 days and (b) the average number of days taken to the first germination event (i.e. any seedling emerging through the soil surface). Results are a mean of 3 runs of the experiment. Bars show standard error of means.

4.4.2 Growth, development and physiology

(a)



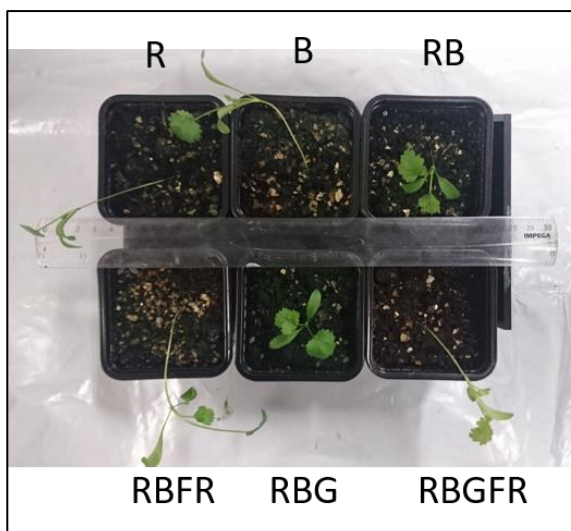
(b)



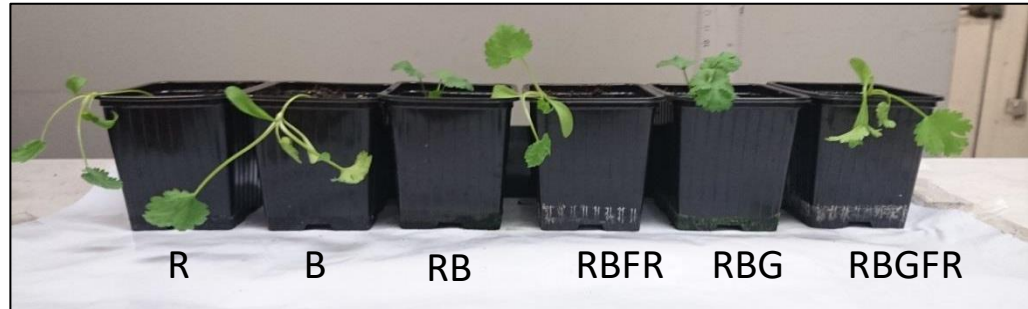
(c)



(d)



(e)



(f)



(g)



Figure 4.4: Photos of example plants (*C. sativum* cv. Standard) grown under each light recipe 7 (a/b), 14 (c/d), 21 (e/f) and 28 (g) days after the first plant germinated. Bars in (g) show scale of 5cm.

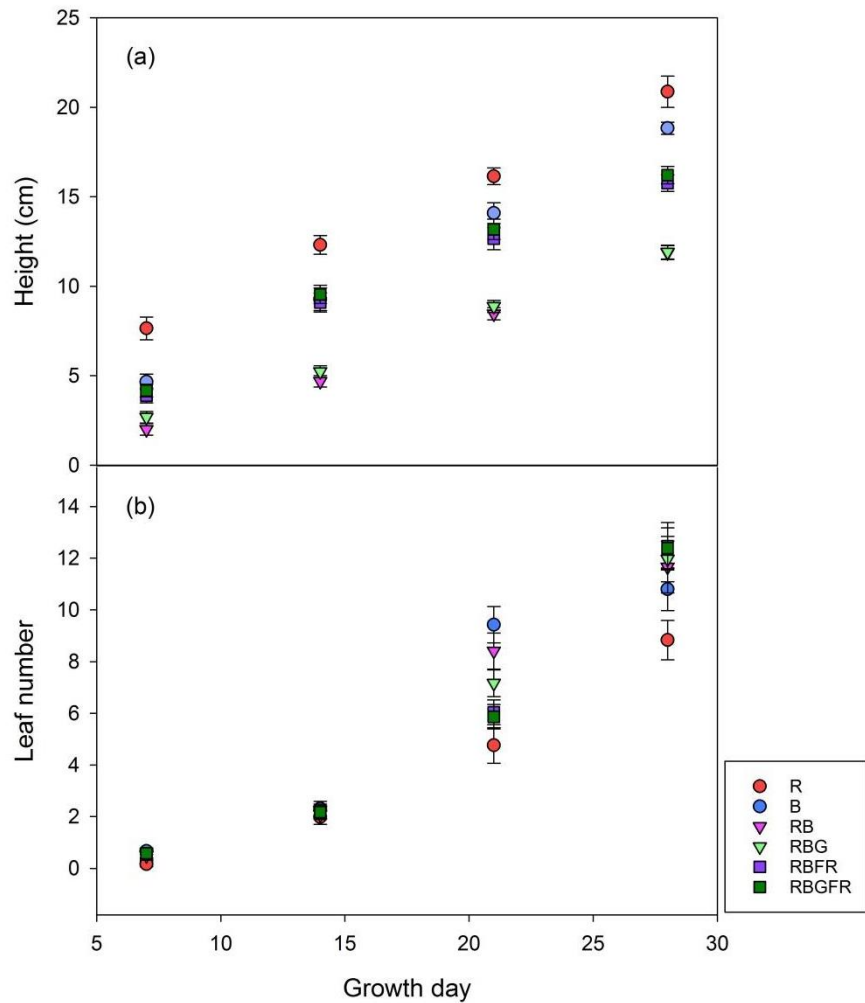


Figure 4.5: Height of the supported plant (a) and true leaf (not including the cotyledons) number (b) of plants grown under each light recipe as measured 7, 14, 21 and 28 days after germination. Measurements were of 8 plants under each light recipe in 3 runs of the experiment (n=24). Bars show standard error of means.

As can be seen in Fig. 4.4 and reflected in Fig. 4.5, ANOVA with post-hoc pairwise comparisons revealed that the R light recipe resulted in significantly longer plants than all other light recipes at all growth stages ($P < 0.001$ for all comparisons apart from with RBGFR on day 28 where $P = 0.024$). RB and RBG light recipes resulted in statistically similar heights on all days measured and they were significantly shorter than all other plants ($P < 0.001$). B, RBFR and RBGFR light recipes resulted in plants with statistically similar heights until day

28 when B recipe plants were significantly taller than RBFR plants ($P=0.033$).

Leaf numbers were statistically similar until day 21, where B light recipe plants had significantly more leaves than R, RBFR and RBGFR light recipe plants ($P<0.001$). R light recipe plants had significantly less leaves than RBFR ($P=0.016$), RB and RBG light recipe plants ($P<0.05$).

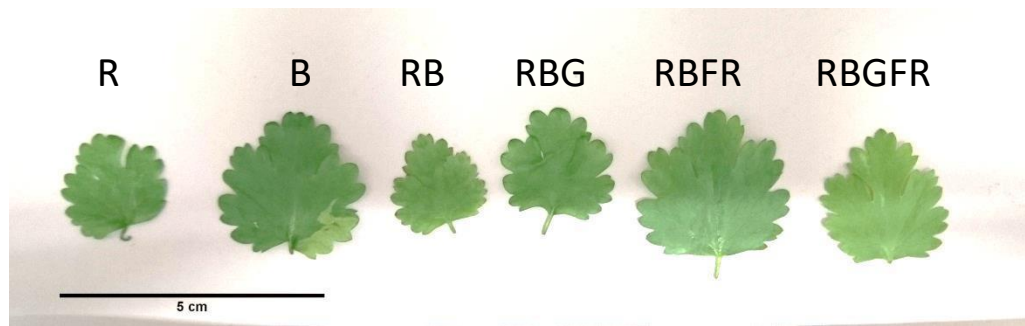


Figure 4.6: Aerial photo of representative fully expanded first true leaves of *C. sativum* cv. Standard grown under each light recipe.

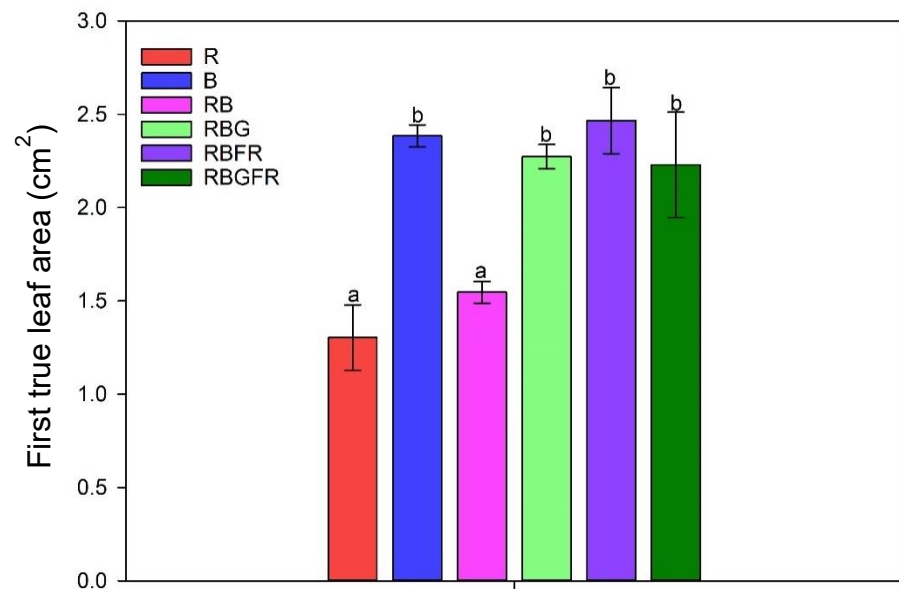


Figure 4.7: Average first true leaf area (cm²) of *C. sativum* cv. Standard grown under each light recipe. Results are a mean of 8 fully expanded leaves under each light recipe in 3 runs of the experiment (n=24). Bars show standard error of means and letters represent statistical differences (P<0.05).

Plants grown under the R and RB light recipes had statistically similar leaf areas and were smaller than those grown under all other light treatments (P<0.001). Plants grown under the B and RBFR light recipes often had larger leaves than

those grown under the RBG and RBGFR light recipes, but this was not statistically significant (Fig 4.6; Fig 4.7).

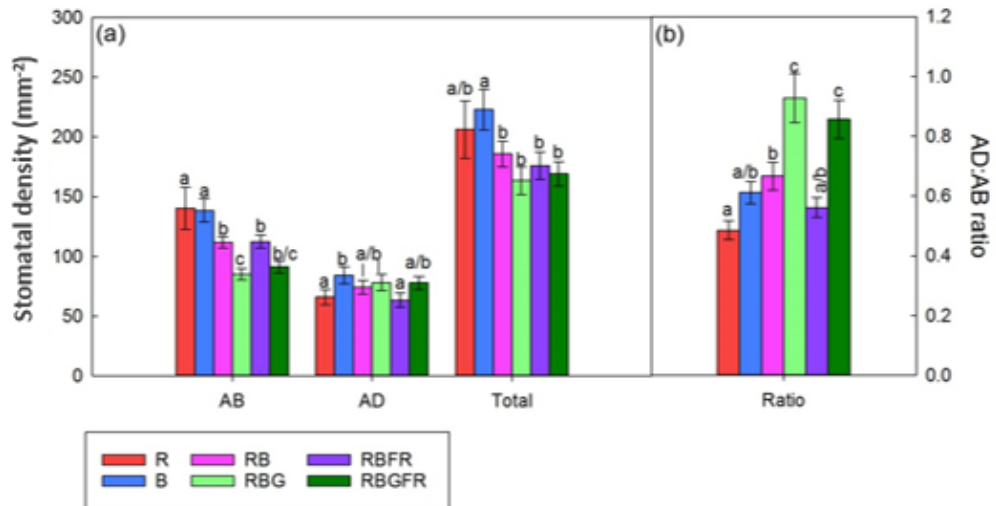


Figure 4.8: (a) Stomatal density per 1 mm² on abaxial and adaxial leaf surface of the third true leaf of *C. sativum*. cv. Standard grown under each light recipe and (b) the ratio of abaxially located stomata to adaxially located stomata. Leaf surface impressions were taken from each side of three leaves in each light recipe in three runs of the experiment (n=9). Bars show standard error of means and letters represent statistical differences (P<0.05).

When combining stomatal counts for both abaxial and adaxial sides of the leaf (Fig. 4.8), ANOVA with post hoc pairwise comparisons revealed that B recipe plants had a higher overall stomatal number than RB (P=0.015), RBG (P<0.001), RBFR (P=0.002) and RBGFR (P=0.001) plants, but statistically similar to R light plants. R recipe plants had a higher overall stomatal number than RBG (P=0.005), RBFR (P=0.046) and RBGFR (P=0.018) plants. RB, RBG, RBFR and RBGFR plants had statistically similar overall numbers of stomata.

When comparing stomatal number on either side of the leaf, R (P=0.001), B (P<0.001), RB (P<0.001), RBFR (P<0.001) and RBGFR (P=0.042) recipe plants all

had a significantly higher number of stomata on the adaxial side of the leaf, whilst the RBG recipe plants had statistically similar numbers of stomata on both sides of the leaf. This was reflected in the adaxial to abaxial leaf surface stomatal ratios (AD:AB), where AD:AB in RBG light recipe plants was 0.9281 (SEM – 0.0818) and significantly higher than that of R, B, RBFR ($P < 0.001$) and RB ($P = 0.001$) light recipe plants. AD:AB in RBGFR light recipe plants was not however significantly lower, being 0.8653 (SEM – 0.064).

When comparing adaxial leaf surface stomatal number between light recipes, B recipe plants had a higher overall stomatal number than RB ($P = 0.015$), RBG ($P < 0.001$), RBFR ($P = 0.002$) and RBGFR ($P = 0.001$) plants, but statistically similar to R light plants. R recipe plants had a higher overall stomatal number than RBG ($P = 0.005$), RBFR ($P = 0.046$) and RBGFR ($P = 0.018$) plants. RB, RBG, RBFR and RBGFR plants had statistically similar overall numbers of stomata. RBG plants had a significantly lower stomatal number than RB ($P = 0.008$) and RBFR ($P = 0.007$) plants but statistically similar to that of RBGFR plants. When comparing abaxial leaf surface stomatal number between light recipes, B light recipe plants had a higher number of stomata than R ($P = 0.042$) and RBFR ($P = 0.022$) light recipe plants. There were no other significant differences. It can be concluded that the more even number of stomata between the adaxial and abaxial leaf surfaces (AD:AB ratios closer to 1:1) of RBG and RBGFR light recipes was due to a decreased number of stomata forming on the adaxial surface of their leaves.

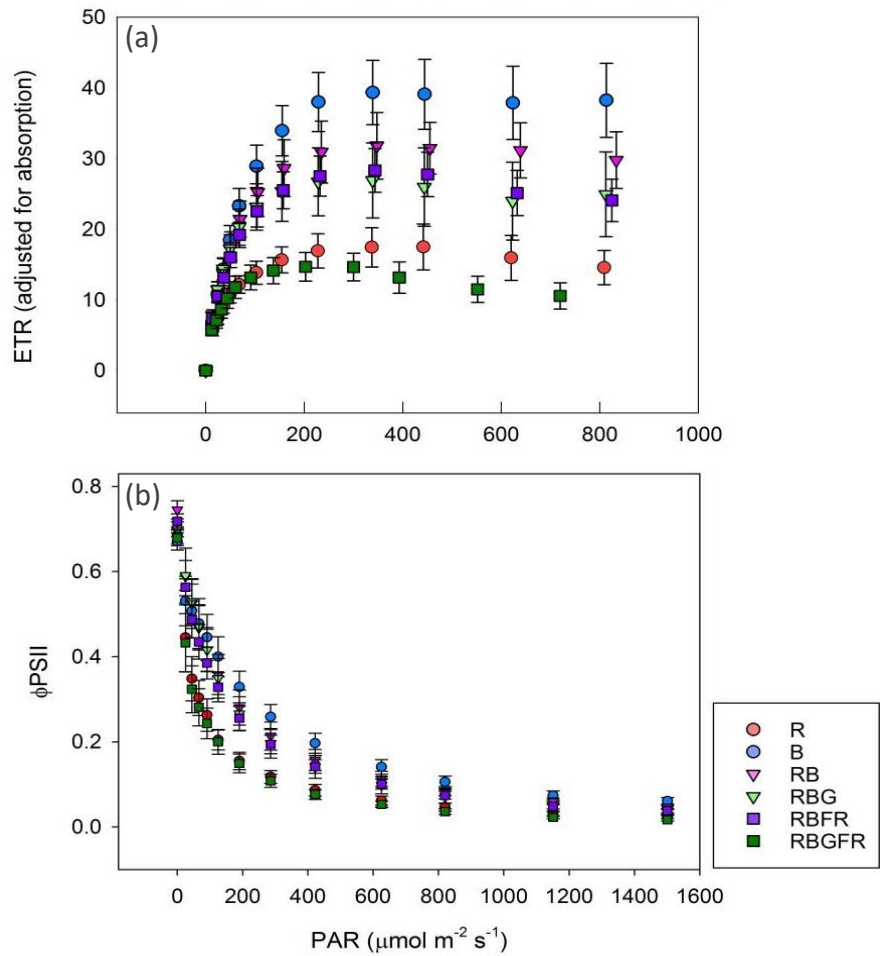


Figure 4.9: Rapid light response curves of chlorophyll fluorescence were taken on the last fully expanded leaf of *C. sativum* cv Standard, grown under each light recipe with the Walz JuniorPAM chlorophyll fluorometer, 14-15 days post germination. Results are from a mean of 3 experiments (n=9). The figure shows (a) electron transport rate (ETR), corrected for absorption of photosynthetically active radiation ($R = 0.53887$; $B = 0.541865$; $RB = 0.55522$; $RBG = 0.541141$; $RBFR = 0.549186$; $RBGFR = 0.479432$) as measured with the FieldSpec HH2 spectroradiometer (ASD) and integrating sphere (LI-COR) as can be seen in Table 4.1 and (b) quantum yield on photosystem II (ϕPSII) under increasing light levels.

When corrected for absorbance, ETRs (Fig. 4.9) appear similar for plants grown under all light recipes up to $90 \mu\text{mol m}^{-2} \text{s}^{-1}$, above which R and RBGFR light recipe plants have lower ETRs than all other light treatments. Above $125 \mu\text{mol m}^{-2} \text{s}^{-1}$, ETR is highest in B light recipe plants, although not significantly higher

than RB light recipe plants. RB, RBG and RBFR light recipe plants have statistically similar ETRs. This is reflected in the decline in ϕPSII where B light recipe plants have the highest quantum efficiency at PAR levels $\geq 125\mu\text{mol m}^{-2}\text{ s}^{-1}$ whilst R and RBGFR plants clearly have the lowest. Differences in ϕPSII became less apparent at PAR levels $> 820\mu\text{mol m}^{-2}\text{ s}^{-1}$ and did not appear statistically significant at 1150 and $1500\mu\text{mol m}^{-2}\text{ s}^{-1}$, indicating equal saturation of photosynthesis in all light recipe plants.

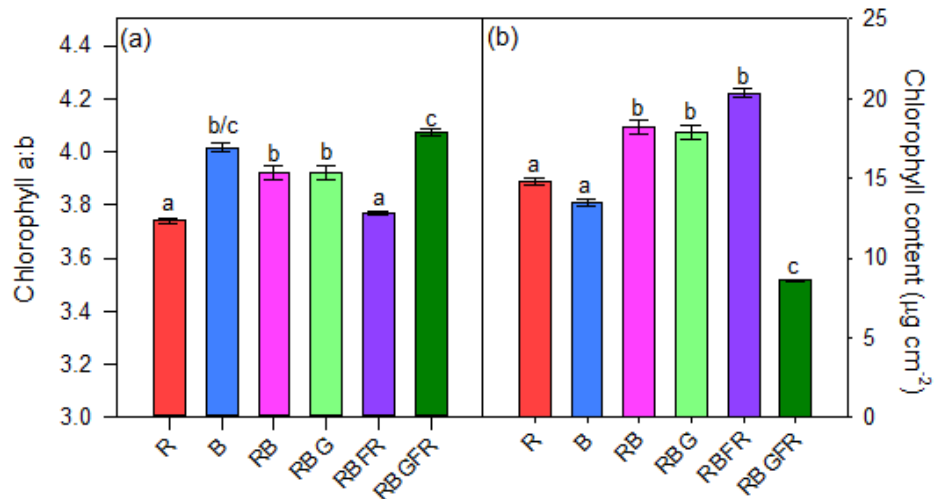


Figure 4.10: Chlorophyll a:b ratio (a) and total chlorophyll content per cm^2 (b) of the third true leaf of *C. sativum* cv. Standard grown under each light recipe. Results are a mean of samples from 3 runs of the experiment (n=9). Bars show standard error of means and letters represent statistically significant differences ($P < 0.05$).

ANOVA with post-hoc pairwise comparisons revealed that, although statistically similar to B light plants, chlorophyll a:b ratio was highest in RBGFR light recipe plants, being significantly higher than R ($P < 0.001$), RB ($P = 0.026$), RBG ($P = 0.023$) and RBFR ($P < 0.001$) plants. B, RB and RBG plants had statistically similar chlorophyll a:b ratios (Fig. 4.10). R light recipe plants had a significantly lower chlorophyll a:b ratio than plants under all other light recipes except RBFR ($P < 0.001$). RBFR light recipe plants also had a significantly lower chlorophyll a:b ratio than B ($P < 0.001$), RB ($P = 0.003$), RBG ($P = 0.004$) and RBGFR ($P < 0.001$) plants.

Total chlorophyll content was significantly lower in RBGFR plants than plants grown under all other light recipes ($P < 0.001$). Total chlorophyll content was also relatively low in B light recipe plants, being statistically similar to that of R light recipe plants but significantly lower than RB, RBG ($P = 0.001$). Red light

recipe plants had significantly lower total chlorophyll content than RB ($P=0.008$), RBG ($P=0.016$) and RBFR ($P<0.001$) light recipe plants. Whilst statistically similar to RB and RBG light recipe plants, RBFR light recipe plants had the highest total chlorophyll content of all light recipes (Fig. 4.10).

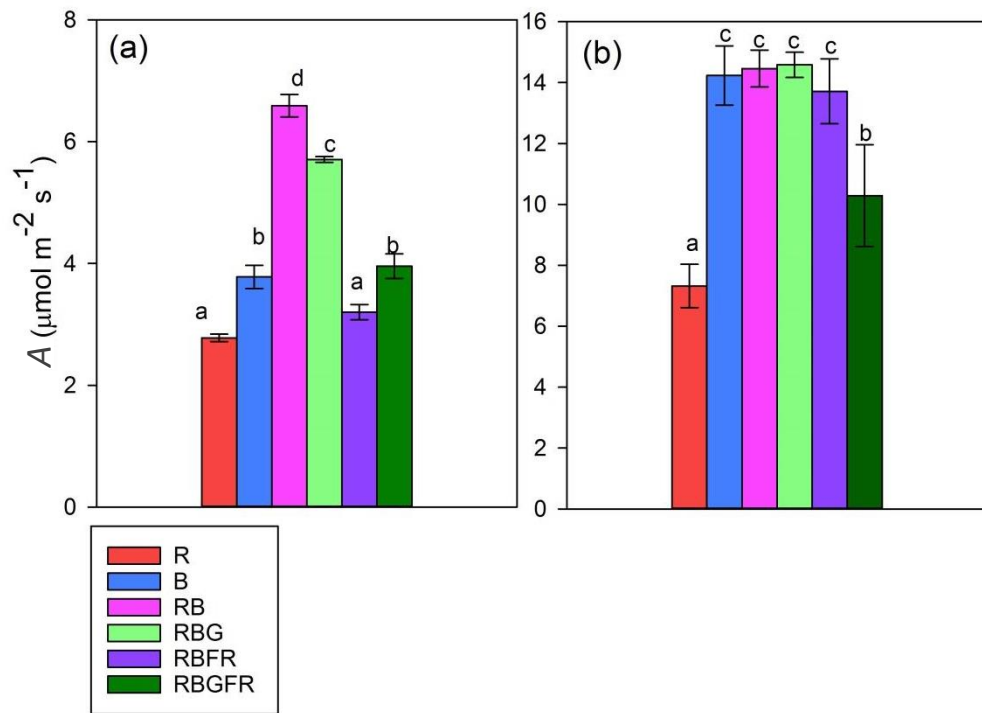


Figure 4.11: Spot measurements of in situ photosynthesis (A) taken on the last fully expanded leaf of plants under each light recipe 21 days after germination using the Licor-LI6400XT with sun and sky clear plastic window. Graphs show photosynthesis per cm^2 (a) and adjusted for leaf area to show whole leaf photosynthesis (b) under $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ of the light in which they were grown. Bars show standard error of means and letters represent significant differences ($P<0.05$). Results are a taken from a mean of three experiments ($n=12$).

As can be seen in Fig. 4.11a, on a per unit leaf area basis, plants grown under the R and RBFR light recipes had significantly lower rates of photosynthesis than plants grown under the other light recipes ($P<0.01$). Plants grown under the B and RBFR light recipes also had a significantly lower rate of

photosynthesis than plants grown under the RB and RBG light recipes ($P < 0.001$) and RB recipe plants had higher rates of photosynthesis than RBG recipe plants ($P < 0.01$). However, photosynthesis of the whole leaf (Fig. 4.11b) was statistically similar in B, RB, RBG and RBFR recipe grown plants and only slightly significantly lower in RBGFR grown plants ($P < 0.05$) suggesting that leaf size evened out the rate of photosynthesis under the conditions in which the plants were grown, apart from in the case of R recipe plants.

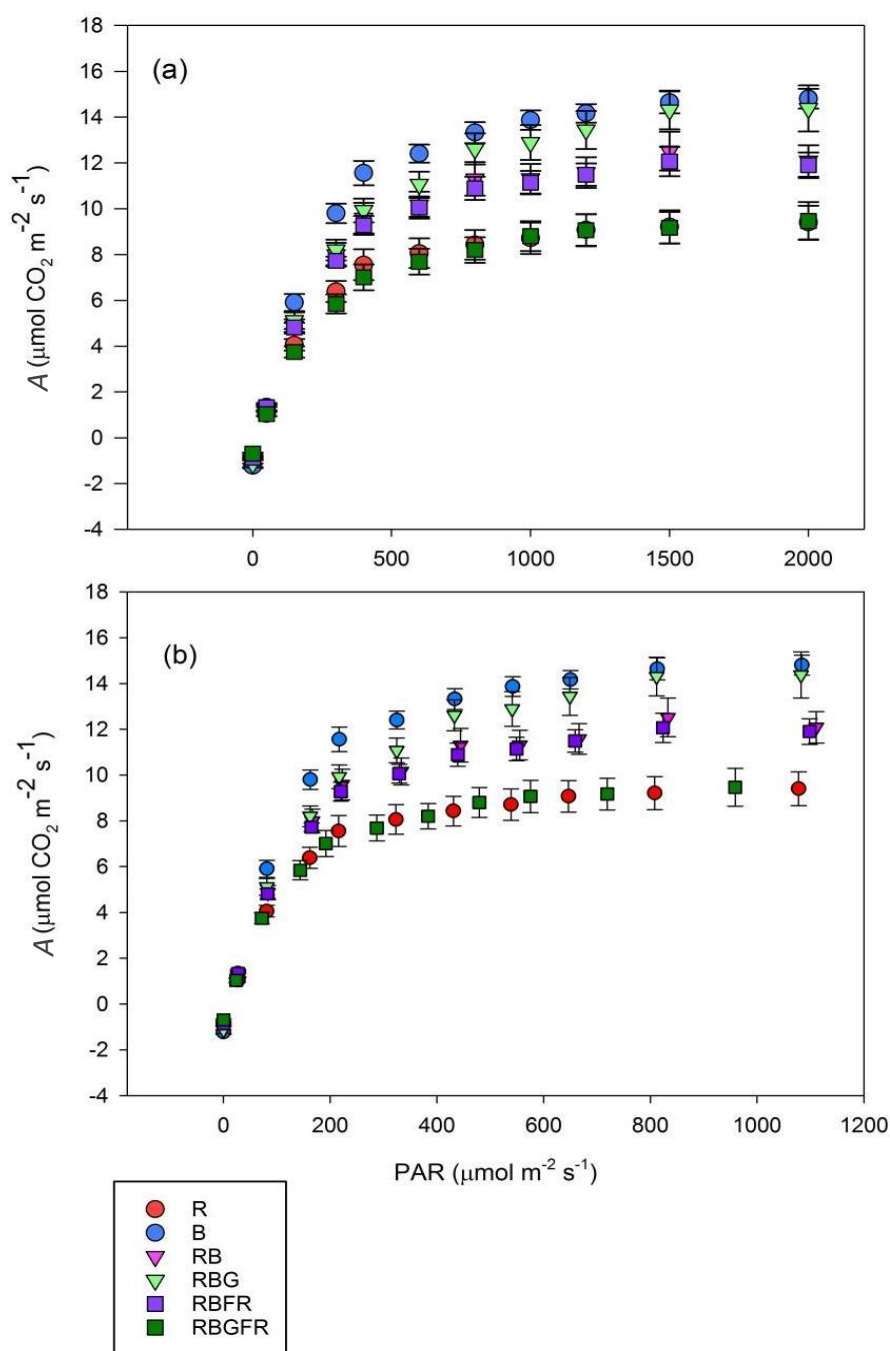


Figure 4.12: Photosynthetic light response curves measured with the GFS-3000 (Walz) showing (a) the carbon assimilation rate (A) of *C. sativum* cv. Standard grown under each light recipe and (b) these values corrected for absorption of PAR (400-750nm). (Absorption values: R = 0.53887; B = 0.541865; RB = 0.55522; RBG = 0.541141; RBFR = 0.549186; RBGFR = 0.479432) as measured with the FieldSpec HH2 spectroradiometer (ASD) and integrating sphere (LICOR) as can be seen in Table 4.1. Bars show standard error of means. Results are a taken from a mean of three experiments (n=9).

On an absorbed light basis (Fig. 4.12b), $\geq 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, there were significant differences in A between light recipes. B light recipe plants consistently had the highest assimilation rates, although $\geq 400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR had a statistically similar rate to RBG light recipe plants. $\geq 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, R and RBGFR consistently had significantly lower carbon assimilation rates than plants in all other light recipes.

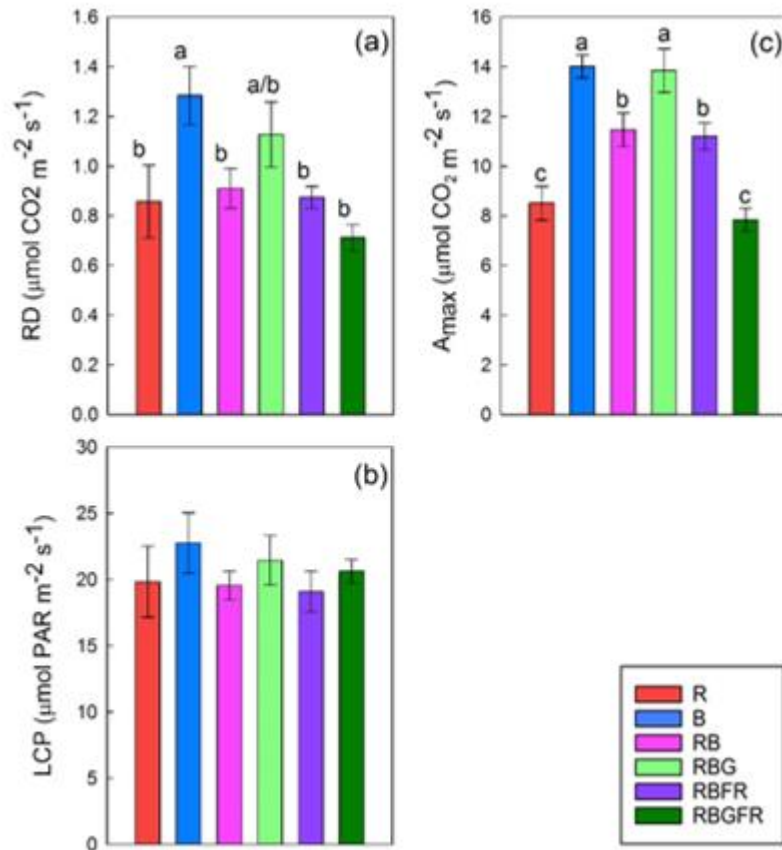


Figure 4.13: (a) Dark respiration (RD), (b) light compensation point (LCP) and (c) maximum rate of carbon assimilation of the third true leaf of *C. sativum* cv. Standard grown under each light recipe 21-24 days after germination. Parameters were calculated by entering the photosynthetic light response curve values (Fig 4.12) into the model described by (Prioul and Chartier, 1977). Results are taken from a mean of three experiments (n=9). Bars show standard error of means and letters represent statistically significant differences (P<0.05).

ANOVA with post-hoc pairwise comparisons revealed that dark respiration (Fig. 4.13a) was highest in B light recipe plants, being significantly higher than R (P=0.035), RB (P=0.014), RBG (P=0.021), RBFR (P<0.001) and RBGFR (P=0.001) light recipe. Plants grown under the B light recipe also had the highest light compensation point (Fig. 4.13b) but this was not statistically significant. Maximum rate of carbon assimilation (Fig. 4.13c) was highest and statistically

similar in B and RBG light recipe plants, being significantly lower in RB and RBFR light recipe plants ($P<0.03$). R and RBGFR light recipe plants had the lowest A_{max} , being significantly lower than RB and RBFR light recipe plants ($P<0.05$).

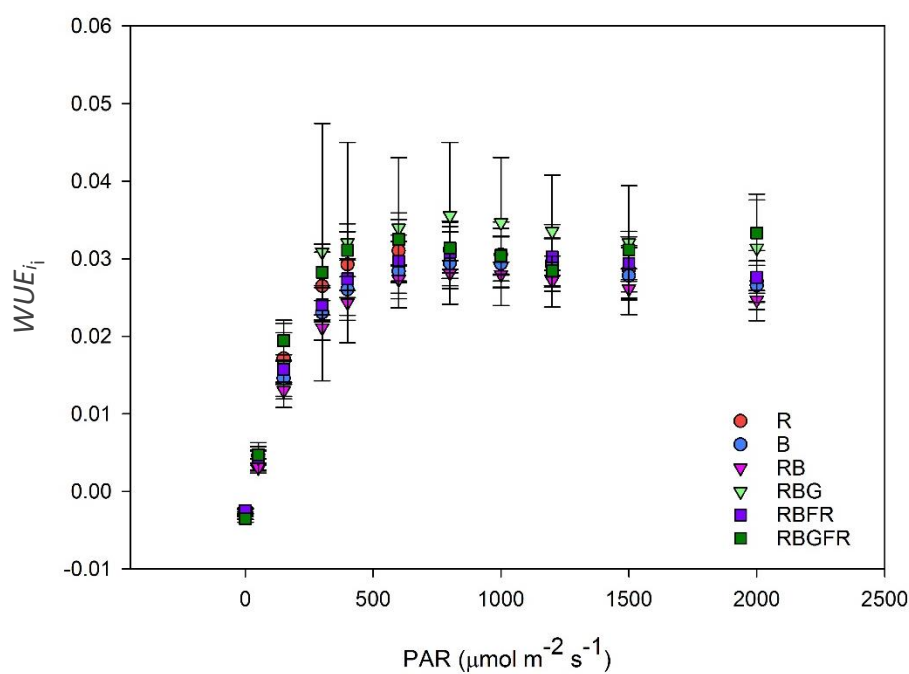


Figure 4.14: Intrinsic water use efficiency (WUE_i) of leaf three of *C. sativum* cv. Standard grown under each light recipe, as calculated with the data from the Walz GFS-3000 photosynthetic light response curves (Fig. 4.12). WUE_i = carbon assimilation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) divided by stomatal conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$). Results are taken from a mean of three experiments ($n=9$). Bars show standard error of means.

Error was too large to report any statistically significant differences in WUE_i ; however, there was a slight trend towards plants grown under recipes containing green and far-red light being more water use efficient.

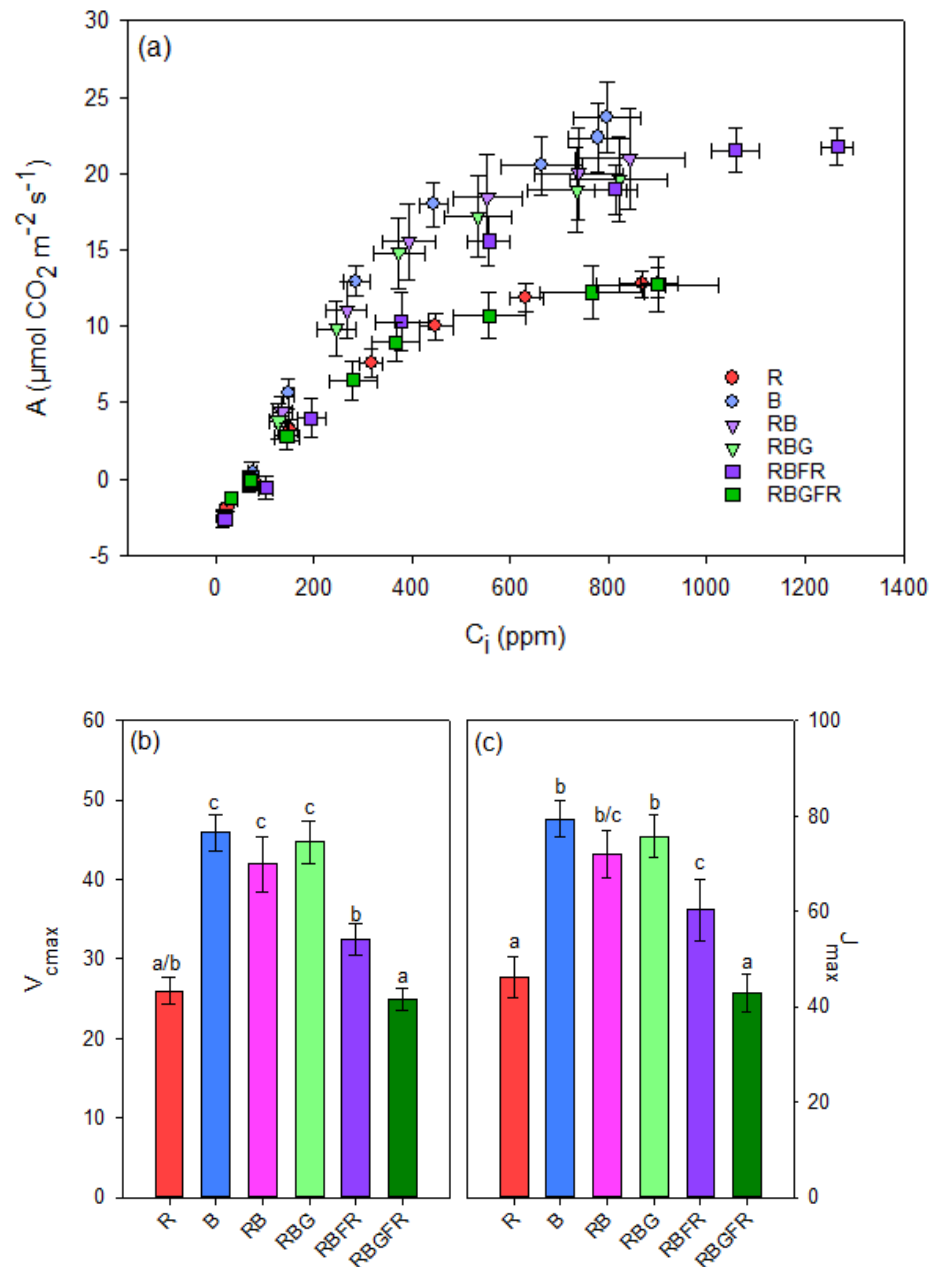


Figure 4.15: Photosynthetic CO_2 response curves were measured with the GFS-3000 (Walz) to show carbon assimilation under increasing carbon dioxide concentrations (a) of the third true leaves of *C. sativum* cv. Standard grown under each light recipe 24-28 days after germination. V_{cmax} (b) and J_{max} (c) were then calculated by entering the values into a two-segment model (Bernacchi, 2013) which used the equations of Farquhar *et al.*, 1980. Results are taken from a mean of three experiments ($n=9$). Bars show standard error of means and letters represent statistically significant differences ($P<0.05$).

V_{cmax} (Fig. 4.15b) was lowest in plants grown under the RBGFR light recipe although statistically similar to those grown under the R light recipe. B ($P<0.001$), RB ($P=0.007$) and RBG ($P<0.001$) had higher V_{cmax} than RBFR light recipe plants which had a statistically similar V_{cmax} to plants grown under the R light recipe. R and RBGFR light recipe plants had lower J_{max} (Fig. 4.15c) than RBFR ($P<0.025$), B, RB and RBG ($P<0.001$) plants. RBFR plants had a lower but statistically similar J_{max} to RB plants.

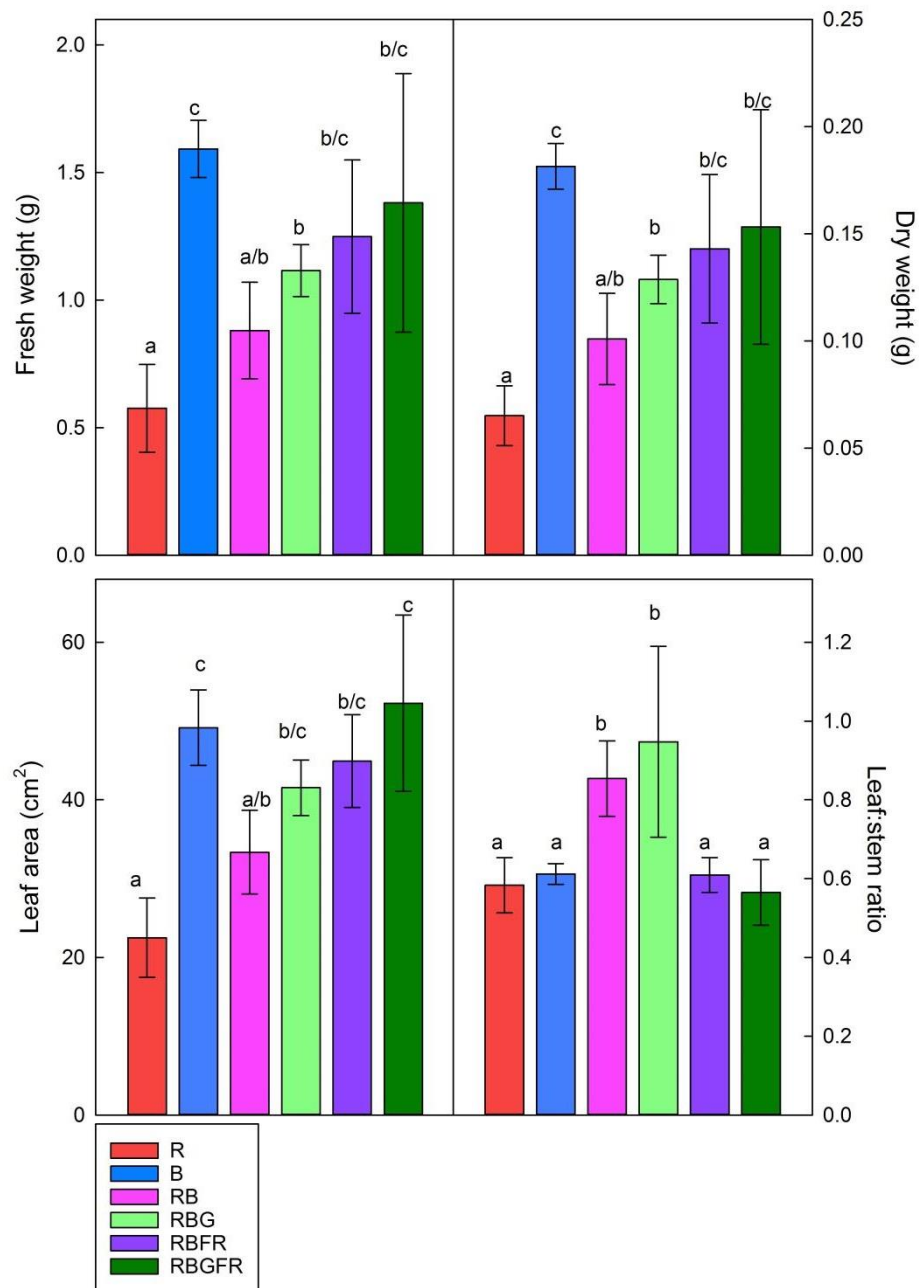


Figure 4.16: Fresh weight (a), dry weight (b), leaf area (c) and leaf:stem fresh weight ratio (d) of *C. sativum* cv. Standard grown under each light recipe for 28 days after germination. Results are a mean from 8 plants in 3 experiments (P=24). Bars show standard error of means and different letters represent statistical differences (P<0.05).

Plants grown in the R light recipe had lower fresh weights (Fig. 4.16a), dry weights (Fig. 4.16b) and leaf areas (Fig. 4.16c) than plants grown under all other light recipes except for RB light plants (P<0.05).

There was the most variation in the RBFR and RBGFR light recipe plants which had statistically similar fresh and dry weights to plants under all recipes except for R ($P=0.002$ and $P=0.006$ respectively). B light recipe plants had significantly higher fresh and dry weights than R ($P<0.001$; $P<0.001$) and RB ($P=0.001$; $P<0.001$) light recipe plants but statistically similar to RBG, RBFR and RBGFR light recipe plants. B light recipe plants also had higher leaf areas than R ($P<0.001$) and RB ($P=0.02$) light recipe plants and RBGFR light recipe plants had a higher leaf area than R ($P<0.001$) and RB ($P=0.011$) light recipe plants. Fresh weight leaf to stem ratio was significantly higher in RB and RBG light recipe plants than plants grown under all other light recipes ($P<0.05$).

4.4.3 Biomass partitioning



Figure 4.17: Pots of *C. sativum* cv. Cruiser 21 days after sowing (a) and individual plants washed from the soil (b) for the R, B, RB and RBG light recipes.

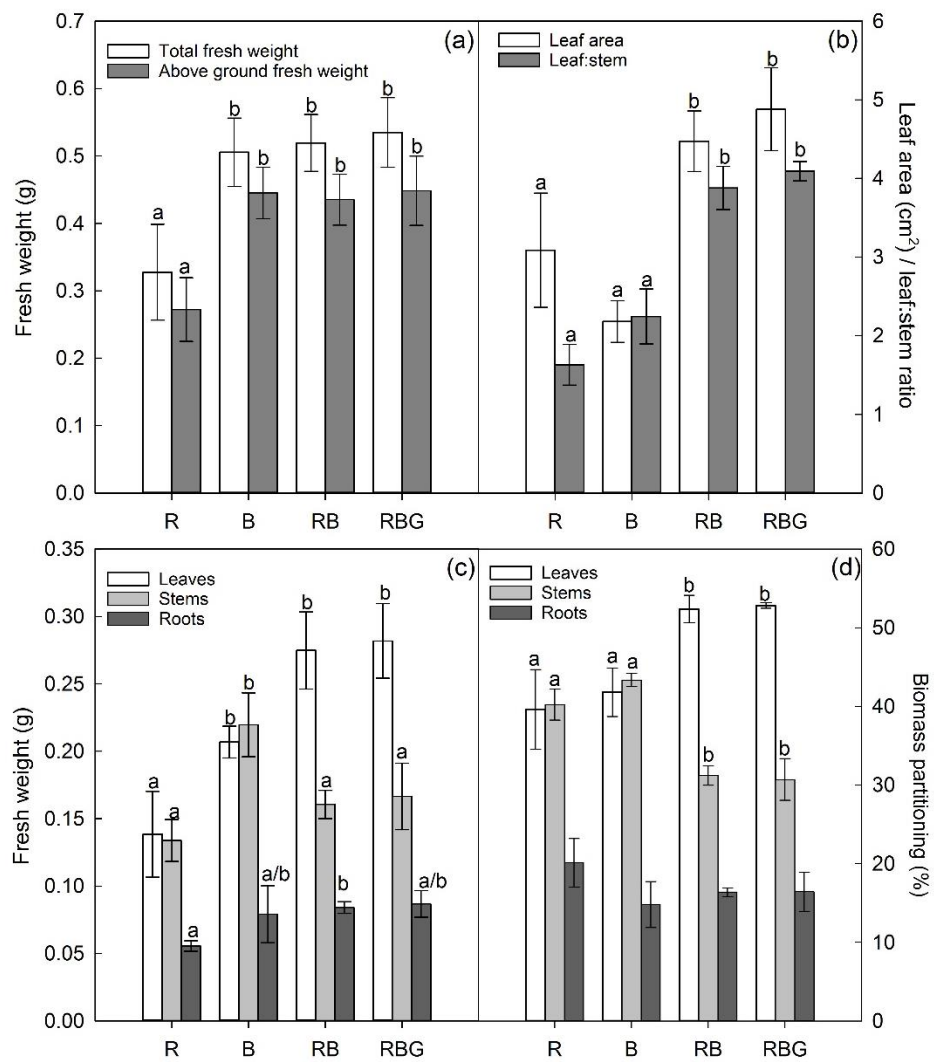


Figure 4.18: Total fresh weight and above ground fresh weight (a), total leaf area and leaf to stem ratio (b), fresh weights of leaves, stems and roots (c) and fresh weight biomass partitioning between leaves, stems and roots (d) of *C. sativum* cv. Cruiser seedlings 21 days after sowing under the different light recipes. Results are from a mean of at least 4 plants in 3 separate experiments (n=12+). Bars show standard error of means and letters indicate significant differences between groups (P<0.05).

ANOVA with post-hoc pairwise comparisons showed that plants grown under the B light recipe had larger total (P=0.003) and above ground (P=0.004) fresh weights than R grown plants (Fig. 4.18a). RB and RBG grown plants also had larger total and above ground fresh weights than R plants (P<0.001). B grown

plants had smaller leaf areas than RB ($P=0.005$) and RBG ($P<0.001$) plants (Fig. 4.18b). R plants had a statistically similar total leaf area to B plants but significantly lower than RB ($P=0.003$) and RBG ($P<0.001$) plants. R and B plants also had significantly lower leaf to stem ratios than RB and RBG plants ($P<0.001$). R plants had lower leaf fresh weights than B ($P=0.025$), RB ($P=0.001$) and RBG ($P<0.001$) plants. B plants had a higher stem fresh weight than R ($P=0.003$), RB ($P=0.023$) and RBG ($P=0.046$) plants. R had a lower root fresh weight than RB ($P=0.03$). There were no significant differences between percentage biomass being partitioned to the roots, however partitioning to leaves and stems did vary (Fig. 4.18b,c,d). Plants under the R and B recipes partitioned more biomass to the stems than plants under the RB and RBG recipes ($P<0.001$). RB grown plants partitioned a significantly higher proportion of biomass into leaves than R ($P=0.003$) and B ($P=0.024$) plants. RBG grown plants also partitioned a significantly higher proportion of biomass into leaves than R ($P<0.001$) and B ($P=0.004$) plants.

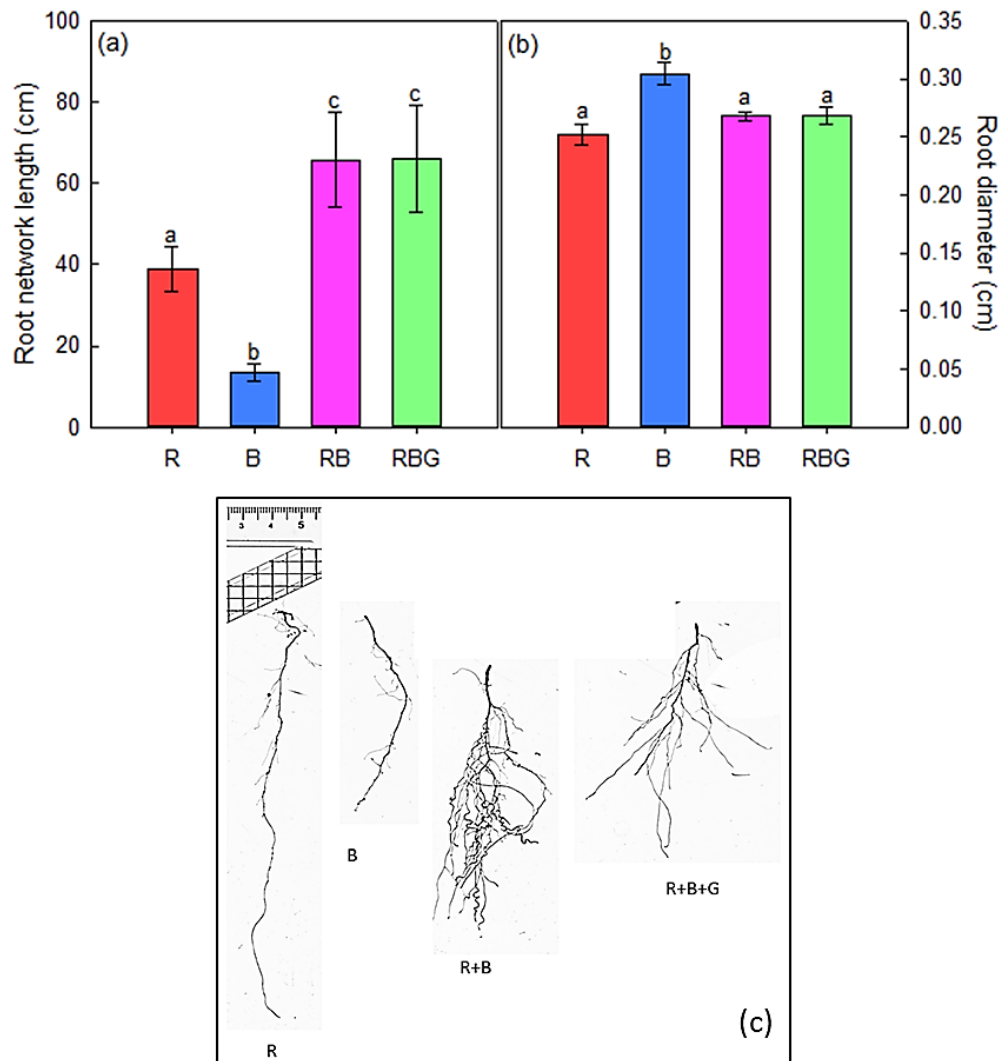


Figure 4.19: Total root network length (a) average diameter of the taproot (b) and example root scans (c) of *C. sativum* cv. Cruiser seedlings 21 days after sowing under the different light recipes. Results are from a mean of at least 4 plants in 3 separate experiments ($n=12+$). Bars show standard error of means and letters indicate significant differences between groups ($P<0.05$). Images were acquired in high resolution with a flat-bed scanner and analysed in the WinRhizo root measuring program (Arsenault *et al.*, 1995).

ANOVA with post-hoc pairwise comparisons revealed that plants grown under the B light recipe had the shortest total root network length (Fig. 4.19), which was significantly shorter than that of plants grown under R ($P=0.042$), RB and RBG ($P<0.001$) light recipes. Plants grown under the R light recipe also had

shorter roots than those grown under RB ($P=0.037$) and RBG ($P=0.043$) light recipes. Average root diameter was however highest in B light recipe plants, being wider than in R ($P=0.005$), RB ($P=0.003$) and RBG ($P=0.009$) recipe plants.

4.4.4 Comparison of absorption spectrum and chlorophyll content

Table 4.1: Average absorption values (and standard error of means – SEM) between 400 and 750nm from the adaxial (AD) and abaxial (AB) side of leaves of *C. sativum* cv. Cruiser ($n=4$) from each light recipe as measured with the FieldSpec HH 2 spectroradiometer (ASD) and integrating sphere (LICOR). Absorption values from cv. Standard ($n=1$) are also presented for purposes of comparison.

Average absorption value of 400 - 750nm						
	<i>C. sativum</i> cv. Standard		<i>C. sativum</i> cv. Cruiser			
Light recipe	AD	AB	AD	SEM	AB	SEM
R	0.05388730	0.44942100	0.46750600	0.00454086	0.34649300	0.00575965
B	0.54186500	0.43164700	0.41342100	0.00499004	0.33107100	0.00328455
RB	0.55522000	0.46558400	0.31487300	0.00346352	0.24701600	0.00577293
RBG	0.54114100	0.47205900	0.30568800	0.00569036	0.20564100	0.00506278
RBFR	0.54918600	0.44723800	0.30518300	0.00546116	0.32777100	0.00449640
RBGFR	0.47943200	0.40213300	0.27525500	0.00453393	0.16186900	0.00524638

All light recipe plants from both cultivars had lower absorption of light from the abaxial side of the leaf (Table 4.1). RBGFR light recipe plants absorbed the least light in both cultivars from both sides of the leaf. RB light recipe plants had the highest absorption from the adaxial side of the leaf in cv. Standard whilst in cv. Cruiser this was R recipe plants. RBG light recipe plants absorbed the most light from the abaxial side of the leaf in cv. Standard whilst in cv. Cruiser this was again R light recipe plants. In both cultivars, B and R light recipe plants appeared to absorb similar amounts of light from the both sides of the leaf.

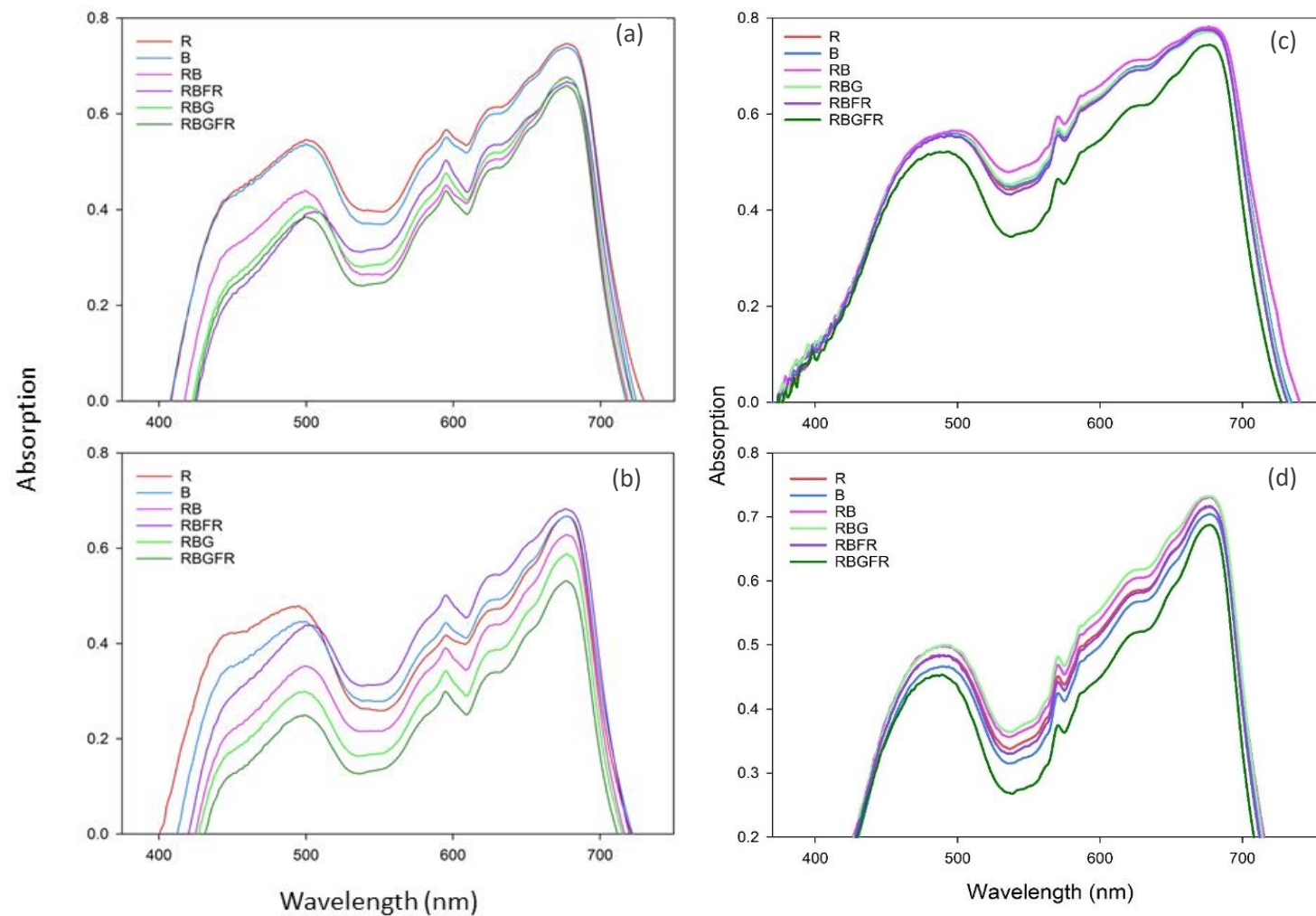


Figure 4.20: The absorption spectrum between 325 and 750nm of the adaxial (a) and abaxial (b) surfaces of the fully expanded third true leaf of *C. sativum* cv. Cruiser (n=4) and the adaxial (c) and abaxial (d) surfaces of the fully expanded third true leaves of *C. sativum* cv. Standard (n=1) grown under the different light recipes as measured with the FieldSpec HH 2 spectroradiometer (ASD) and integrating sphere with broad-wavelength white light illuminator (LI-COR).

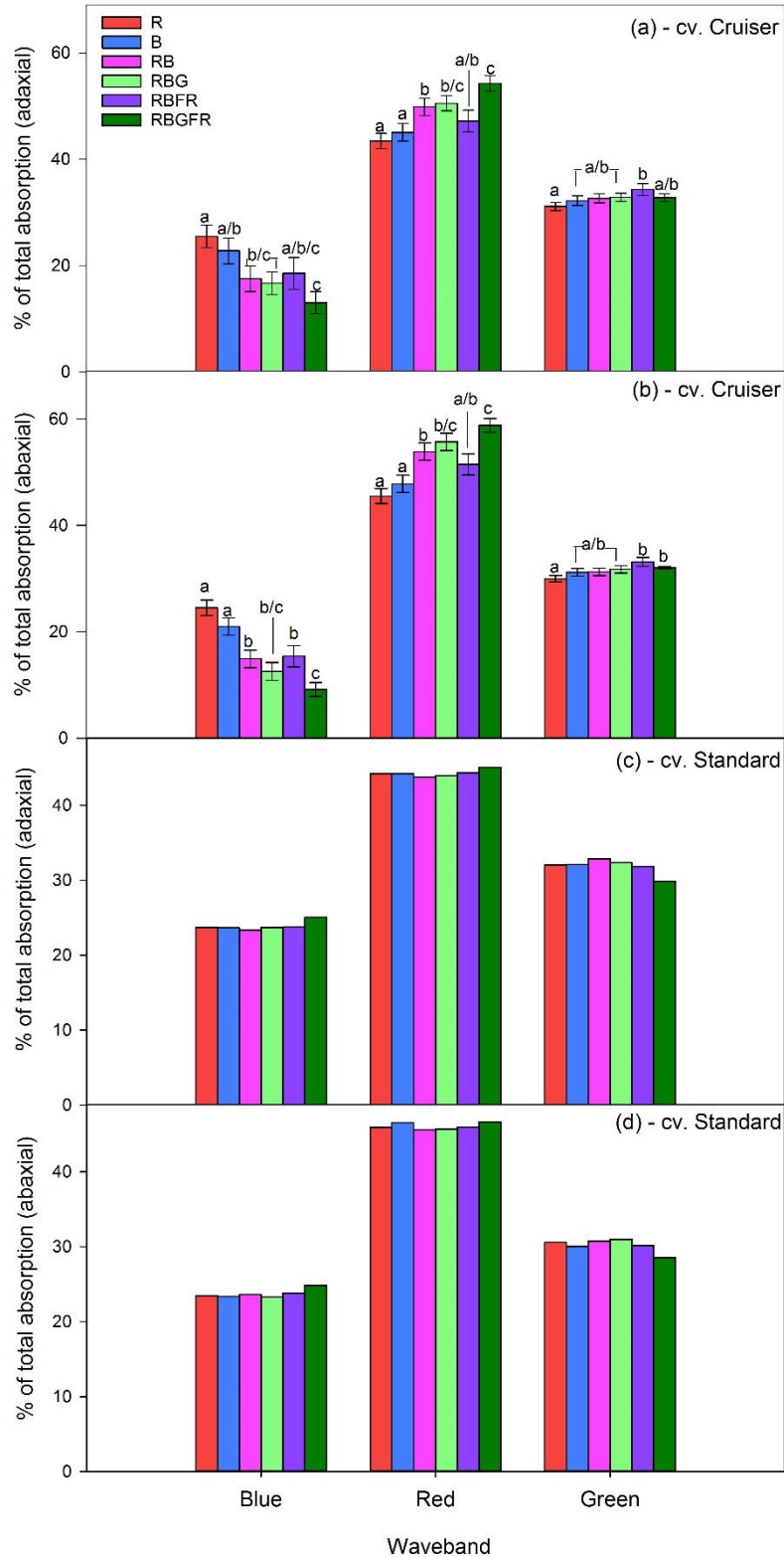


Figure 4.21: Percentage of total absorption between 400 and 750nm made up of each waveband (blue = 400-499nm; red = 600-699nm; green = 500-599nm) of adaxial (a) and abaxial (b) leaf surfaces of the third true leaf of *C. sativum* cv. Cruiser (n=4) and the adaxial (c) and abaxial (d) leaf surfaces of the third true leaf of *C. sativum* cv. Standard (n=1) grown under the different light recipes. Bars show standard error of means and letters indicate significant differences between groups (P<0.05).

ANOVA with post-hoc pairwise comparisons revealed that, in cv. Cruiser, from the adaxial side of the leaf, plants grown under the R light recipe absorb more blue light as a percentage of total light absorbed than RB ($P=0.026$), RBG ($P=0.01$) and RBGFR ($P=0.001$) grown plants (Fig. 4.21). B recipe leaves also absorb more blue light as a as a percentage of total light absorbed than RBGFR ($P=0.009$) grown plants. RBGFR grown plants absorbed the least blue light as a proportion of total absorbed light and significantly lower than R ($P<0.001$) and B ($P=0.001$) grown plants. Absorbance in the green as a percentage of total light absorbed was significantly higher in RBFR plants than R plants ($P=0.032$). From the abaxial side of the leaf, R light recipe grown plants absorbed the most blue light as a percentage of total light absorbed, being significantly higher than RB ($P=0.001$), RBFR ($P=0.003$), RBG and RBGFR ($P<0.001$). B light recipe plants also absorbed more blue light as a percentage of total light absorbed than RB ($P=0.02$), RBG ($P=0.003$) and RBGFR ($P<0.001$) grown plants. RB ($P=0.016$) and RBFR ($P=0.02$) plants also absorbed significantly more blue light as a percentage of total light absorbed than RBGFR grown plants. In terms of red light as a percentage of total light absorbed, R ($P<0.001$), B ($P<0.001$) and RBFR ($P=0.008$) grown plants again absorbed significantly less than RBGFR grown plants. R and B grown plants also absorbed significantly less red light than the other light recipe plants ($P<0.03$). R grown plants also absorbed significantly less green light than RBFR ($P=0.009$) and RBGFR ($P=0.023$) plants. In cv. Standard, differences are not as pronounced, although RBGFR light recipe plants appear to absorb more blue and less red waveband light as a percentage of total light

absorbed than plants grown under the other light recipes from both the adaxial and abaxial side of the leaf.

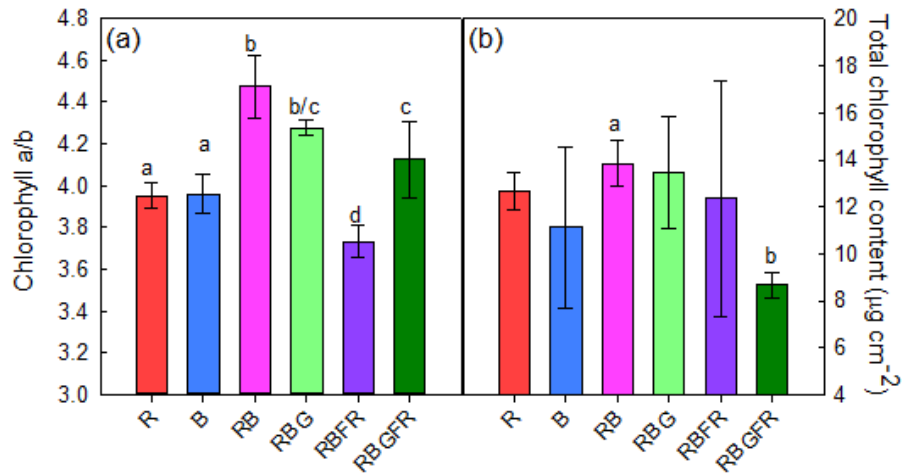
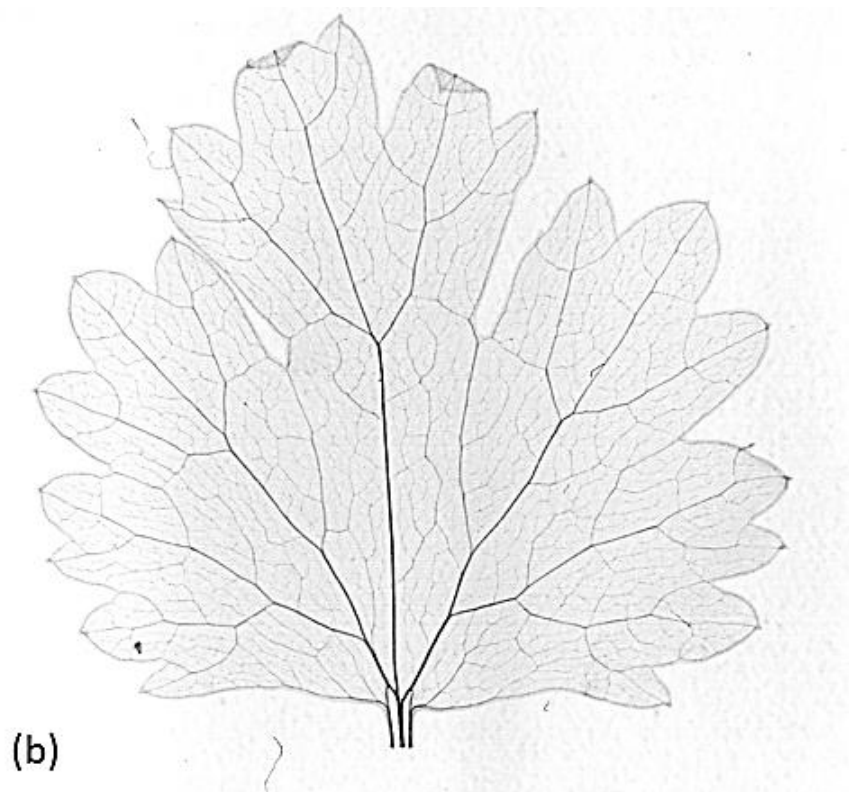
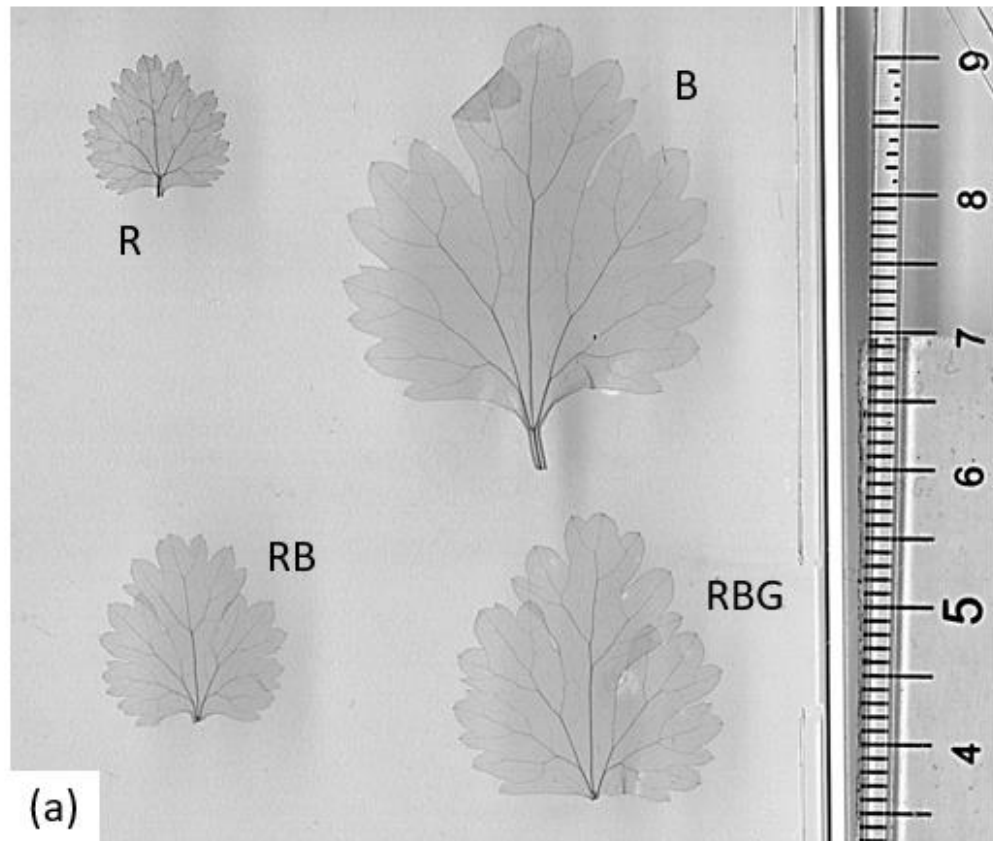


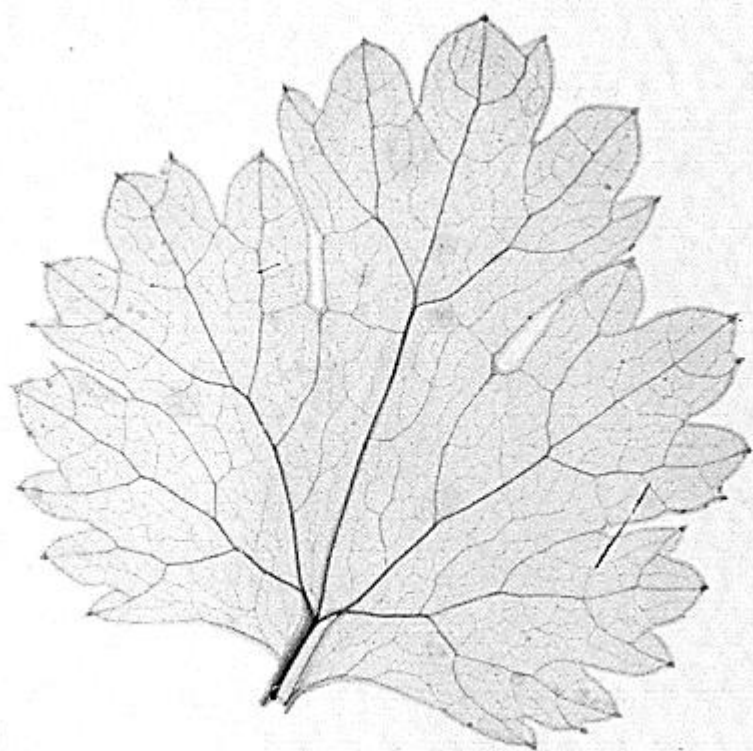
Figure 4.22: Chlorophyll a:b ratio (a) and total chlorophyll content per cm^2 (b) of the third true leaf of *C. sativum* cv. Cruiser grown under each light recipe (n=4). Bars show standard deviation from the mean and letters represent statistically significant differences ($P < 0.05$).

Leaves of the RBFR light recipe plants had the lowest chlorophyll a to b ratio (chlorophyll a/b) which was significantly lower than R ($P=0.015$), B ($P=0.013$), RB ($P<0.001$), RBG ($P<0.001$) and RBGFR ($P=0.001$) grown plants (Fig. 4.22a). RB recipe plants had leaves with the highest chlorophyll a/b ratio, significantly higher than R, B, RBFR ($P<0.001$) and RBGFR ($P=0.001$) grown plants. RBG plants also had leaves with significantly higher chlorophyll a/b ratios than R ($P=0.003$), B ($P=0.004$) and RBFR ($P<0.001$) plants. Total chlorophyll content (Fig. 4.22b) was higher in RB than RBGFR grown plants ($P=0.024$).

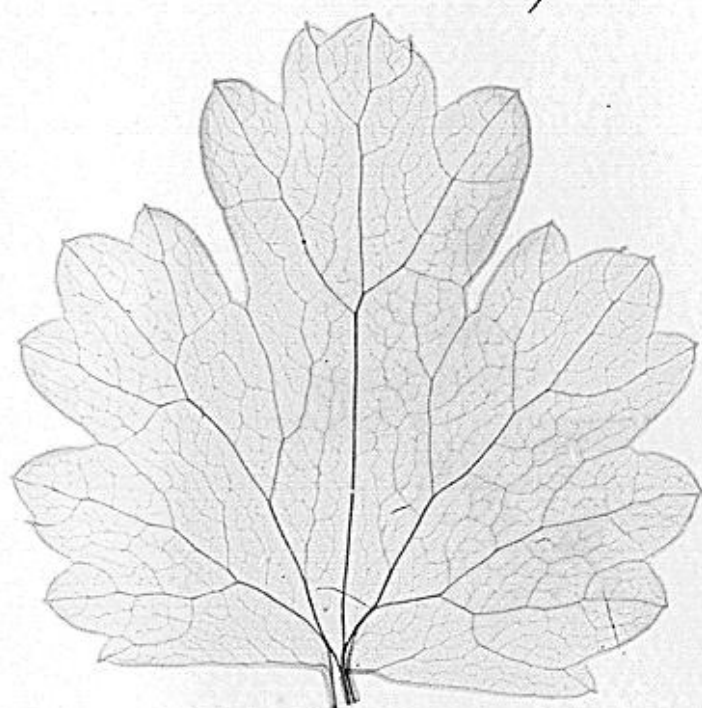
4.4.5 Visualisation of vein networks in *C. sativum* cv. Cruiser grown under combinations of red, blue and green light



(c)



(d)



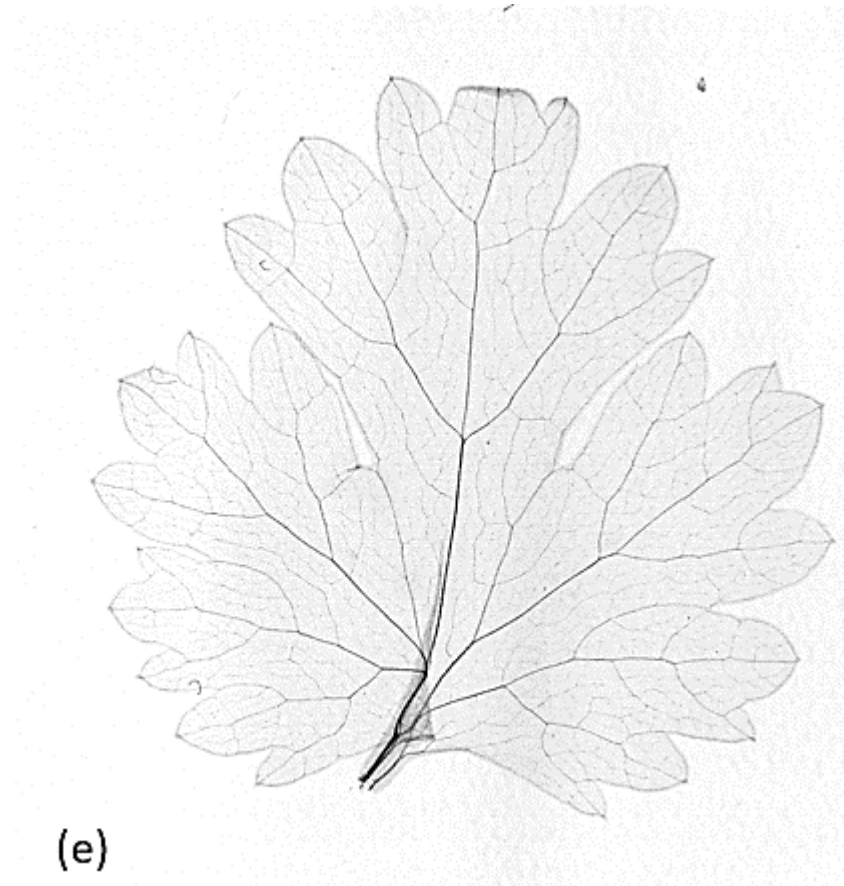


Figure 4.23: The fully expanded first true leaf from a plant in each light recipe (R, B, RB and RBG) cleared of pigment to allow for visualization of the vein network. Images were scanned at 1200dpi with a ruler for scale (a). Expanded, high resolution images (not to scale) of the individual B (b), R (c), RB (d) and RBG (e) light recipe leaves are also shown for better image clarity.

Leaves under all recipes have a similar number of veins and vein-branches (Fig. 4.23), suggesting that an increase in leaf area is down to inter-vein cell expansion and/or division rather than production of more vasculature.

4.4.6 Investigation into the differences in fresh-weight yield of cv. Standard and cv. Cruiser grown under the B light recipe

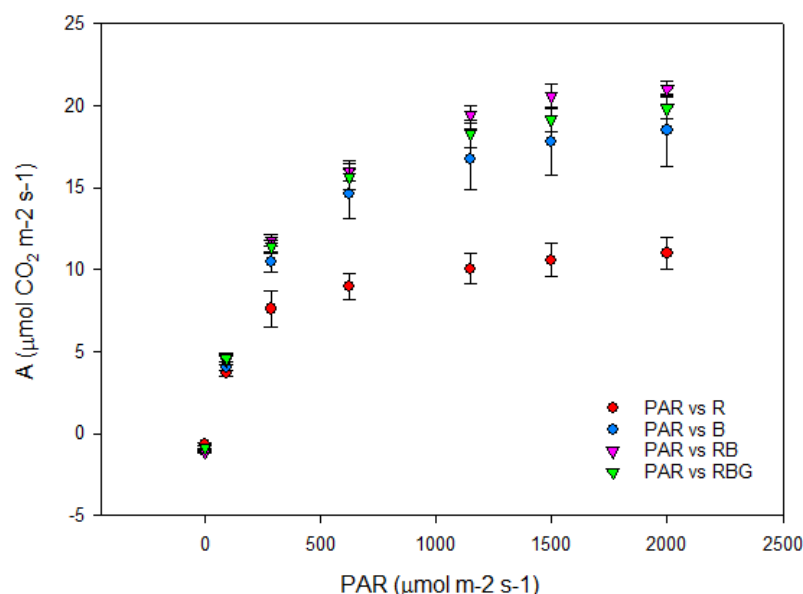


Figure 4.24: Photosynthetic light response curves measured with the LI-6400XT (LICOR) showing the carbon assimilation rate (A) of the third true leaves of *C. sativum* cv. Cruiser grown under each light recipe (n=4).

In cv. Standard B light recipe plants had significantly higher total fresh weights than RB and RBG light recipe plants (see Fig. 4.16), however in cv. Cruiser, they had statistically similar fresh weights to RB and RBG light recipe plants (Fig. 4.19a). As carbon assimilation rate was not previously measured in cv. Cruiser and root development had not been investigated in cv. Standard, further experimental work was carried out to find if either of these were the cause of the difference in the growth of the plants. As in cv. Standard (Fig. 4.12), B light recipe plants had statistically similar carbon assimilation rates to RB and RBG light recipe plants, however unlike in cv. Standard they were not the highest. The smaller stature of cv. Cruiser plants as compared to the cv. Standard plants

grown under the B light recipe could be therefore due to a slight decrease in photosynthetic capacity.

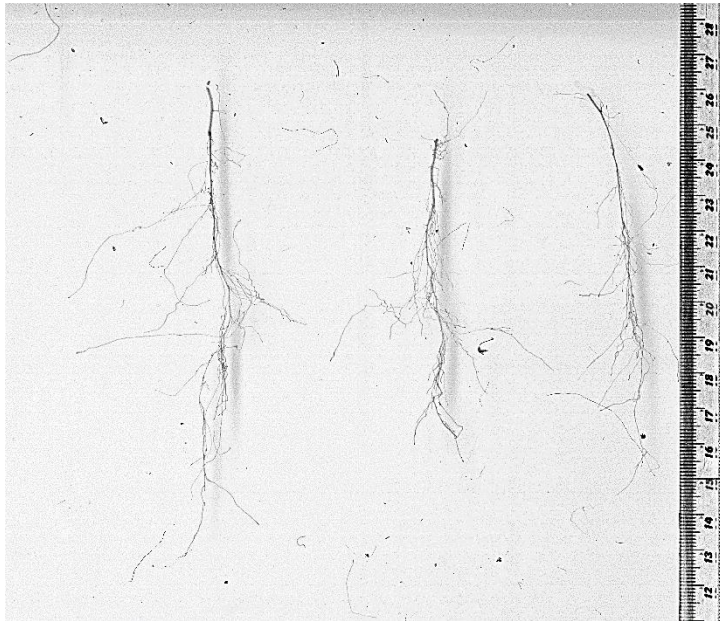


Figure 4.25: Roots of *C. sativum* cv. Standard grown under the B light recipe imaged with a flat-bed scanner at high resolution 21 days after sowing.

Roots of cv. Standard grown under the B light recipe appeared more developed than those of cv. Cruiser (see Fig. 4.19c), especially with regards to lateral root development. The better establishment of the root system in cv. Standard as compared to cv. Cruiser under the B light recipe could be the main cause of the differences in fresh weight yield between the two.

4.5 Discussion

4.5.1 Overview of novel observations

Wavelength-specific effects were observed in hypocotyl elongation, leaf expansion, stomatal distribution, photosynthetic stoichiometry, pigment content and absorption of different wavelengths, root architecture, photosynthetic capacity, whole-plant morphology (in terms of biomass partitioning between leaves, stems and roots) and fresh weight yield of stems and leaves and all are discussed in detail below. First, a summary of the truly novel observations is presented.

Monochromatic red light is unsuitable for plant growth due to a combination of factors. First, due to an absence of blue light, plants grown under only red light experience rapid elongation of the hypocotyl, producing long, thin stems which makes them susceptible to lodging (see section 4.5.3). The collapsing of the stem moves their leaves away from the growth lamp and reduces the amount of light they are exposed to. Although the leaves of R recipe plants are capable of absorbing a similar amount of PAR to B light recipe plants on a per unit leaf area basis (section 4.5.9), likely due to a lack of blue light, they only have a small surface area (see section 4.5.4) over which to absorb light and photosynthesise. This is compounded by the fact that due to a decreased photosynthetic capacity (see sections 4.5.7 and 4.5.8) leaves grown under monochromatic red light are incapable of using much absorbed light in photochemistry, all resulting in low fresh-weight yields (see section 4.5.10).

Novel data for the effects of quality of light applied to the shoots on root development is presented (section 4.5.6). There have been few studies into how light quality affects root growth in a natural growth environment, with light often being applied directly to the roots (see chapter 1.17). It is demonstrated here that a combination of red and blue wavelengths is essential for healthy root establishment in soil, with recipes containing multiple wavelengths (RB and RBG) producing better established root systems than those grown under monochromatic red or blue light.

Dark respiration but not the light compensation point appeared to acclimate to light quality (see section 4.5.8). Plants grown under all light recipes exhibited relatively low light compensation points, suggesting that they instead acclimated to the low intensity growth light, suggesting a decoupling of the acclimation of these two parameters.

Finally, green light had some interesting effects that are not yet reported in the literature. Recipes containing green light had a more even distribution of stomata between the adaxial and abaxial side of the leaf due to a decrease of stomata on the adaxial side. Also, the addition of far-red light to the red and blue light background caused a fall in chlorophyll a:b ratio, yet this was completely reversed by the addition of green light (see section 4.5.9). The increased height of the plants, which brought them closer to the growth light, combined with their large leaf surface area for photon capture and photosynthesis likely made the RBGFR light recipe plants the most ideally

acclimated to their growth light environment, resulting in high fresh weight yield (see section 4.5.10).

4.5.2 Germination

As there were no differences in germination rate and very little difference in germination success (Fig. 4.3), it can be assumed that coriander does not have a light requirement for the breaking of seed dormancy, or that this requirement was fulfilled by the red/far-red/blue light present in every recipe activating the phytochromes. Although phytochromes absorb most strongly in the red part of the spectrum, PHYA has also been shown to absorb weakly in the blue and mediate some responses associated with red light (Lin, 2000). As discussed in Chapter 1.15, the phytochromes are the only photoreceptors which have been shown to be directly involved in inducing the light-dependent seed germination process (Hennig *et al.*, 2002) by causing the increased production of the phytohormone gibberellin (GA), which acts antagonistically against another phytohormone, abscisic acid (ABA) to break seed dormancy (Oh *et al.*, 2007). A simple additional experiment attempting to germinate seeds in complete darkness and comparing germination rate and success with those under the light recipes could be conducted to clarify the response in *C. sativum*. It can be assumed that any of the light recipes are suitable for and would not be detrimental to germination.

4.5.3 Stem elongation

Hypocotyl elongation was most pronounced in plants grown under monochromatic red light, from seedling stage until harvest. Plants grown under monochromatic blue light and those under recipes containing far-red light also

produced longer stems, whilst those under combinations of red and blue, and red + blue + green light without far-red light were shorter (Fig. 4.4; Fig. 4.5). Increased blue light exposure has been shown to decrease stem and internode length by inducing cryptochrome mediated inhibition of cell extension in the short term and cell division in the long term (Dougher and Bugbee, 2004) however this was in combination with other wavelengths. Pronounced elongation of the hypocotyl under monochromatic red or blue light but not under the combination of red and blue suggests that stem elongation in coriander is under the control of multiple red and blue light photoreceptors and interaction of their downstream signalling pathways. Perhaps activation of both phytochrome and cryptochrome is needed to inhibit rapid elongation. Suppression of hypocotyl growth during de-etiolation has been shown to involve both CRY1 and PHYB (Cashmore *et al.*, 1999). They may also act additively in suppressing stem growth in mature plants and therefore only activation of one or the other would cause an elongated morphology. Far-red light is the main signal in shade avoidance syndrome (see Chapter 1.20) – where increased stem elongation occurs in response to a decrease in red to far-red light ratio in an attempt to break through the canopy and out of a low-light environment unfavourable for photosynthesis (Franklin and Whitelam, 2005). Therefore, the elongated morphology of plants grown under additional far-red light is likely an acclimation response co-ordinated by the phytochromes. As plants grown under combinations of red and blue and red, blue and green light would have experienced the additive effects of phytochrome and cryptochrome stem elongation suppression coupled with no red to far-red light

ratio, they developed a short-stemmed morphology. It appears the proportion of green light added when comparing the red and blue and red, blue and green light recipes was not enough to cause a green light induced shade avoidance response of hypocotyl elongation such as that reported by Sellaro *et al.*, 2010 and Wang and Folta, 2013, as height of plants under the RB and RBG light recipes were statistically similar throughout growth. The RB and RBG light recipes are therefore ideal for keeping the plant relatively short and upright – a characteristic required for coriander which is to be sold in pots.

4.5.4 Leaf expansion

Difference in leaf expansion of plants grown under the different light recipes can be observed from the very beginning. There were significant differences in the sizes of fully expanded first true leaves, which were likely due to differences in rate of intervein cell division and/or expansion (Fig. 4.6; Fig. 4.7). Plants grown under monochromatic red light produced smaller leaves than all other light recipes, although not significantly smaller than under the red and blue combination recipe (Fig. 4.7). Plants grown under monochromatic blue light likely had larger leaf areas due to increased blue light activation of PHOT1 in the leaf epidermal cells, promoting cell expansion (Sakamoto and Briggs, 2002). The additional blue light in the red and blue light combination recipe increased leaf cell expansion but was not enough to increase it significantly. With the addition of green and/or far-red light however leaves did become significantly larger. This is likely a shade response mediated by the phytochrome (Franklin and Whitelam, 2005) and cryptochrome (Sellaro *et al.*, 2010) photoreceptors.

Leaves grown under shade conditions produce fewer layers of palisade tissue (Kim *et al.*, 2005b) in order to spread the chloroplasts over a larger surface area rather than waste resources on producing them deep in the leaf where weak shade light will not reach (Tateno and Taneda, 2007). As mentioned previously, far-red light provides the main shade signal to the plant (Franklin and Whitelam, 2005) whilst green light has been implicated in the induction of mild shade avoidance responses (Wang and Folta, 2013). The thinning and expansion of the leaf in order to capture more PAR over a greater surface area is here likely an acclimation response being induced by the lowered blue to green light ratio and/or the decreased red to far-red light ratio. Interestingly, the addition of both green and far-red portions of the spectrum to the light recipe (RBGFR) did not produce a more pronounced shade response than the addition of green or far-red alone (RBG/ RBFR).

In conclusion, the RBG and RBGFR light recipes induced the most leaf expansion and would therefore be the most suitable for the growth of commercial coriander in terms of producing plants with a large leaf area. However, as mentioned in section 4.5.3, the far-red light in the RBGFR light recipe also increases stem elongation as part of the shade avoidance response, whilst the green light does not, meaning the RBG light recipe produces plants with a higher leaf to stem ratio. If plants are to be sold in pots, the RBG recipe which produces shorter, more upright plants would be the most suitable. However, if plants are to be sold dried or bagged, the RBGFR recipe, which produced a higher overall fresh and dry weight yield (Fig. 4.16) would be ideal.

4.5.5 Stomatal distribution and water use efficiency

Blue, red and far-red light via the CRY1, CRY2, PHYA and PHYB photoreceptors have been demonstrated to additively promote the production and maturation of stomata (Kang *et al.*, 2009), however there have been few studies on the effect of varying light quality in terms of proportions of wavelengths on the number and distribution of stomata over the leaf surfaces. Leaves of plants grown under monochromatic blue light produced the highest number of stomata overall (although statistically similar to the R light recipe) and on the adaxial (upper) leaf surface, and a statistically similar number to those grown under the RB, RBG and RBGFR light recipes, whilst those under the R and RBFR recipes produced the fewest (Fig. 4.8a). A study by Gay and Hurd, (1975) demonstrated that tomato plants grown under high light intensities produced more stomata on their adaxial leaf surfaces than a control grown under a lower light intensity. As discussed in chapter 1.21, blue light is often the signal to the plant that induces high-light acclimation responses (Hogewoning *et al.*, 2010). In high-light, it would be beneficial for the plant to increase the number of stomata in the upper leaf surface in order to increase gas exchange to be able to take advantage of this additional PAR and channel it into photosynthesis. The addition of blue light via the cryptochromes likely amplifies the red light induced phytochrome induction of stomatal development on the upper leaf surface. As far-red light is a shade signal (Franklin and Whitelam, 2005), the addition of far-red light in the RBFR light recipe likely caused an acclimation response whereby fewer stomata were produced on the upper leaf surface as there would be no advantage to using energy to producing them where little

gas exchange would be taking place under low light conditions. The addition of green light however in the RBGFR light recipe seemed to reverse this acclimation reaction, suggesting green light here is actually causing a more high-light acclimation reaction, possibly through the increased activation of red light absorbing Pr over the far-red light absorbing Pfr (see chapter 1.12).

On the abaxial (lower) surface of the leaf, plants grown under the monochromatic red light recipe (R) produced a higher but statistically similar number of stomata to the plants under the monochromatic blue light recipe (B), leaving them with the lowest adaxial to abaxial stomatal ratio (Fig. 4.8b). This ratio was statistically similar to the ratio produced by leaves of plants grown under the RBFR recipe, which produced a statistically similar number of stomata on the abaxial surface to plants under the RB light recipe. Increased localisation of the stomata to the lower leaf surface is likely a low-light acclimation response to position the portals for gas exchange where the leaf can capture diffuse light reflected from the leaves below. The addition of green light in the RBG and RBGFR light recipes decreased the number of stomata on the abaxial leaf surface, resulting in the green light containing recipes having adaxial to abaxial stomatal ratios of closest to 1:1. Again, green light seems to be having the unusual effect of causing a more high-light acclimated response, although it could simply be inhibiting stomatal production on the abaxial leaf surface by reversing the blue-light cryptochrome response (as described in chapter 1.11). The RBG and RBGFR light recipes also appeared to have increased (although not significantly) increased intrinsic water use efficiency from the adaxial surface under high light levels (Fig. 4.14). Therefore, the

inclusion of green light in the growth-light recipe may not only have the advantage of increasing leaf surface area (as discussed in section 4.5.5) but also could benefit the grower in terms of water savings.

4.5.6 Root development

There were clear differences in root establishment between plants grown under different combinations of red, blue and green light (Fig. 4.19). In *C. sativum* cv. Cruiser, plants grown under monochromatic blue light had the shortest root network length and rather than elongation or the production of many lateral roots appeared to form a shorter, thicker primary. Plants grown under monochromatic red light produced a longer, thinner tap root and plants under the red and blue (RB) and red, blue and green (RBG) combination light recipes produced the largest root networks with the best developed lateral roots. As discussed in chapter 1.17, the above-ground light environment is known to directly influence root architecture (Lee *et al.*, 2016). Evidence suggests that although blue light activation of the phototropins and cryptochromes can initiate primary root formation (Canamero *et al.*, 2006), activation of PHYA/ PHYB is required to initiate lateral root branching (Salisbury *et al.*, 2007) and primary root elongation (Correll and Kiss, 2005). This may be why plants grown in the absence of red light (the B light recipe) had reduced root growth. The additive effects of phototropin, cryptochrome and phytochrome induced root growth in the plants grown under the RB and RBG light recipes are likely to have resulted in the significantly increased root network length. Root growth was not so inhibited under the monochromatic blue light recipe in *C. sativum* cv. Standard, which is likely why the plants were

able to grow so large (giving equivalent yields to the plants grown under combinations of wavelengths – see section 4.4.6) as they developed a healthy root network for the acquisition of water and nutrients. This highlights the complexity of the plant response to light and that even plants of the same species may react differently to different light qualities. An ideal light recipe for healthy root growth and development would therefore appear to need to include multiple wavebands of light as monochromatic red light is not and monochromatic blue light may not always be suitable.

4.5.7 Efficiency of photosystem II and electron transport

Light response curves of chlorophyll fluorescence revealed that plants grown under the monochromatic blue light recipe (B) had the highest quantum efficiency of photosystem II (Φ_{PSII}) and also ETR on both a total and absorbed light basis, whilst plants grown under the R and RBFR light recipes had the lowest (Fig. 4.9). As discussed above and in chapter 1.21, plants exposed to high levels of blue light often develop leaves acclimated to high light intensities. Mainly due to activation of the phototropins, they have been demonstrated to develop a thicker, ‘sun-leaf’ morphology with more layers of chloroplast packed palisade cells (Kim *et al.*, 2005a; Tatenos and Taneda, 2007). They have also been observed to have a higher photosynthetic capacity (Hogewoning *et al.*, 2010; Hoffmann *et al.*, 2015) possibly due to this increased ability to capture light coupled with a greater capacity for development and maintenance of the molecules which make up the photosynthetic electron transport chain, which is induced by activation of CRY1, CRY2 and PHYA (Thum *et al.*, 2001; Tsunoyama *et al.*, 2004). The plants grown in the absence of blue light (the R light recipe)

were likely unable to develop and maintain parts of the photosynthetic electron transport chain and those under the recipe with the addition of both green and far-red light likely acclimated their electron transport chain to low-light conditions. As mentioned above and in chapter 1.20, both green and far-red light provide shade signals to the plant. Shade grown plants often reduce the production of unnecessary components of the electron transport chain (Walters, 2005). The efficiency of photosystem II in plants with either additional green or far-red light was lower than that of blue, but not significantly different from plants grown under the RB recipe, indicating it is the additive response of the two that caused this low-light acclimation response of the photosynthetic apparatus. Green light has been shown to down-regulate some of the genes that code for proteins which support the photosynthetic apparatus (Dhingra *et al.*, 2006), which would explain this additional layer of reduced photosynthetic capacity. As the plants in this study were grown under relatively low light intensities, the plants grown under light recipes containing green and far-red light (RBG and RBGFR) were well acclimated to their light environment and produced relatively high yields. It would be beneficial to investigate further, with increasing growth-light intensities, to find the intensity at which shade acclimation signals induced by the green and far-red light become detrimental to crop yield.

4.5.8 Carbon assimilation rate and photochemistry

The calculation of $J_{\max}$ from gas analysis of carbon dioxide response curves is another way to determine maximum rate of photosynthetic electron transport (Farquhar *et al.*, 1980). Again, plants grown under the B light recipe, along with

plants grown under the RB and RBG light recipes, were shown to have high rates of photosynthetic electron transport whilst plants grown under the R and RBGFR light recipes had significantly lower rates (Fig. 4.15). The RBFR recipe resulted in plants with a J_{max} lower than B, RB and RBG plants but higher than those under the R and RBGFR recipe, suggesting that the far-red light caused a slight low light acclimation of the ETC whilst green light did not, and that when far-red and green light were combined this caused a strong low light acclimation response.

V_{cmax} is the maximum rate of carboxylation given by the amount of active ribulose 1,5-bisphosphate carboxylase (rubisco), which catalyses carbon dioxide into the PCR cycle (Chapter 1.6; Farquhar *et al.*, 1980; Sharkey, 1989) and followed a similar pattern (Fig. 4.15b). V_{cmax} appears to have acclimated in the same way as the ETC, only this time plants grown under monochromatic red light had a response similar to those grown under the RBFR light recipe and not as pronounced as those grown under the RBGFR light recipe. Indeed, gas analysis of light response curves revealed that maximum carbon assimilation rate (A_{max}) was highest in plants grown under the B and RBG light recipes and up to 75% higher than plants grown under the R and RBGFR light recipes (Fig. 4.13c).

R_d is the background respiration from plant cells i.e. CO_2 evolution from mitochondria not associated with the photosynthetic carbon reduction cycle (Farquhar *et al.*, 1980) and is generally higher in high-light acclimated plants (Bazzaz and Carlson, 1982) due to the higher availability of carbohydrates in the

leaves that is assimilated during the periods that the plant is exposed to the light and the greater amount of leaf tissue per unit leaf area (Loveys et al., 2003). Therefore, it would be expected that plants grown under the B and RBG light recipes, with higher rates of photosynthesis, would have higher dark respiration rates. Dark respiration (R_d) rates were higher in plants grown under the B light recipe ($P < 0.05$) and the RBG (not statistically significant) light recipes (Fig. 4.13a). Plants grown under the monochromatic blue light recipe also had higher (but not statistically significantly) light compensation points (LCPs) than all other light treatments. Plants acclimated to low light intensities often have lower LCPs in order to take advantage of all available light for photosynthesis (Walters, 2005). It might be possible that plants used light quantity rather than light quality to acclimate here, as $110\mu\text{mol}$ is a low light intensity, similar to that found at the middle/ bottom of a canopy (see chapter 3). This links in with the theory that acclimation to light intensity and light quality can be decoupled, as reported by Hogewoning *et al.*, 2010 and discussed further in section 4.5.9.

One thing to consider is that the GFS-3000 used to measure carbon dioxide and light response curves uses a mixture of 10% blue light and 90 % red light and the JuniorPAM used to measure the efficiency of photosystem II uses only monochromatic blue light to excite the photosynthetic apparatus. Due to differences in pigment concentrations and light spectral absorption profiles (see section 4.5.9 below) plants may react differently to this light than the light under which they are grown. For this reason the spot measurements of photosynthesis under the growth room light spectral conditions (Fig. 4.11) may

be more indicative of the actual rate of carbon assimilation of plants during growth.

The spot measurements revealed that under the light intensity and quality that the plants were exposed to during development, plants under the RB recipe had the highest carbon assimilation rate per unit leaf area, followed by plants grown under the RBG and then the B and RBGFR light recipes (Fig.4.11a). Plants grown under the R and RBFR light recipes had the lowest carbon assimilation rates per unit leaf area. However, after using leaf area to adjust the values to the whole leaf scale (Fig. 4.11b), leaves of plants grown under B, RB, RBG and RBFR light recipes had statistically similar carbon assimilation rates whilst those developed under the RBGFR recipe had slightly lower rates and those developed under the R light recipe had the lowest. The larger leaf areas of plants under the B, RBG, RBFR and RBGFR light recipes made up for their lower photosynthesis per unit leaf area than RB plants, whilst plants grown under monochromatic red light had both small leaves and low photosynthesis per unit leaf area.

4.5.9 Leaf chlorophyll content and absorption spectra

As discussed in chapter 1.18, the leaf photosynthetic apparatus acclimates to light quality in order to balance photosystem excitation with carbon assimilation and best optimise photosynthetic efficiency. Increasing levels of far-red light are known to 'over-excite' chlorophyll *a* containing PSI and this imbalance induces the production of more chlorophyll *b* containing PSII, resulting in a lowered chlorophyll *a*:*b* ratio (Chow *et al.*, 1990; Anderson *et al.*, 1995; Murchie and Horton, 1998; Murchie *et al.*, 2002; Walters, 2005). This

phenomenon can be observed in Fig. 4.7 where the addition of far-red light to the blue and red light background (the RB versus RBFR recipe) resulted in a significantly lowered chlorophyll a:b ratio ($P < 0.05$). Also discussed in chapter 1, due to the different absorption profiles of the chlorophylls, an increase in chlorophyll *b* leads to higher absorption in the green (400-500nm) area of the spectrum (Fig. 1.4; Rabinowitch 1969). This can be observed in Fig. 4.21, where the RBFR light recipe leaf absorption spectrum notably shifts further into the green than those of the other light recipes.

As expected, the monochromatic blue light recipe (B) used in both parts of the experiment resulted in a high chlorophyll a:b ratio. Blue light is known to be involved in the process of photoacclimation to high light intensities (Hogewoning *et al.*, 2010; Hoffmann *et al.*, 2015) and would be preferentially absorbed by PSII resulting in production of more chlorophyll *a* containing PSI. The reaction of the chloroplasts to the monochromatic red light recipe (R) however differed between the experiments using *C. sativum* cv. Standard and *C. sativum* cv. Cruiser. In cv. Standard, growth under monochromatic red light resulted in a lowered chlorophyll a:b ratio when compared to all other light recipes, except the RBFR recipe, to which it was statistically similar (Fig. 4.7). However in cv. Cruiser, chlorophyll a:b ratios higher than those under the RBFR recipe and statistically similar to the monochromatic blue light were observed (Fig. 4.22). This was reflected in the similar absorption profiles of the leaves of plants grown under monochromatic red and blue light (Fig 4.20). Perhaps cv. Cruiser contains more red light absorbing accessory pigments than Standard which are passing red photonic energy to PSII and promoting production of

more chlorophyll *a* containing PSI. Due to the lack of an integrating sphere during the experiment using cv. Standard, and then a lack of seed, the absorption profile of only one leaf per light recipe of cv. Standard was measured and differences in absorption spectra were unclear (Fig. 4.20; 4.21). It would be interesting to repeat the experiment to see if how more accurate absorption spectra of leaves of cv. Standard compare to those of cv. Cruiser and if they also reflect the chlorophyll *a*:*b* ratios observed.

Perhaps the most interesting results observed however related to the addition of green wavelengths to the light recipes. As discussed at length in chapters 1 and 3, green wavelengths appear to provide positional signals via the non-photosynthetic photoreceptors to the plant which help it acclimate to low light, shaded conditions (Folta, 2004; Zhang *et al.*, 2011). However, green light is also preferentially absorbed by chlorophyll *b* and therefore will excite PSII. The addition of green light to both red and blue and red, blue and far-red light backgrounds (RB and RBFR vs RBG and RBGFR) resulted in significantly increased chlorophyll *a*:*b* ratios (Fig 4.7; Fig. 4.22) suggesting that the opposite reaction of that to the addition of far-red light occurs – PSII over-excitation in response to green light causes production of more chlorophyll *a* containing PSI. As chlorophyll *a*:*b* ratios of the RBG and RBGFR light recipe leaves were statistically similar, this also suggests that the fall in chlorophyll *a*:*b* ratio resulting from the addition of far-red light to the red and blue light background was completely reversed by the addition of green light which is a novel observation.

As shown in chapter 3, the ratios of light in the RBG and RBGFR light recipes are actually similar to those found in natural sunlight at the top of the canopy in the field or glasshouse (Fig 3.1), with the RBGFR recipe most closely matching the sunlight spectrum. Despite the relatively low light intensity of $110\mu\text{mol m}^{-2} \text{s}^{-1}$, the photosystem stoichiometry appears to have acclimated to the light quality and therefore the chlorophyll a:b ratio closely relates to that of a leaf which has developed at a high natural sunlight intensity at the top of a canopy in field conditions.

Recent work by (Hogewoning *et al.*, 2012) confirms that leaves grown in an artificial shade light spectrum (with the highest intensity light being above 680nm) have a higher quantum yield for CO₂ fixation when exposed to PSI - exciting wavelengths than plants grown in an artificial full sunlight spectrum or a spectrum enriched in blue light (all of the same intensity). Chlorophyll a:b ratios were lowest in the shade light grown plants but these plants also had the lowest PSI:PSII ratio. Antenna size of the LHCs of the shade spectrum plants were similar to those of the sunlight spectrum grown plants however. As plants exposed to low light intensities often acclimate by increasing LHC antenna size in order to absorb more of the available light (Walters, 2005), this suggests that the acclimation processes mediated by overall light intensity and light quality are distinct. It could be possible that, like one enriched with far-red light, a growth spectrum enriched in green light is causing changes in photosystem stoichiometry distinct from those caused by light intensity.

In terms of absorption spectrum however, in cv. Cruiser, the two recipes containing green light appear to only start absorbing light quite far into the blue region of the spectrum. In the case of the RBGFR recipe leaves this may be due to their lowered overall chlorophyll content (Fig. 4.7; Fig 4.22) lowering their absolute absorption across the spectrum added to the fact that the light source used in for the integrating sphere containing too little blue light meaning that the measurements did not register properly with the spectroradiometer. However, a similar phenomenon was observed in leaves grown under the RBG recipe which had relatively high overall chlorophyll content (Fig. 4.7; Fig 4.22) and therefore it is likely that additional physiological factors are at play. The addition of green light may be causing the production of less blue light absorbing carotenoids for example. As discussed in chapter 1.21, blue light signaling via the cryptochromes is known to stimulate photoprotective mechanisms such as the production of pigments such as zeaxanthin which enable the plant to safely remove excess excitation energy through the process of non-photochemical quenching. Green light however is known to alter the redox state of one of cryptochromes' chromophores, deactivating the photoreceptor and reversing many blue light induced physiological responses (Frechilla *et al.*, 2000; Talbott *et al.*, 2002; Bouly *et al.*, 2007; Banerjee *et al.*, 2007). In this way, green light seems to be a sort of paradox, causing both high-light acclimation related reactions via the photosynthetic photoreceptors and low-light acclimation related reactions via the non-photosynthetic photoreceptors.

4.5.10 Assimilate portioning and yield

The highest total fresh and dry weight yields of *C. sativum* cv. Standard were observed in plants grown under monochromatic blue light (B) RBFR and RBGFR light, although yields of RB and RBG plants were not significantly different from RBFR or RBGFR plants. Plants grown under the monochromatic red light recipe produced the lowest yields (Fig. 4.16). It is likely that the RBFR and RBGFR plants gave high yields despite having a lowered photosynthetic capacity due to their increased leaf surface area over which to photosynthesise and their increased stem elongation which allowed them to grow closer to the light, and therefore some of their leaves to receive a higher light intensity than the RB or RBG light recipe plants. An example of excellent acclimation to the light environment came from the RBGFR light recipe plants which were grown under low light intensity but in a light quality which would be similar to that found at the top of the canopy in high light. The plants developed a lower chlorophyll content per unit leaf area and spread the LHCs out over a wider surface area in order to capture more light, which is characteristic of acclimation to low light intensity. The chlorophyll a:b ratio however appeared to acclimate to light quality and not quantity, remaining high and therefore still enabling the most efficient delivery of photons into the ETC.

In *C. sativum* cv. Cruiser, total above ground fresh weight of plants grown under monochromatic blue light was not higher but statistically similar to RB and RBG light recipe plants (Fig. 4.18). Upon further investigation it was determined that this was not due to a decrease in photosynthetic capacity, which remained high (Fig. 4.24), but likely due to the roots of cv. Standard being significantly more

developed than those of cv. Cruiser (Fig. 4.25). This highlights the complexity of the plant response to light by showing that physiological characteristics of plants even of the same species grown under monochromatic blue light can be variable.

Leaf to stem ratio was highest in RB and RBG light recipe plants, giving them a more desirable morphology (as discussed in section 4.1) however resulting in them receiving a lower light intensity for growth due to being further from the lamps. Plants grown under the monochromatic red light recipe (R) also experienced rapid stem elongation but had thin stems making them susceptible to lodging (Fig. 4.3) and therefore not bringing them much closer to the light. They were also shown to absorb as much light per unit leaf area as blue light (Fig. 4.20), but were likely unable to use this light in photochemistry due to their decreased capacity for photosynthesis. This coupled with their inhibited leaf expansion is likely what resulted in them giving such a small overall yield.

4.6 Conclusions

The aim of the study was to identify an ideal 'light recipe' for the commercial growth of coriander by identifying the wavelengths which must be provided to enable specific aspects of plant growth and development which increase crop quality and yield. Both suitable and unsuitable light recipes were identified and the reasons are justified with physiological data.

As hypothesised, light recipes containing combinations of wavebands rather than single wavebands produced the highest yielding plants and those of the best quality when looking for commercially desirable characteristics such as

high leaf to stem ratio. Photosynthetic capacity per unit leaf area was also shown to be able to be influenced by light quality alone.

It can be concluded that monochromatic red light was unsuitable for plant growth as blue light was crucial for many aspects of healthy growth and development, such as leaf expansion, stomatal development and stem and root elongation and branching. Blue light was also a key determinant in increasing photosynthetic capacity as it greatly affected photosynthetic stoichiometry/ leaf pigment content, enzymes such as rubisco and components of the electron transport chain.

However, the addition of blue light was not the only factor that influenced quality and yield. In fact, the best yields came from plants which successfully acclimated to the low-light environment in which they were grown. Far-red light gave plants in the RBFR and RBGFR light recipes an advantage, increasing their yields when compared to plants in the RB and RBG recipes, as it enabled better acclimation to the low relatively light intensity. A combination of both green and far-red light enabled the best acclimation of the photosynthetic stoichiometry by allowing both acclimation to light intensity and light quality.

Based on the conclusions of the study, a number of recommendations for growers can be made:

- Monochromatic red light is only suitable as a sole light source before the seedlings emerge from the soil, after which, blue light is crucial for healthy development of roots and shoots.

- Recipes containing green light, i.e. RBG or RBGFR may be preferable over those not containing this waveband, i.e. RB and RBFR, due to increased leaf expansion and possible increases in canopy water use efficiency.
- If growing coriander at low light intensities for sale in bags or for drying and sale of ground coriander, as the stems also have a strong flavour, the best recipe to use would be the highest yielding. Due to variable response of the root development to monochromatic blue light and therefore differences in yield, the RBGFR light recipe would be recommended.
- However, if coriander is being grown for sale in pots, a suitable morphology for a robust, upright plant with a high leaf to stem ratio is needed, and the RBG light recipe would be preferential.

In future work, it may be beneficial to further investigate the effects of green light on crop water use efficiency, and to determine whether the ideal light recipe would change at different growth-light intensities.

5 Red LED lamps boost photosynthesis and fresh-weight yield for more crop per watt than conventional HPS

5.1 Introduction

Much of the world's food is produced in glasshouses. For example in 2015, over 25% percent of total recorded UK yield of fruit and vegetables came from glasshouse cultivated crops (Defra, 2015a). As rising world population puts an increasing strain on natural resources such as arable land, fossil fuels and water there is a growing pressure to produce more food in less space with minimum inputs i.e. less gas, electricity, water and fertilisers (Murchie *et al.*, 2009; Pinho *et al.*, 2012). It is therefore important to find areas of glasshouse horticultural production that can be further optimised in terms of energy and plant resource capture efficiency. Glasshouse supplementary lighting is crucial in the horticultural industry, allowing the continuous year-round production of protected (non-field) crops of a high yield and quality to meet consumer demands. Growth lamps are often needed to supplement natural light, particularly during the winter months, to extend day length to lengthen the growing season (Heuvelink *et al.*, 2006). Electricity used for supplementary lighting currently contributes, depending on species and environmental conditions, approximately 20-30% of energy usage and yearly glasshouse crop production costs (Brumfield, 2007; Ozkan *et al.*, 2011) and is therefore a prime target for energy and cost reduction through optimisation.

For the purpose of adding light to promote biomass accumulation commercial growers have commonly used broad-waveband, white light sources of artificial

lighting such as tubular fluorescent (TF), metal halide (MH), high-intensity discharge (HID) and, most popular, high-pressure sodium (HPS) lamps (Pinho *et al.*, 2012). It could be argued that these light sources are inefficient as they produce wavelengths that the plant does not need for improved growth or development. In all higher plants, the photosynthetic photoreceptors chlorophyll a and b along with accessory pigments such as the carotenoids and carotenes absorb wavelengths between 400 and 740nm and convert this photonic energy to chemical energy for use in the process of photosynthesis enabling accumulation of biomass (Croce *et al.*, 2003; Zhu *et al.*, 2008; Walla *et al.*, 2014). Photons of wavelengths between 400 and 700nm are therefore referred to as photosynthetically active radiation (PAR). However different wavelengths of PAR are absorbed with varying efficiency, with peaks in the blue (400-500nm) and red (600-700nm) (Zhu *et al.*, 2008) and, depending on species, complete reflection of up to 50% of the green (500-600nm) (Terashima *et al.*, 2009).

Conventional broad-band white lights emit a high percentage of green light; for example, HPS lamps emit approximately 31% green light when taken as a percentage of total plant photoreceptor detectable wavelengths (see figure 2.11b) or 48% green light when taken as a percentage of total PAR. Although there is evidence from experiments in controlled environment growth rooms that growing plants in PAR made up of up to 24% green light can be beneficial to growth, mainly due to the unique ability of green photons to penetrate deep into the crop canopy or folded leaf structures and drive photosynthesis (Kim *et al.*, 2004), the amount provided by HPS lamps is excessive. Furthermore, a large

amount of higher wavelengths are produced by conventional white-light lamps (Pinho *et al.*, 2011; Figure 2.11a) meaning that much of the photonic energy is wasted as heat despite it having a heating effect on plants and soil. To prevent heat damage to the crop, excessive heat from the lamps often has to be actively vented from the glasshouse requiring additional energy costs (Nelson and Bugbee, 2014b) and lamps must be suspended high above plant level. As commercial glasshouses often leave wide gaps between growth bays to enable workers and machinery to efficiently monitor, maintain and harvest the crops, the height and the wide beam angle of the light causes an inability to focus supplementary lighting directly onto the plants which often results in the wasting of useful light to the surroundings (Nelson and Bugbee, 2014b).

Light-emitting diodes (LEDs) however are narrow band devices capable of emitting light at wavelengths that specifically target the photosynthetic photoreceptors to drive photosynthesis and increase biomass. They are fast becoming popular with growers and researchers due to a variety of advantages that they have over conventional artificial light sources, including higher energy efficiency, longer life and high degree of programmability (Morrow, 2008; Massa *et al.*, 2010). and red and blue LEDs are set to surpass conventional forms of lighting in terms of photon flux efficacy ($\mu\text{mol/J}$) and luminous efficacy (lm/W) by the year 2020, and already have a higher electrical efficiency for producing photosynthetically active radiation (PAR) (Pinho *et al.*, 2011). As LEDs emit very little radiant heat (see figure 2.11) this allows them to be placed nearer to biological material than conventional lighting (Massa *et al.*, 2010). Coupled with the ability to focus the light beams with customisable lenses, this

ensures that more light is intercepted by the canopy, further increasing the overall energy efficiency of the system and decreasing power costs.

When connected to control systems with ambient (natural) PAR level sensors, conventional lamps are typically switched either on or off when ambient PAR falls below or rises above a set threshold determined to be adequate to saturate photosynthesis. This threshold can be determined by measuring a photosynthetic light response curve and determining the level of PAR above which carbon assimilation rate no longer rises.

Conventional filament bulbs such as those found in HPS lamps have a warm up time to reach full output and once switched off require a long cool down (typically 30 minutes) before it is safe to switch them back on without the risk of damage (Massa *et al.*, 2010), meaning that they cannot always quickly react to changes in ambient PAR, often leaving plants in suboptimal light conditions.

LEDs however are solid state, semiconductor devices which do not burn a filament (Humphreys, 2008). This not only greatly increases their longevity but makes them the only light source able to switch almost instantaneously between 0 and 100% output without change to their spectral output or damage to the hardware (Morrow, 2008). The advantages explained above provide a compelling case for the movement away from glasshouse crop cultivation

under conventional light sources towards LED lighting systems. However, it must first be determined that crops of the same or higher quality and yield can be produced with LED lamps with an energy saving in comparison to conventional lighting systems.

Most growers use supplementary lighting during the winter, when day photoperiods are short and need extending, and light levels are low and the photonic energy for use in photosynthesis needs topping up (Heuvelink *et al.*, 2006). However, PAR received from natural sunlight in the spring/summer months might not always saturate photosynthesis, especially in the early morning or late afternoon. Natural light levels constantly fluctuate and may range from incredibly low to short bursts of intense sunlight known as sunflecks (Murchie and Niyogi, 2011). Supplementing a constant, low level of PAR throughout summer days could increase yield by keeping photosynthesis closer to saturation, the photosynthetic apparatus acclimated to high light intensities over cloudy/ less bright days and the stomata open ready for gas exchange in shorter periods of cloud, better enabling the plant to take advantage of sudden increases in available solar radiation.

Over the past 30 years (1985-2015), glasshouse area designated to the cultivation of lettuce has been consistently higher than all other glasshouse

vegetable crops (Defra, 2015*b*). In 2015, *Lactuca sativa* L (lettuce) was grown across 352 out of a total of 861 hectares dedicated to protected (not field) vegetable crop production, with a marketed yield of 38 tonnes per hectare or 13.4 thousand tonnes. However, whereas marketed yield of crops such as tomatoes has nearly doubled since 1985 (218.1 – 419.7 thousand tonnes), lettuce yields appear inconsistent and have changed relatively little fluctuating between 31.6 and 43.8 thousand tonnes over the same time period. One reason for the increase in tomato yield may be due to advances in growing practices which allow increased capture of water, nutrients, light and carbon such as a move towards hydroponically, wire grown tomatoes with intra-canopy lighting in glasshouse compartments supplied with additional CO₂ (Mitchel *et al.* 2012). Lettuce however is still typically grown flat on benches with conventional overhead high-pressure sodium (HPS) lighting. This coupled with the fact that lettuce is so widely grown makes lettuce an ideal candidate for testing LED systems.

5.2 Aims and hypotheses

This experiment was therefore designed to directly compare lettuce growth under conventional HPS lighting and LED lighting in a realistic glasshouse environment, paying particular attention to effects on yield, conversion of electrical energy to biomass (crop per watt) and differences in plant physiology and photosynthetic efficiency that may account for changes in intrinsic conversion efficiency. HPS lamps were replaced with low powered LED floodlights providing the same level of PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$), which are widely available, requiring little additional infrastructure to be added to the glasshouse for installation – a setup which could easily be mimicked in a small commercial glasshouse for a low cost. Red LEDs were chosen as they are currently the most energy efficient colour available known to drive photosynthesis (Pinho *et al.*, 2012) and blue LEDs were tested due to the knowledge that they both contribute to photosynthesis and also to acclimation of plants to high light intensities (Hogewoning *et al.*, 2010). For full details of the materials and methods used in this chapter please refer to chapter 2.3.

The main aims of the experiment were to:

- a) determine whether a similar quality and yield can be produced with supplementary monochromatic LED red or blue lighting as with conventional supplementary HPS lamps in summer glasshouse grown lettuce
- b) determine whether supplementing $50\mu\text{mol m}^{-2} \text{ s}^{-1}$ of HPS, or monochromatic red or blue LED light in summer lettuce can increase yield in comparison to a sunlight-only control
- c) investigate the photomorphogenic/physiological effects that different qualities of supplementary lighting may have on the plants
- d) determine the level of light required and when to apply supplementary light to keep photosynthesis near saturation and boost yield
- e) compare the efficiency of the supplementary lighting - 'crop per watt'

Initial hypotheses were:

- a) The small amount of supplementary light, particularly blue light, will keep the plants acclimated to higher light intensities, allowing them to take advantage of sunflecks.
- b) Supplementation with LED lamps will be more cost effective than supplementation with the HPS lamp.

- c) Even during the summer months, supplementary light will increase yield.

5.3 Methodology

Two varieties of summer lettuce, *Lactuca sativa* L. cv. Lobjoits Green (further referred to as 'cv. L.G.') and *Lactuca sativa* L. cv. Match (referred to as 'cv. Match') were grown in a south facing glasshouse in Leicestershire, UK. During daylight hours plants were supplemented with a constant $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ of either high-pressure sodium, monochromatic red LED or monochromatic blue LED light. The first run of the experiment which took place between May and June 2014 is further referred to as 'run 1' whilst the second run of the experiment which took place between June and July 2014 is referred to as 'run 2'. Light levels at plant height and sunlight levels at the local weather station, as well as a range of physiological measurements, were recorded during growth of the plants. Fresh and dry weight yields were compared at harvest. For full details of the materials and methods used in this chapter, please refer to chapter 2.3.

5.4 Results

5.4.1 Measured light levels

There was a significantly higher average natural sunlight level (detected by the Sutton Bonington weather station) over the growth period in run 2 ($P=0.025$). However natural sunlight levels as measured by the MONITORING-PAMs at plant height in the glasshouse were not significantly different in run 1 and 2 over the 3-6 days measured and were in fact slightly higher in run 1 (see Fig. 5.1b). The lamps appear to be adding slightly over $50\mu\text{mol m}^{-2} \text{s}^{-1}$, closer to $60\mu\text{mol m}^{-2} \text{s}^{-1}$, which can be attributed to natural light variation across the glasshouse due to its position, with less light naturally reaching the sunlight control bench. However, in both runs overall PAR received at plant height at each supplementary light treatment bench was statistically similar, and all supplementary light treatment benches received significantly more PAR than the sunlight only control bench ($P<0.001$).

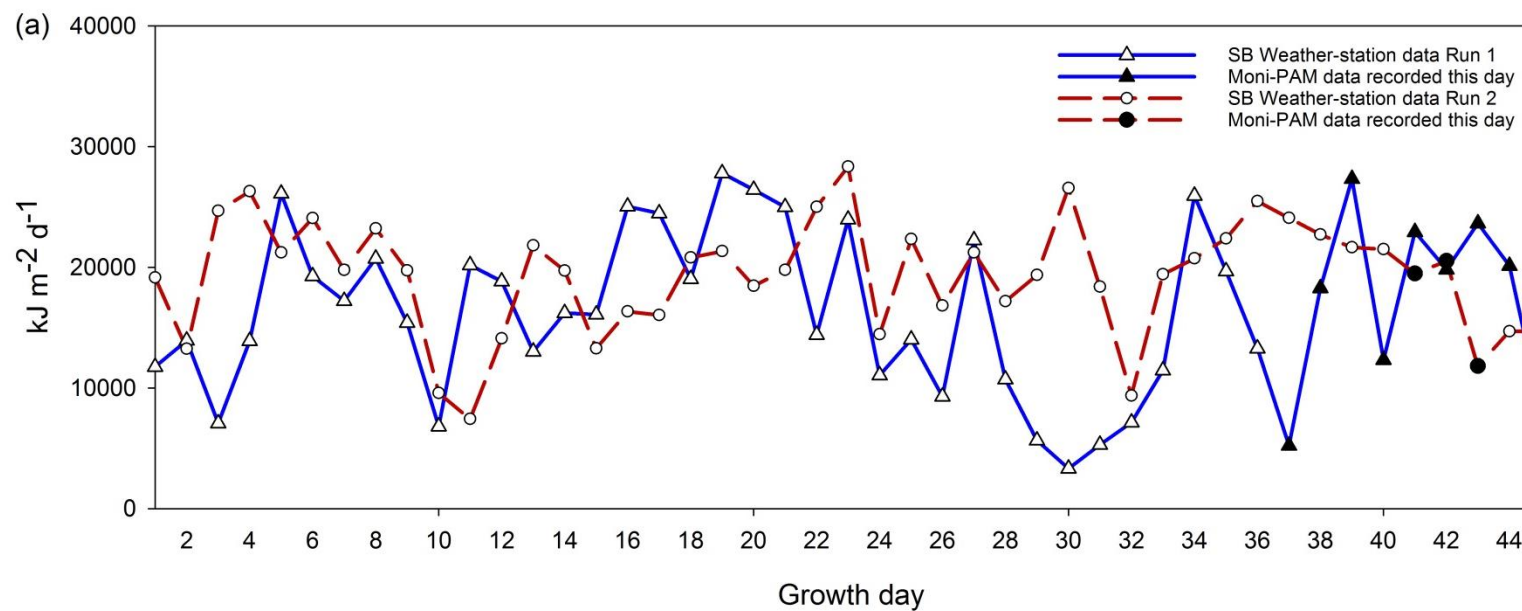
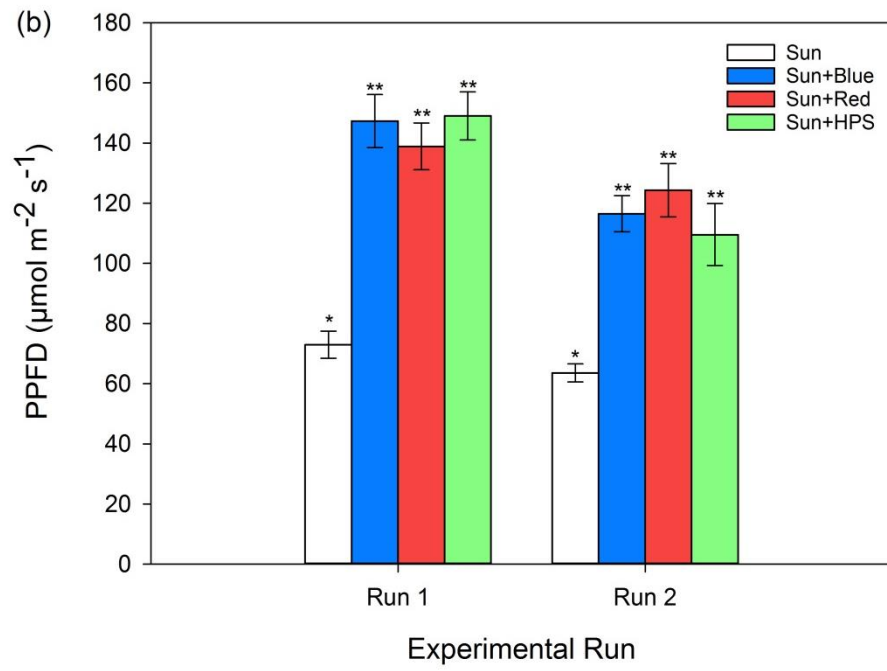


Figure 5.1: (a) Daily average PAR (kJ m⁻² d⁻¹) as measured by the Sutton Bonington Met Station from a rooftop over the two 45-day growth periods in Summer 2014. Filled in data points indicate Moni-PAM (Walz) data was also recorded on this day (see Fig 5.1b.). Results indicate a high level of variation between bright and cloudy days over the growth period.



(b) Average daily PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$) as measured by MONITORING-PAM chlorophyll fluorometers (Walz) at plant height in the glasshouse over 6 (run 1) or 3 (run 2) days during growth.

5.4.2 Physiological effects

5.4.2.1 *Leaf number and green area over time*

There was very little difference between leaf number and green area, as measured by aerial photographs, over time. The only light treatments that significantly increased leaf number when compared to the sunlight control were red and HPS (Fig. 5.2). In run 2 on days 14 ($P=0.02$), 29 ($P=0.009$) and 31($P<0.001$) red light and on day 20 ($P=0.001$) HPS light supplemented plants had significantly more leaves than the sunlight control.

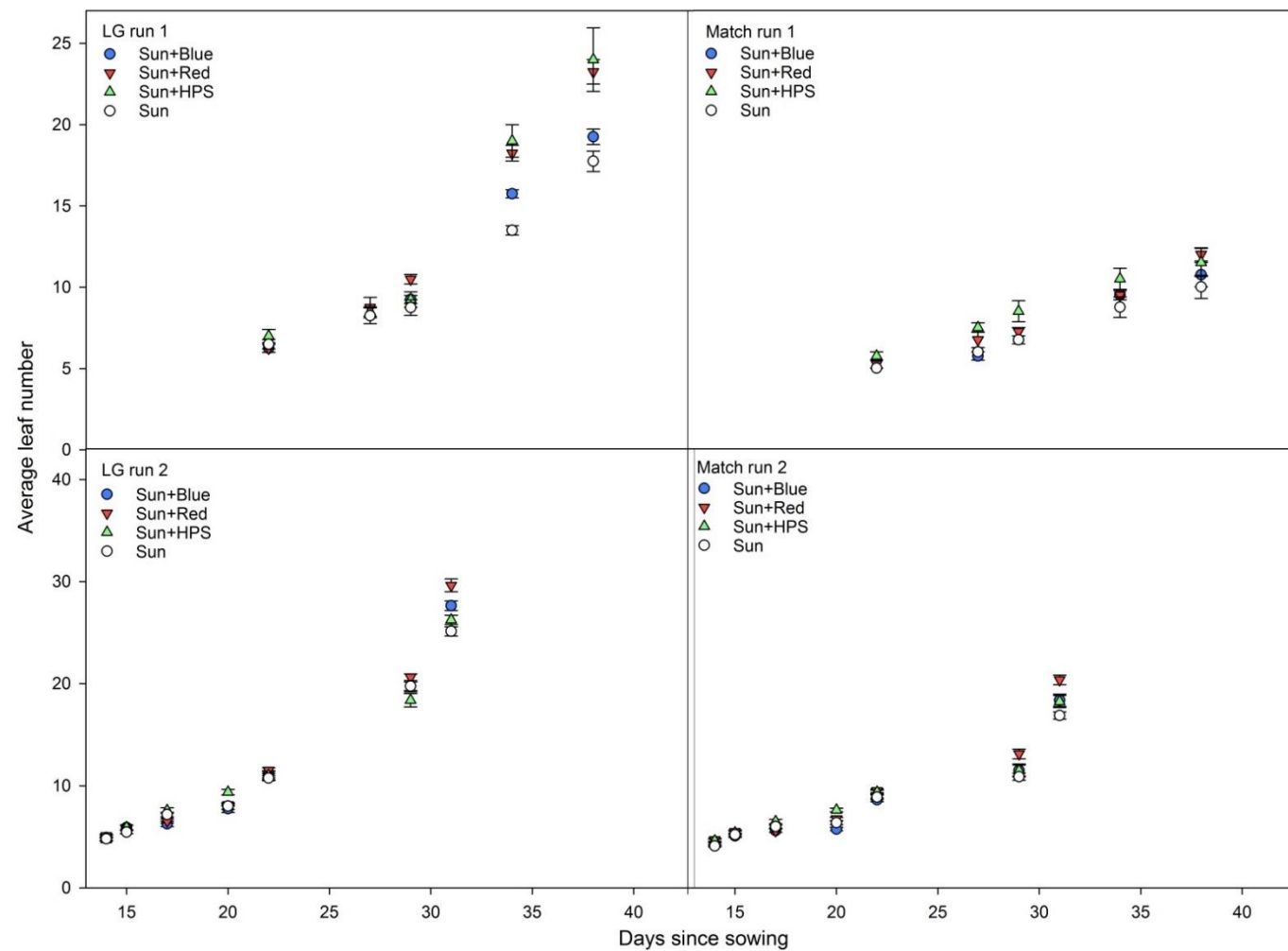


Figure 5.2: Leaf number over time of two cultivars lettuce – L.G. (*L. sativa* L. cv. Lobjoits Green) and Match (*L. sativa* L. cv. Match) – grown under a sunlight only control, and with either $50\mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. N=4 (run 1) and n=8(run 2).

5.4.2.2 Leaf chlorophyll fluorescence measurements

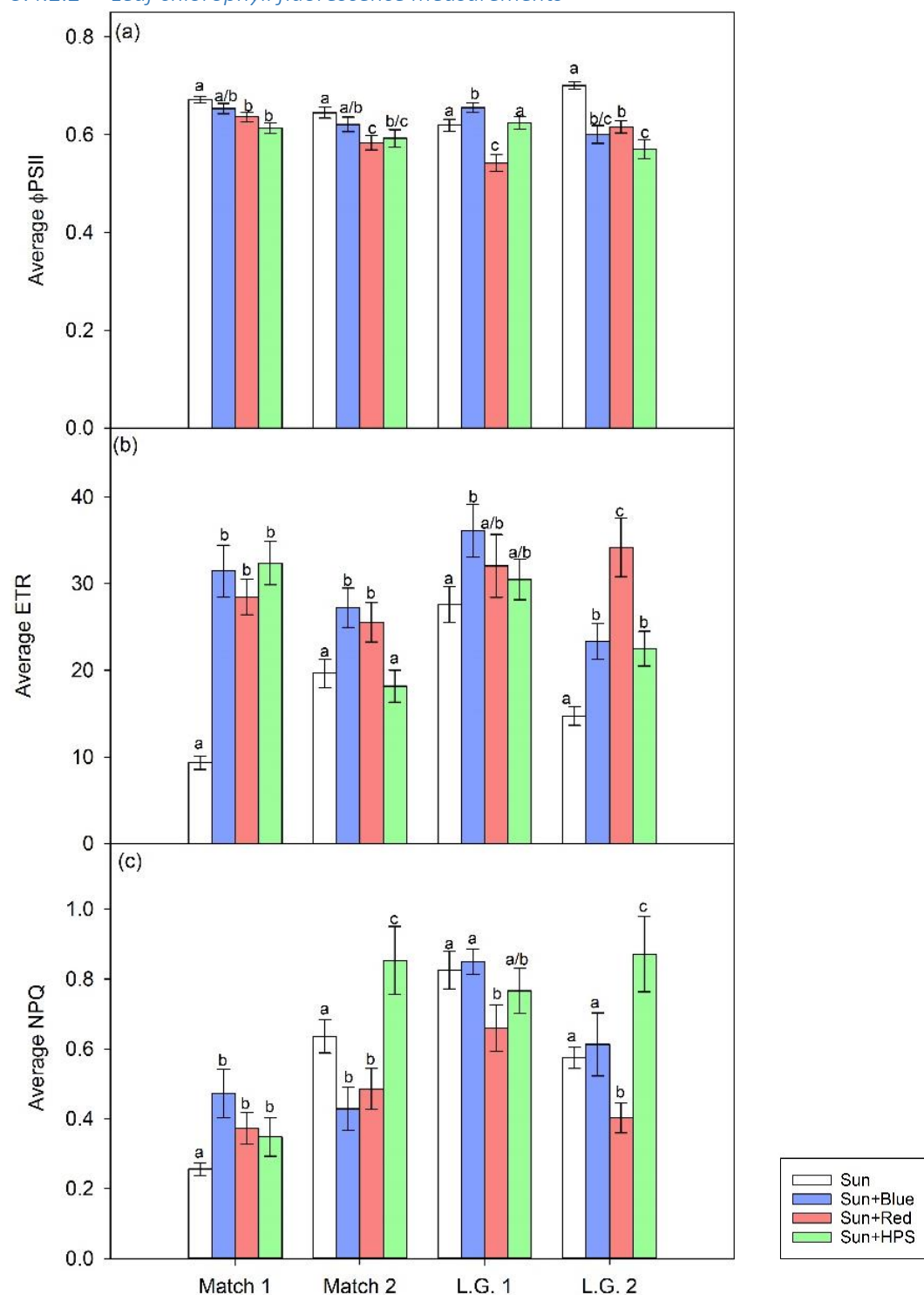


Figure 5.2: (a) Average daily photochemical yield of photosystem II (ϕ PSII), (b) electron transport rate through PSII (ETR, corrected for absorbance) and (c) level of non-photochemical quenching (NPQ) as measured with Walz MONITORING-PAM chlorophyll fluorometers in two cultivars lettuce – L.G. (*L. sativa* L. cv. Lobjoits Green) and Match (*L. sativa* L. cv. Match) – grown under a sunlight only control, and with either $50\mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. Letters indicate significant differences between groups ($P < 0.05$).

In cv. Match run 1, daily average ETR (Fig 5.3a) was higher than in the sunlight control plants in all light supplemented plants ($P < 0.001$). Daily average NPQ was highest in blue light supplemented plants, but statistically similar to HPS and red light supplemented plants (Fig. 5.3c). Blue ($P = 0.001$), red ($P = 0.005$) and HPS ($P = 0.05$) light supplemented plants all had higher daily average NPQ than the sunlight control (Fig. 5.3c). Daily average $\phi PSII$ was highest in the sunlight control plants but this was statistically similar to the blue light supplemented plants (Fig. 5.3a). Red ($P < 0.001$) and HPS ($P < 0.001$) supplemented plants had lower daily average $\phi PSII$ than the sunlight control (Fig 5.3a). Daily average $\phi PSII$ of red light supplemented plants was significantly lower than the sunlight control ($P < 0.001$) and blue light ($P < 0.001$) supplemented plants but statistically similar to that of the HPS light supplemented plants (Fig. 5.3a).

In run 2 in cv. Match, blue ($P < 0.001$) and red ($P = 0.008$) light supplemented plants had higher daily average ETRs than the sunlight control plants (Fig. 5.3b). Blue ($P < 0.001$) and red ($P < 0.001$) light plants also had higher daily average ETRs than HPS light supplemented plants (Fig. 5.3b). HPS light supplemented plants had a higher average daily NPQ than sunlight control plants ($P = 0.003$), which had a higher NPQ than the red ($P = 0.004$) and blue ($P = 0.003$) light supplemented plants (Fig 5.3c). Daily average $\phi PSII$ is again highest in the sunlight control plants but statistically similar to that of the blue and HPS light supplemented plants (Fig. 5.3a).

As can be seen in Fig 5.3b, in cv. L.G. in run 1, daily average ETR in blue light supplemented plants was higher than in sunlight control plants ($P = 0.001$). Daily

average ϕ PSII of blue light plants was also higher than sunlight control ($P=0.003$), red ($P<0.001$) and HPS light supplemented plants ($P=0.013$). Average daily NPQ was lower in red light supplemented plants than the sunlight control ($P=0.001$) and blue light supplemented plants ($P<0.001$) but statistically similar to HPS light supplemented plants (Fig. 5.3c).

As can be observed in Fig. 5.3b, in cv. L.G. in run 2, average daily ETR was higher in red light supplemented plants than the sunlight control as well as blue and HPS light supplemented plants ($P<0.001$). Daily average NPQ was significantly higher in HPS plants than the sunlight control ($P<0.001$), blue ($P=0.001$) and red ($P<0.001$) light supplemented plants (Fig. 5.3c). Daily average ϕ PSII was higher in the sunlight control plants than blue, red or HPS light supplemented plants ($P<0.001$) and in red light plants was higher than in HPS plants ($P=0.007$) (Fig. 5.3a).

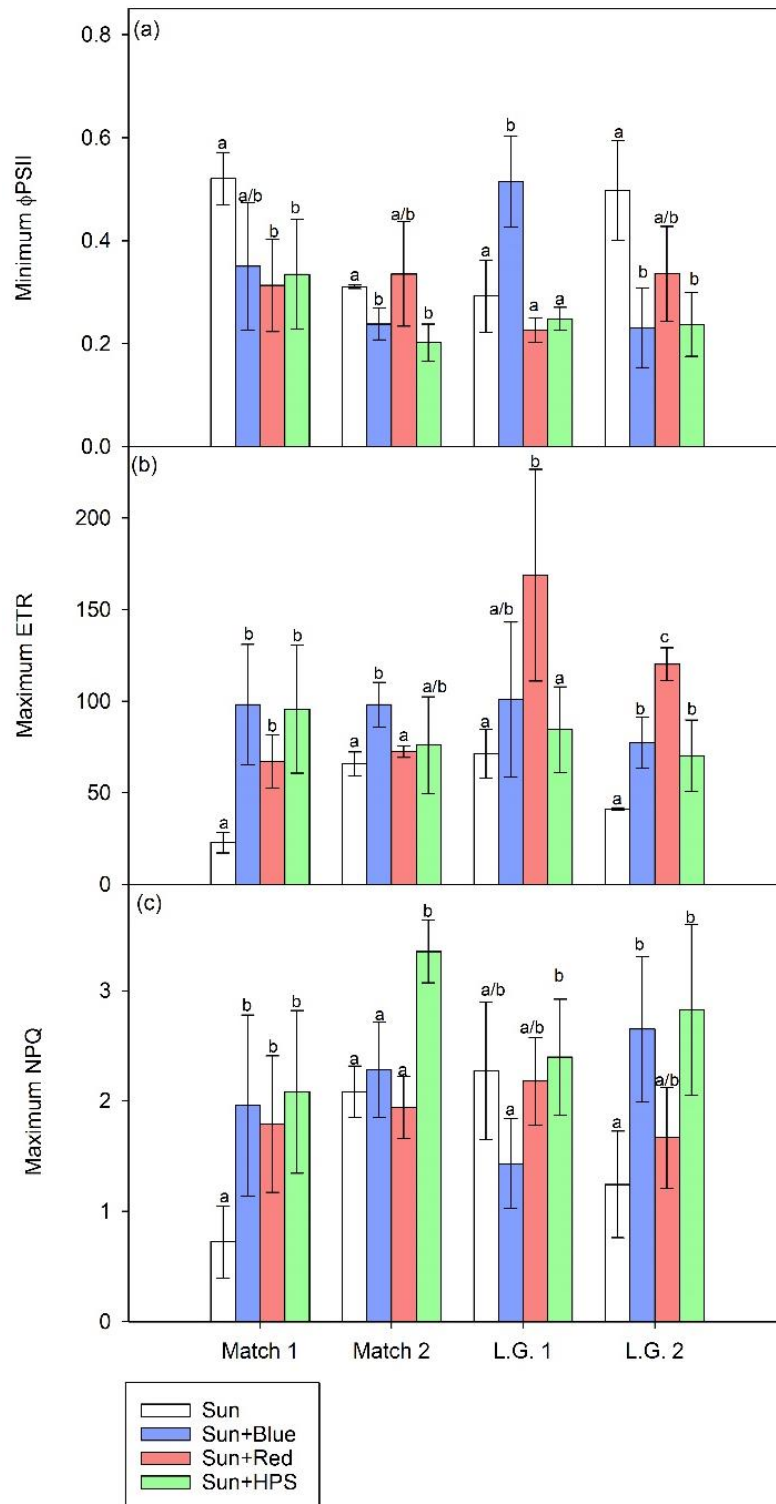


Figure 5.3: (a) Minimum daily photochemical yield of photosystem II (ϕ_{PSII}), (b) maximum daily electron transport rate through PSII (ETR, corrected for absorbance) and (c) daily maximum level of non-photochemical quenching (NPQ) as measured with Walz MONITORING-PAM chlorophyll fluorometers in two cultivars lettuce – L.G. (*L. sativa* L. cv. Lobjoits Green) and Match (*L. sativa* L. cv. Match) – grown under a sunlight only control, and with either $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. Letters indicate significant differences between groups ($P < 0.05$).

In cv. Match run 1, daily maximum ETR was higher than in the sunlight control plants in all light supplemented plants ($P < 0.05$) (Fig. 5.4b). Daily maximum NPQ was highest in blue light supplemented plants, but statistically similar to HPS and red light supplemented plants. Blue ($P = 0.04$), red ($P = 0.036$) and HPS ($P = 0.024$) light supplemented plants all had higher daily maximum NPQ than the sunlight control (Fig. 5.4c). Daily minimum $\phi PSII$ was highest in the sunlight control plants but this was statistically similar to the blue light supplemented plants. Red ($P = 0.036$) and HPS ($P = 0.045$) supplemented plants had lower minimum average $\phi PSII$ than the sunlight control but statistically similar to blue light (Fig. 5.4a).

In cv. Match run 2, maximum daily ETR was highest in blue light supplemented plants which was statistically similar to that of HPS light supplemented plants and higher than the sunlight control ($P = 0.025$) and red ($P = 0.036$) light supplemented plants (Fig. 5.4b). Daily maximum NPQ was higher in HPS light supplemented plants than the sunlight control, blue, and red light supplemented plants ($P < 0.01$) (Fig. 5.4c). Minimum daily $\phi PSII$ was higher in the sunlight control plants than in blue ($P = 0.02$) or HPS ($P = 0.034$) light supplemented plants (Fig. 5.4a).

In cv. L.G. run 1, maximum daily ETR was higher in red light supplemented plants than in the sunlight control ($P = 0.02$) and HPS light supplemented plants ($P = 0.047$) (Fig. 5.4b). Daily maximum NPQ was lower in blue light supplemented plants than HPS light supplemented plants ($P = 0.05$) (Fig. 5.4c). Minimum daily $\phi PSII$ was also higher in blue light supplemented plants than in the sunlight

control ($P=0.024$), red ($P=0.003$) and HPS ($P=0.004$) light supplemented plants (Fig. 5.4a).

In cv. L.G. run 2, maximum daily ETR was higher in red light supplemented plants than the sunlight control ($P=0.012$) as well as blue ($P=0.024$) and HPS ($P=0.36$) light supplemented plants (Fig 5.4b). Maximum daily NPQ was lower in the sunlight control than in blue ($P=0.038$) and HPS ($P=0.047$) light supplemented plants (Fig.5.4c). Daily minimum $\phi PSII$ was higher in the sunlight control than in blue ($P=0.037$) and HPS ($P=0.026$) light supplemented plants (Fig. 5.4a).

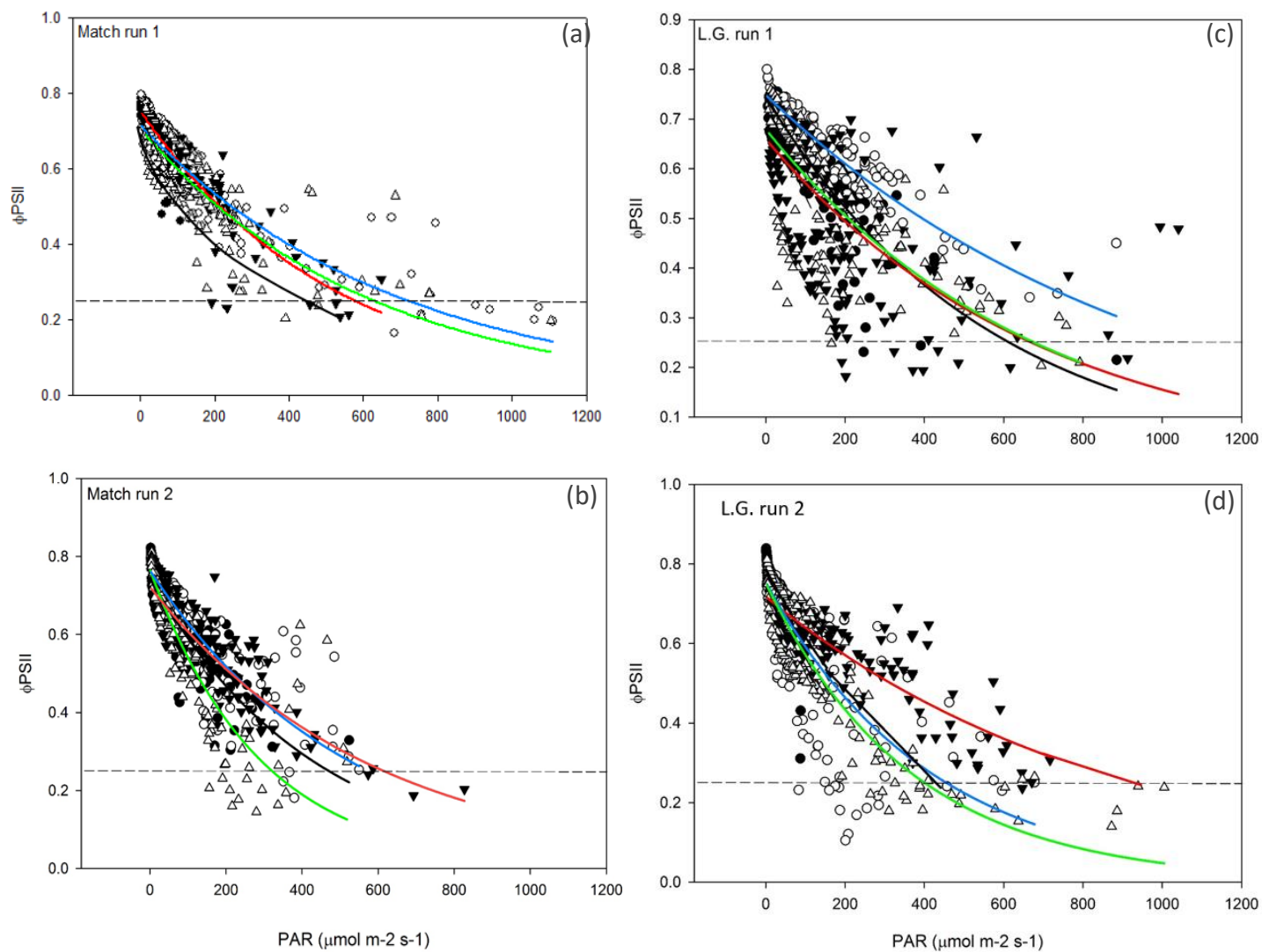


Figure 5.5: (a-d) Photochemical yield of photosystem II (ϕ_{PSII}) plotted against level of photosynthetically active radiation for two cultivars lettuce – L.G. (*L. sativa* L. cv. Lobjoits Green) and Match (*L. sativa* L. cv. Match) as measured with Walz MONITORING-PAM chlorophyll fluorometers in two cultivars lettuce – L.G. (*L. sativa* L. cv. Lobjoits Green) and Match (*L. sativa* L. cv. Match) – grown under a sunlight only control, and with either $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. Lines show points fitted with the non-linear regression exponential decay $f = a \cdot \exp(-b \cdot x)$. Dotted lines indicate the target ϕ_{PSII} .

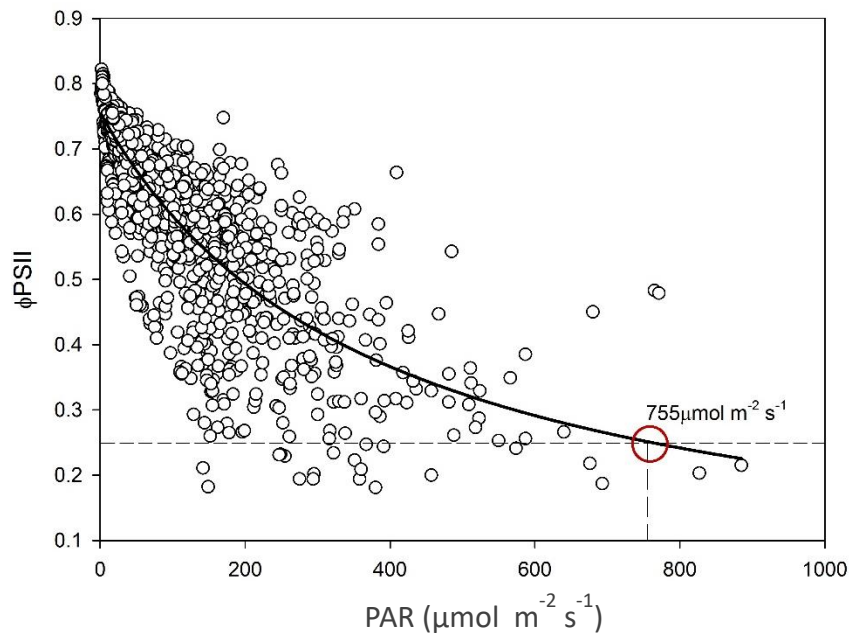


Figure 5.4: (e) Combined data from all treatments combined in order to determine average light level required to saturate photosystem II.

In both cultivars in run 1, plants provided with supplementary light all met the target level of photosynthetic saturation at higher light intensities than the sunlight control (Fig 5.5 a-d). The sunlight control plants were saturated in cv. Match at approximately $450 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 5.5a) and in cv. L.G. at approximately $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 5.5c). However, plants grown with additional blue light saturated at closer to $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ in cv. match (Fig. 5.5a) and over $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ in cv. L.G. (Fig. 5.5c). In run 2, both cultivars supplemented with HPS light saturated at lower levels than the sunlight control, although both blue and red light supplemented plants needed increased levels of PAR to reach the saturation target, with plants supplemented with red light saturating at particularly high light levels (Fig 5.5b; Fig 5.5d). When all data was combined, average light level for a ϕPSII of 0.25 was $750 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 5.5e).

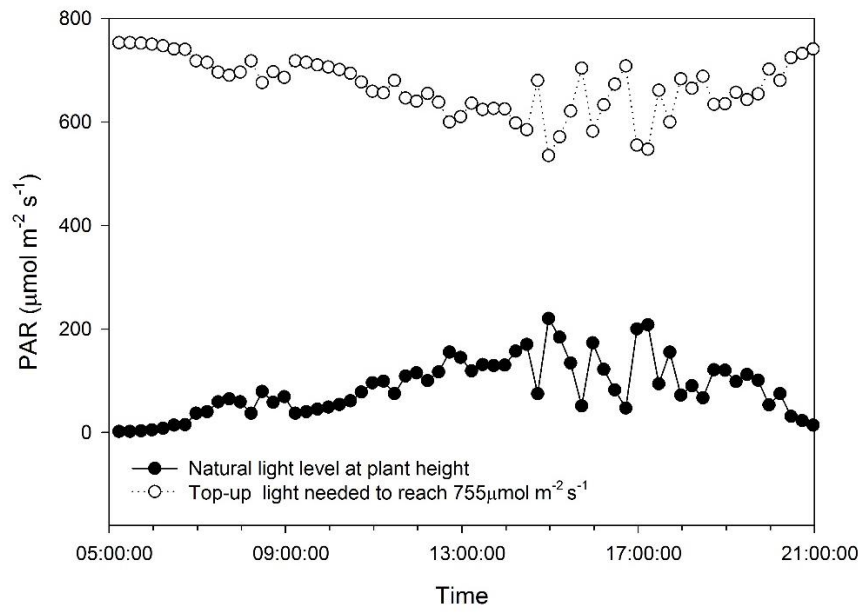
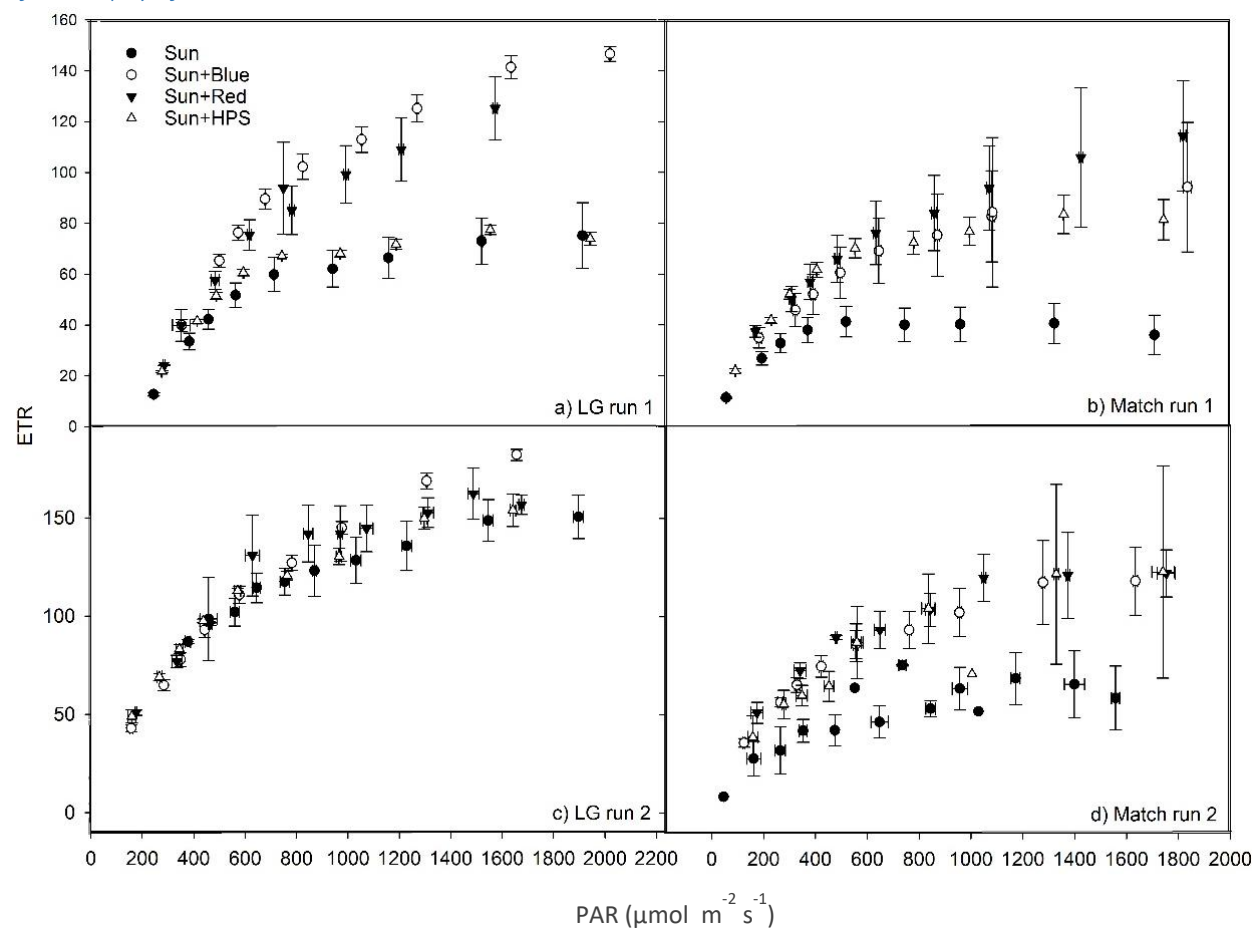


Figure 5.5: natural sunlight levels at plant height over a typical bright day, and the amount of supplementary light that would need to be added to reach a target level of $755\mu\text{mol m}^{-2} \text{s}^{-1}$ for near maximum saturation of photosynthesis.

5.4.2.3 Junior-PAM rapid light response curves of chlorophyll fluorescence

Figure 5.6: Relative electron transport rate (ETR) as measured by the Walz JuniorPAM chlorophyll fluorometer in of two cultivars of lettuce – L.G. (*L. sativa* cv. Lobjoits Green) and Match (*L. sativa* L. cv. Match) – grown under a sunlight only control, and with either 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. Bars show standard error of means.



In cv. Match, rapid light curves of chlorophyll fluorescence show that, from PAR levels of $400\text{-}1800\mu\text{mol m}^{-2}\text{ s}^{-1}$ (run 1) (Fig.5.7b) and $200\text{-}1000\mu\text{mol m}^{-2}\text{ s}^{-1}$ (run 2) (Fig. 5.7d), the outer leaves of plants under all supplementary light treatments have a statistically higher ETR than those of the sunlight control ($P<0.05$). In cv. L.G. in run 1, the outer leaves of plants grown under red and blue supplementary lighting had higher ETRs than those supplemented with HPS light or the sunlight control above PAR levels of $600\mu\text{mol m}^{-2}\text{ s}^{-1}$ (Fig. 5.7a).

5.4.3 Crop yield at harvest

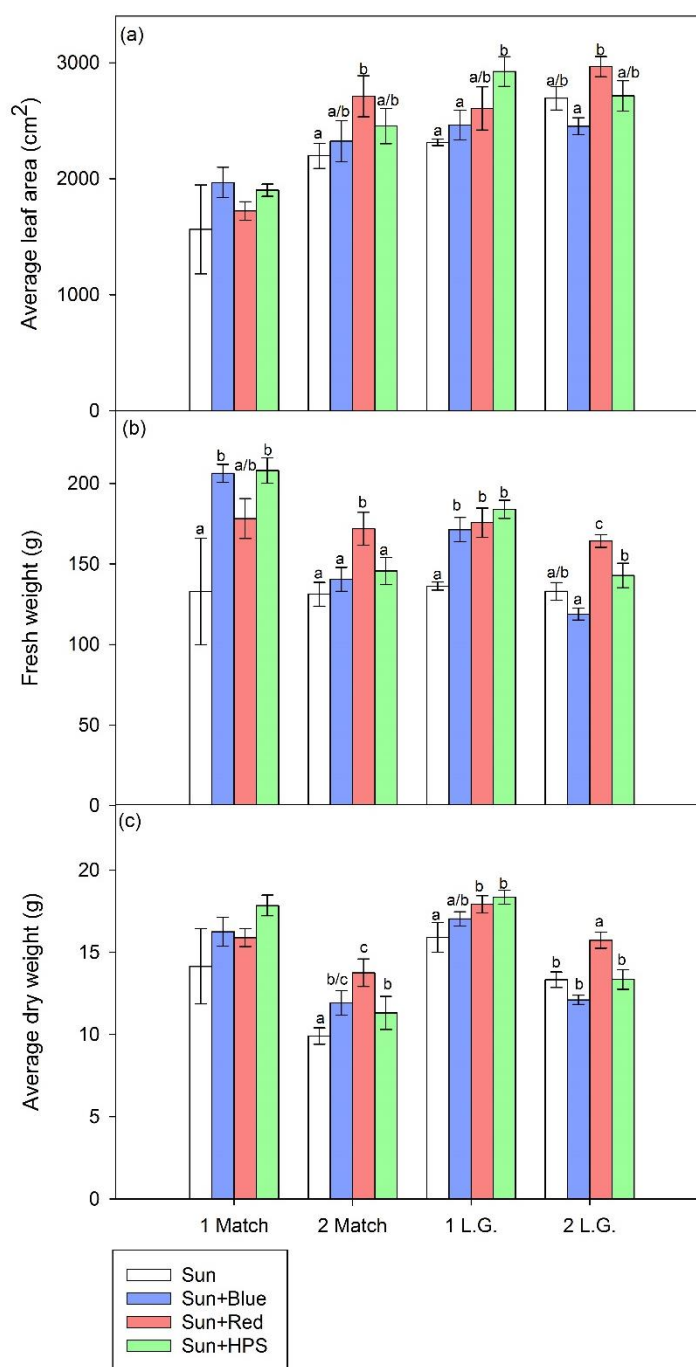


Figure 5.7: Average leaf area (cm²), fresh weight (g) and dry weight (g) 45 days after sowing of two cultivars of lettuce – L.G. (*L. sativa* cv. Lobjoits Green) and Match (*L. sativa* L. cv. Match) – grown under a sunlight only control, and with either 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. For dry weights, fresh plant material was dried for 1 week at 80°C. N=4 (run 1) or n=8(run 2). Bars show standard error of means and letters represent statistically significant differences (P<0.05).

There was no significant effect of light treatment on leaf area (Fig. 5.8a) in run 1 ($P=0.076$) however it did show that effect of light treatment was highly dependent on cultivar ($P<0.001$). In run 2, there was a significant effect of light treatment ($P=0.002$) and also cultivar ($P=0.001$) on leaf area. When pooling data from both cultivars, in run 2, multiwise comparisons showed that red light supplementation resulted in significantly higher ($P=0.009$) and blue light supplementation resulted in significantly lower ($P=0.002$) leaf areas than the sunlight control. Post-hoc pairwise comparisons for individual runs showed that in run 1 in L.G., HPS light supplemented plants had a significantly higher leaf area than the sunlight control ($P=0.004$) and blue light supplemented plants ($P=0.022$), however had a statistically similar leaf area to red light supplemented plants. In run 2 in Match ($P=0.012$), red light supplemented plants had a higher leaf area than the sunlight control plants ($P=0.012$) and in L.G. they had a higher leaf area than blue light supplemented plants ($P=0.001$).

There was a significant effect of light on fresh weight (Fig.5.8b) in run 1 ($P=0.001$) and run 2 ($P<0.001$). When pooling data from both cultivars, in run 1, multiwise comparisons showed that blue, ($P=0.004$), red ($P=0.031$) and HPS ($P=0.001$) light supplementation significantly increased average fresh weight when compared to the sunlight control. In run 2, multiwise comparisons showed that only red light supplementation significantly increased fresh weight ($P<0.001$) with blue ($P=0.735$) and HPS ($P=0.092$) average fresh weights being not significantly different to the control. Post-hoc pairwise comparisons for individual runs showed that in run 1 in cv. Match, blue ($P=0.015$) and HPS

($P=0.013$) light supplementation resulted in significantly higher fresh weight than the sunlight control. Fresh weight of red light supplemented plants was however higher than the sunlight control and statistically similar to the other light supplemented groups. In run 1 in cv. L.G., blue ($P=0.003$), red ($P=0.001$) and HPS ($P<0.001$) light supplemented plants all had significantly higher fresh weights than the sunlight control. In run 2 in cv. Match, red light supplementation resulted in significantly higher yields than the sunlight control ($P=0.002$), blue ($P=0.014$) and HPS ($P=0.036$) light supplemented plants. In run 2 in cv. L.G., red light again resulted in significantly higher fresh weights than the sunlight control ($P<0.001$), blue ($P<0.001$) and HPS ($P=0.009$) light supplemented plants. Blue light supplemented plants had a lower but statistically similar fresh weight to the sunlight control plants, and a significantly lower fresh weight than the HPS light supplemented plants ($P=0.004$).

There was a significant effect of light treatment on dry weight (Fig. 5.8c) in run 1 ($P=0.044$) and run 2 ($P<0.001$). When pooling data from both cultivars, in run 1, multiwise comparisons showed that HPS supplemented plants had significantly ($P=0.006$) and red plants almost significantly ($P=0.074$) larger dry weights than the sunlight control. In run 2, red light supplemented plants had larger dry weights than the sunlight control ($P<0.001$), as well as blue ($P<0.001$) and HPS ($P=0.001$) supplemented plants. Post-hoc pairwise comparisons for individual runs showed that in run 1 in cv. L.G., red ($P=0.035$) and HPS ($P=0.014$) light supplementation resulted in higher dry weights than the sunlight control. In cv. Match run 2 red light supplementation resulted in a

higher dry weight than the sunlight control ($P=0.002$) and HPS light supplemented plants ($P=0.014$). In cv. L.G. run 2 red light supplementation resulted in higher dry weights than the sunlight control ($P=0.001$), blue ($P<0.001$) and HPS ($P=0.001$) light supplemented plants.

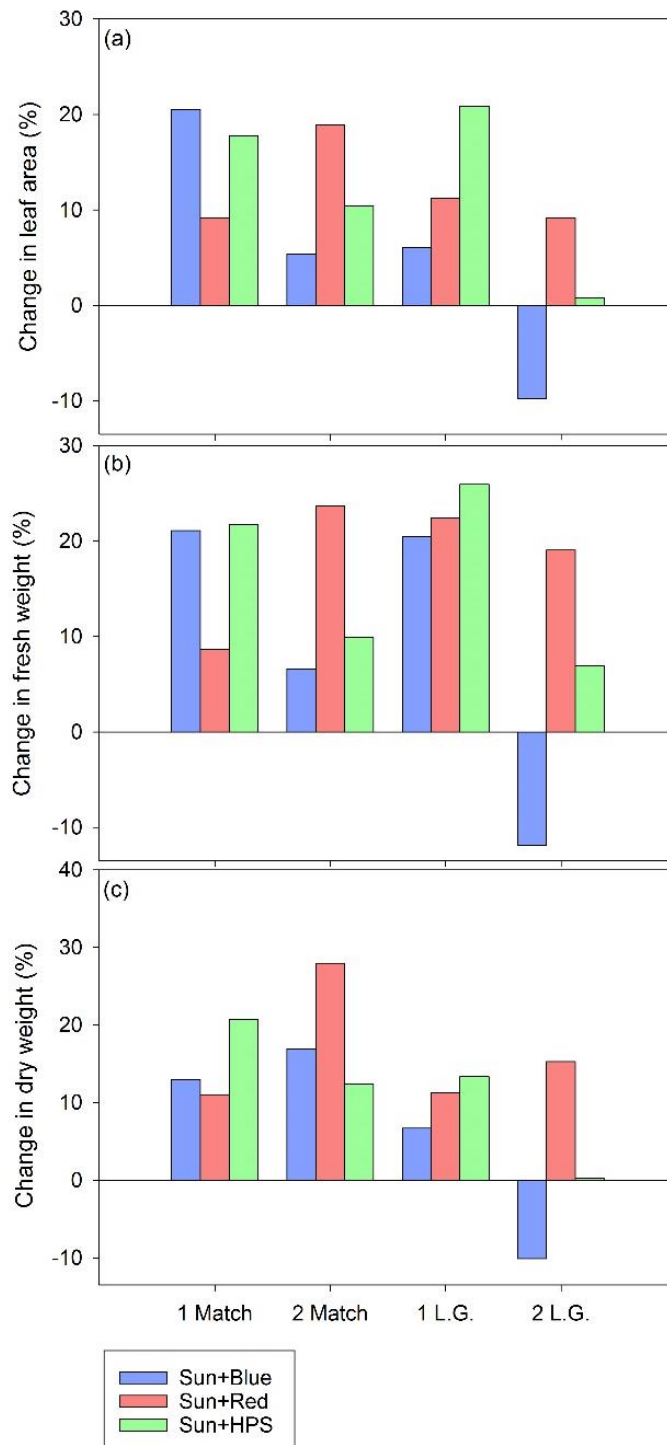


Figure 5.8: Total percentage change in leaf area, fresh weight and dry weight of plants of each cultivar in each run under each supplementary light treatment as compared to the sunlight control 45 days after sowing of two cultivars lettuce – L.G. (*L. sativa* L. cv. Lobjoits Green) and Match (*L. sativa* L. cv. Match) – grown under a sunlight only control, and with either 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ supplementary blue LED light, red LED light or high-pressure sodium white light. N=4 (run 1) or n=8(run 2). For dry weights, fresh plant material was dried for 1 week at 80°C.

Red LED and high pressure sodium light supplementation consistently resulted in increased leaf area, fresh and dry weight at harvest as compared to the control (Fig. 5.9). Blue LED light however resulted in a decreased leaf area, fresh and dry weight yield in cv. L.G. in run 2 of the experiment.

5.4.4 Cost analysis

Table 5.1: A simple cost analysis was carried out based on the cost per plant of providing supplementary light divided by the average increase in fresh weight in run 1 and 2 combined. This was scaled up using the information in chapter 2.1.3 to determine how many plants could have been grown in the area under 1 HPS lamp or 2 red or blue LED lamps illuminated with $50\mu\text{mol m}^{-2} \text{s}^{-1}$ of supplementary PAR. Based on current electricity prices (EoN, Sept 2016), the cost per kilowatt hour (kWh) of electricity was assumed to be £0.13. The energy in kilowatt-hours is equal to the power in watts (W), times the time-period in hours divided by 1000. Lamps were run for a total of 16 hours per day for 35 days and therefore time period in hours equated to 560.

Lamp	HPS	Red LED	Blue LED
W	400	60	60
kW	0.4	0.06	0.06
kWh	6	0.9	0.9
kWh total (35 days)	210	31.5	31.5
Total cost (£)	27.03	4.095	4.095
Area covered (cm ²)	17172	7722	7722
No. plants covered (13cm pots; 5cm spacing)	48	18	18
Cost per plant (£)	0.56	0.23	0.23
Cost (£) per % increase in fresh weight (LG)	0.04	0.01	0.05
Cost (£) per % increase in fresh weight (Match)	0.04	0.01	0.02

The red LED lamps were most cost effective in terms of conversion of electrical energy to biomass, i.e. 'crop per watt', giving up to 4 times as much percentage increase in fresh weight for the same energy input as the conventional HPS lamps (Table 5.1). The blue LED lamps were twice as efficient in terms of 'crop

per watt' as the HPS lamps for growth of cv. Match, but were slightly less efficient when growing cv. L.G (Table 5.1). It can therefore be concluded that red LED lights were the most energy efficient and cost effective form of supplementary lighting.

5.5 Discussion

5.5.1 The benefit of supplementary light

As hypothesised, even during the summer months, supplementary light increased yield in terms of both fresh and dry weight at harvest, with increases as high as 25% (Fig. 5.8; Fig 5.9). Photosynthesis was not generally fully saturated by natural light levels in the glasshouse (Fig. 5.5). The optimal light level for photosynthesis and lettuce growth is likely well above that which is provided by natural sunlight, with ETR still increasing well above $1000\mu\text{mol m}^{-2} \text{s}^{-1}$ in some cases (see Fig. 5.7). The lowest minimum average daily ϕPSII recorded by the MONITORING-PAMs was approximately 0.25 (Fig. 5.3). It would be safe to assume that photosynthesis would be highly saturated at this point. In Fig. 5.5e, a 2 parameter hyperbolic decay equation [$f = (a*b)/(b+x)$] was used to fit a curve through the compiled MONITORING-PAM ϕPSII vs PAR data from all treatments in both cultivars in both runs of the experiment. The target light level for near maximum saturation of photosynthesis was then estimated by determining where the fitted curve dissected the 0.25 point of the Y axis - $755\mu\text{mol m}^{-2} \text{s}^{-1}$. Fig. 5.6 shows natural light levels at plant height over a typical bright day, and the amount of supplementary light that would need to be added to reach a target level of $755\mu\text{mol m}^{-2} \text{s}^{-1}$ at plant level. Even during the summer supplementary light would have to be applied throughout the day to fully saturate photosynthesis.

5.5.2 Comparison of growth and physiology under conventional HPS and new LED lighting

Most likely due to the highly variable natural light environment within the glasshouse, there was much variation in the chlorophyll fluorescence data, although a number of conclusions can be drawn. Firstly, the data did confirm that, in most cases, red and blue light either produced plants with statistically similar or higher photosynthetic capacities than HPS light (Fig. 5.5a-d; Fig. 5.7) - i.e. there was no detrimental effect on photosynthesis when switching from a broad waveband conventional HPS lamp to the new LED lamps.

HPS light supplemented plants had consistently high levels of NPQ (Fig. 5.3; Fig. 5.4), and in some cases (Match and L.G. run 2) photosynthesis appeared to become saturated at lower light levels than in the sunlight control (Fig. 5.5a-d). Fresh weight yield in these cases was significantly lower than in red light supplemented plants (Fig. 5.8) showing that this increase in NPQ had a detrimental effect on yield. As HPS lamps emit more longer-wavelength photons, this could be having a heating effect on the leaves that was not detected by the TinyTag data loggers which were logging air temperature at plant height. This may have caused a wavelength specific induction of higher NPQ.

Of all the supplementary light treatments, only blue light in L.G. run 2 failed to increase fresh weight yield (Fig. 5.8), producing a lower fresh/ dry weight and leaf area to the sunlight control (although the difference was not statistically significant). Cv. L.G. run 2 was the only time that maximum NPQ of blue light supplemented plants was statistically similar to that of HPS plants and it could

be that this increase in NPQ caused the loss of yield. Photosynthesis did appear to saturate at a lower light level (Fig.5.5a-d) and ETR was significantly lower than that of red light supplemented plants (Fig. 5.3; Fig. 5.4) although statistically similar to that of HPS plants.

The MONITORING-PAMs only measure the outer leaves and it could be possible that this decrease in ETR caused by increase in NPQ was more detrimental to fresh-weight yield in blue light supplemented plants than HPS light supplemented plants because of the absorption qualities of the supplementary lights. As discussed in detail in chapter 1.8 and chapter 3, blue light is least effective and green light (which makes up almost 50% of HPS spectral output) is most effective at penetrating through the leaf/ canopy structure in order to drive photosynthesis. Therefore, if the outer leaves of blue light supplemented plants are photoinhibited, this is likely to have more of an effect on growth, as the leaves inside the folded lettuce head structure of HPS light supplemented plants are likely photosynthesising more due to the additional green photons which are reaching their chloroplasts.

5.5.3 Red LED light for increased 'crop per watt'

It can be determined that the red LED lamps were the best form of supplementary lighting for plant growth and development. They gave the highest percentage increase in fresh weight yield in terms of 'crop for watt' (see table 5.1) and were up to 4 times more efficient than HPS lamps. Red LED light consistently boosted leaf area, fresh and dry weight yield (Fig. 5.8; Fig. 5.9) and photosynthetic capacity in the outer leaves (Fig. 5.5a-d; Fig. 5.7).

5.6 Conclusions

Supplementary light in the summer months significantly increased yield. The natural light levels, even at the brightest time of year, were not sufficient to saturate photosynthesis and drive growth at maximum rate. It was determined that red LED light supplementation produced increases in yield higher or statistically similar to HPS supplementation. Only blue light supplementation resulted in a small (though not statistically significant) decrease in yield when compared to the sunlight control in cv. L.G. in run 2. This could perhaps be attributed to an increase in NPQ to the detriment of photosynthesis coupled with the inability for blue light to penetrate far into the lettuce head structure. NPQ was also consistently high in HPS supplemented plants but the broad wavelength spectrum containing wavelengths (such as those in the green), which are capable of driving photosynthesis in the deeper layers of the folded lettuce structure, may have negated any negative effects on yield in the outer leaves. Light supplementation with just $50\mu\text{mol m}^{-2} \text{s}^{-1}$ red light increased electron transport rate and lowered ϕPSII in the mature outer leaves. However, it was impossible to measure the effect on leaves inside the lettuce head structure. Cost analysis revealed that, 'crop per watt', red LED light was the most energy efficient form of supplementary lighting.

6 General discussion

6.1 Summary of findings

Measurements of natural sunlight spectral quality through crop canopies in the field and glasshouse environment (chapter 3) highlighted the importance of the interaction of signals induced by multiple wavelengths of light as positional signals to the plant. The variation in distribution of blue, green, red and far-red photons through the vertical canopy profile and plant sensing of the proportions of these different wavelengths is likely crucial for optimisation of acclimation to levels of photosynthetically active radiation (PAR). Not only far-red light but also green light increased as a percentage of total plant detectable light (TPDL) from top to base of the canopy profile.

It was concluded that the declining blue to green (B:G) light ratio observed in all crop canopies, especially in deeper, more heavily shaded regions of the canopy, may act as a more stable, extended source of positional information to the relatively steep declining red to far-red (R:FR) light ratio. The novel hypothesis was presented that, under low TPDL conditions, the B:G light ratio fine-tunes stomatal aperture and contributes to whole canopy water use efficiency. Additionally, leaves in these areas of low PAR and high far-red light are primed - through the production of more chlorophyll *b* (chl *b*) containing light-harvesting complex (LHC) II – to better absorb green light. Therefore, the ability of green wavelength photons to penetrate deep into the canopy profile distributes energy more evenly throughout the crop and more efficiently drives photosynthesis where other wavelengths of PAR are more severely depleted.

Based on the information gained in chapter 3, it was concluded that, as plants have evolved under a broad-waveband spectrum, each part of that spectrum has a role in optimisation of plant growth, development and resource use efficiency in the natural light environment. It is therefore unlikely that narrow-band, monochromatic LED light would be a suitable alternative for the conventional, broad-waveband 'white' light sources currently used in controlled environment growth rooms or tissue culture labs.

Growth under combinations of red, blue, green and far-red LED light (chapter 4) confirmed that, indeed, growth lamps which emitted photons of multiple wavebands of light produced herb crops of a higher quality, in terms of desirable morphology for commercial production and sale, and fresh-weight yield. Growth under monochromatic red light produced plants with long, thin, weak stems, small leaves with a low photosynthetic capacity and low fresh-weight yield. Monochromatic blue light in one cultivar resulted in the under-development of the root system and also lowered fresh-weight yield. Plant response to addition of green light to a red + blue + far-red light background was particularly interesting. RBGFR light recipe plants produced thin, broad leaves with LHCs spread over a larger surface area, but a high chlorophyll a:b ratio. They successfully acclimated to both the low PAR environment and light quality, which was similar to that found in high light at the top of a canopy, resulting in a high fresh-weight yield.

Monochromatic LED light was however found to be a suitable replacement for conventional horticultural lighting in a glasshouse environment (chapter 5).

Here, any additional light is supplementary to an already full sunlight spectrum, so the aim is to provide PAR to further saturate photosynthesis and wavelengths that will potentially increase whole plant photosynthetic capacity. There were no detrimental effects on photosynthetic capacity or fresh-weight yield of summer lettuce when exchanging supplementary high-pressure sodium (HPS) light for red LED light. Red LED lamps also gave the highest percentage increase in fresh weight yield in terms of 'crop for watt', being up to 4 times more efficient than HPS lamps, confirming huge potential energy savings for commercial growers.

The following recommendations for growers can therefore be made:

- In controlled environment conditions for example a vertical farming system where artificial lighting is the sole source of light, providing multiple wavelength spectra will result in higher yields than monochromatic light.
- If the crop is being grown for overall fresh weight yield, for example in the case of bagged herbs, a blue + green + red + far-red light recipe is ideal. The additional stem elongation caused by the far-red light is not a problem.
- If it is important for the crop to have a high leaf to stem ratio, for example in the case of potted herbs, a blue + green + red light recipe is more suitable, as this recipe promotes leaf expansion without boosting stem elongation.

- In the glasshouse environment where the sole aim is to increase yield, and supplement natural light during daylight hours, providing monochromatic red LED light is the most energy efficient way to achieve this. There is no detrimental effect on quality or yield when switching from supplementary conventional high pressure sodium lighting to red LED lighting.

6.2 Perspectives on green light

The addition of light in the green waveband clearly has interesting effects on plant growth and development and is worth further investigation. Green light as part of the natural sunlight spectra found in plant canopies acts as a shade signal and causes acclimation to low light intensities (Wang and Folta, 2013). However, green light, at the proportion of TPDL used in the experiments in chapter 4, did not result in induction of low light characteristics, and, as mentioned above, in some cases appeared to induce high-light characteristics. In this way, green light seems to be a sort of paradox, causing low-light associated physiological characteristics via the non-photosynthetic photoreceptors (but only when the proportion of green light is higher than that of blue light), and high-light associated characteristics via the photosynthetic photoreceptors when added (in a proportion where additional green light does not exceed intensity of blue light) to a red + blue + far-red light background.

The experiments in chapter 4 were limited to looking at the effects of addition of mid-waveband (545nm) green wavelengths, however work by Johkhan et al (2012) suggests that different portions of the 'green light' waveband have different effects on plant growth. They grew *Lactuca sativa* L. cv Banchu Red

Fire under four different light qualities; white fluorescent, green LED $\lambda 510 \pm 18 \text{ nm}$ (G510), green LED $\lambda 524 \pm 30 \text{ nm}$ (G520) and green LED $\lambda 532 \pm 30 \text{ nm}$ (G530). For each of the four light qualities, plants were grown at photon flux densities (PPFs) of 100, 200 and $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Plants grown under G520 and G530 had lower fresh and dry shoot weights than white fluorescent grown plants at all light intensities. Plants grown under $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ G510 however had significantly higher fresh and dry weights than plants in all other light treatments. G510 plants grown at this intensity also had the highest leaf transpiration and photosynthetic rates, followed by the white fluorescent grown plants. This suggests that the long-wavelength blue and short-wavelength green light emitted from the G10 LEDs stimulates growth more effectively than the longer green wavelengths found in the G520 and G530 LEDs, and that short and long wavelength green light affects plants differently. With the continual progress in green light LED technology (as discussed in chapter 4.1) it will be possible in future to further elucidate the effects of individual portions of the green light waveband on plant physiology, and possibly use narrow band green-wavelength LEDs to target specific responses which improve plant quality and yield.

Another beneficial effect of the addition of green light to the light recipes was not on plant physiology but on the appearance of the light quality to the human eye. The addition of green light to the red and blue containing light recipes (RB and RBFR) changed the light from a harsh purple looking colour to a natural 'white' light colour that appears similar to that produced by the sun. NASA scientists observed similar effects when adding as little as 5% green light to a

red + blue light background. When developing energy efficient LED lighting systems for bioregenerative life support systems in space (which will be discussed in more detail later) Kim et al., (2006) found that when combined, the red and blue wavelengths needed to support photosynthesis produced a harsh purple light that caused plants to look grey/black making visualisation of plant health status difficult for workers in the growth areas and causing them discomfort. By adding green light wavelengths to the light recipe, plants appeared green and visualisation of any pests, disease or nutrient deficiency was much easier. The addition of small amounts of green light to LED light recipes to balance out the spectrum is therefore also beneficial for human welfare.

6.3 Improvements and further work

Time, space, equipment and operational issues with lamp, growth room and glasshouse control limited reliable data to that which is presented in this thesis. However, there were results that raised additional questions. For example, results suggested differences in intrinsic water use efficiency (WUE_i) due to the spectrum of the growth light (Fig. 4.14), which is worth further exploration. As fresh water is a valuable resource in many growth environments (see sections 6.5.1 and 6.5.2), it is important to find light recipes where an increase in yield is not to the detriment of WUE_i , and determine which combinations of wavelengths may improve WUE_i as compared to conventional white light sources.

It is also important to note that rapid light response curves of chlorophyll fluorescence, which were used in much of the work carried out, have a number of limitations. Although they were a quick and non-invasive means of comparing relative photosynthetic efficiency of PSII, allowing data from large amounts of replicates to be collected over the course of plant growth, the rapid pulsing of light may not allow for the complete relaxation of fluorescence induced by the previous pulse. The fibre-optic may also be positioned at the same distance from the leaf for each measurement to be comparable, and the area of leaf measured is very small and may therefore not be representative of the leaf as a whole. When comparing photosynthetic efficiency and capacity it is therefore important to also refer to the time intensive but more accurate infra-red gas analysis measurements presented.

6.4 Future research

There are many more areas on the topic of plant developmental/ physiological response to light quality which could be explored, some of which are now discussed in detail.

6.4.1 UV-B light for the improvement of nutrition, pest and disease resistance

The effects of UV-B light on plant physiology were not explored as part of this thesis. As mentioned previously, the spectroradiometer used detected light at wavelengths of 325-1075nm, and this does not include the UV-B waveband range of 280-315nm (Rizzini *et al.*, 2011). Although improvements are being made, UV-B LEDs are currently highly inefficient as compared to those that emit longer wavelengths, with external quantum efficiencies, i.e. the efficiency with which the LED converts electrons to photons and allows them to pass through

the device, being less than half that of LEDs which emit light of wavelengths above 365nm (Pernot *et al.*, 2011). In addition, use of LED UV-B light could have posed a health and safety risk during the conduction of the experimental work (Goodsell, 2001).

UV-B radiation is a highly-energised portion of the electromagnetic spectrum, capable of having damaging effects on the tissues, proteins and even DNA of plants and animals. Fortunately, the Earth's stratosphere absorbs strongly in the in the UV-B, so only a small fraction of this radiation from the sun passes through to the Earth's surface (Goodsell, 2001). Even less UV-B light passes through the glass of the average glasshouse (Gitz *et al.*, 2005). Plant stress responses to UV-B such as decreased metabolism, photosynthesis and yield are well documented (Mackerness, 2000). However, recently it has become clear that UV-B light has a useful role as an informational signal with a range of effects on photomorphogenesis (as extensively reviewed by Heijde and Ulm, 2012; Jiang *et al.*, 2012; Li *et al.*, 2013; Jenkins, 2014) and can even increase resistance to pests and pathogens.

Demkura and Ballaré, (2012), found that, through increased levels of flavonoids and sinipates, *A. thaliana* exposed to levels of UV-B light found in nature had increased resistance to *Botrytis cinerea* than those exposed to no UV-B. *Botrytis cinerea* is a necrotrophic fungus that causes soft-rot pre and post-harvest damage to over 200 crop species (Williamson *et al.*, 2007). UV-B light may also increase resistance to herbivory due to its stimulation of the production of

secondary metabolites or defensive phenolic compounds which are toxic or repellent to pests (Demkura *et al.*, 2010).

Some of these protective compounds which the plant produces in response to damaging UV-B light may also be beneficial to the consumer. Although at a cost to yield, UV-B light has been found to increase the production of secondary metabolites which are potentially beneficial to health. For example, a study by Tsormpatsidis *et al.*, (2008) found that anthocyanin contents in the red coloured lettuce *Lactuca sativa*. cv. Lollo Rosso increased by up to 8-fold when exposed to natural levels of UV-B light. In conclusion, the addition of UV-B light to a supplementary glasshouse light recipe may be therefore be a useful preventative measure against pests and disease, and exclusion of UV-B light wavelengths in artificial light environments may leave plants more susceptible to pests and disease and with less nutritious quality than if they had been grown under natural sunlight. In an automated system or a system that only applied low levels or brief flashes of UV-B light, ensuring the safety of researchers or workers, there could be real advantages to the inclusion of UV-B wavelength photons in light recipes, and this is worth further investigation.

6.4.2 Light for control of flowering

Another area that may be explored is the manipulation of the plant circadian clock, and flowering response. It may be possible to control time to harvest and even fruiting and fruit yield with light alone. Light is the dominant environmental signal involved in the entrainment of the circadian clock – an endogenous molecular oscillator which enables the plant to synchronise its biochemical, physiological and developmental processes with the light and

dark cycle of over a 24-hour period and also the annual cycle of the seasons (Yu *et al.*, 2011). The sense of time conferred by this evolutionary mechanism increases the chance that plant processes will occur in the most favourable environmental conditions and therefore increases the likelihood of the plant's survival and propagation.

Both photoperiod and light spectral quality have a pronounced effect on the timing of transition from the vegetative to the reproductive stage of plant development (Endo *et al.*, 2005). In terms of photoperiod, each plant species has what is known as a critical day length (CDL) for induction of flowering. This is the day length most likely to induce the response. If the CDL is shorter than 12 hours, the plant is known as a short-day plant (SDP). If the CDL is longer than 12 hours, the plant is known as a long-day plant (LDP) for the flowering response. Day neutral plants (DNP) do not respond to photoperiod. (Jackson, 2009).

Light spectral quality may however over-ride this photoperiod requirement. Exposure to far-red light for example has been shown to stimulate flowering as part of the shade avoidance response (Tao *et al.*, 2008) – a response that likely evolved to give the plant the best chance at propagation before lowered levels of photosynthesis result in decreased metabolism to such an extent that causes plant death. Low level intra-canopy supplementary far-red LED light has also been shown to increase yield of tomato in the early stages of fruiting (Hao *et al.*, 2016), suggesting that light in this waveband not only induces, but increases strength of the flowering response. Far-red light could therefore be used, for

example in the commercial production of any fruit crop, or even cut flowers, to induce uniform and rigorous flowering across the crop at the grower's time of choosing.

In herb crops grown for their leaves and stems, growers will likely want to delay time to flowering, to allow the plant to accumulate as much vegetative biomass as possible before harvest and sale. It may also be possible to achieve this, under controlled light conditions. CDL may change throughout the life-cycle of a plant. Plants have a juvenile period of growth in which even inductive day lengths do not cause flowering. The juvenile and adult phases are controlled by micro-RNAs and the levels of these are known to be affected by daylength and light quality, with longer days and blue light increasing the mRNA responsible for transition into the adult phase (Jung *et al.*, 2007). For example, under a broad white-light or natural sunlight spectrum, *Coriandrum sativum* (coriander) appears to be an LDP for 'bolting', i.e. end of the vegetative phase. If, with the use of altered levels of blue light, herbs such as coriander could be kept in this juvenile phase and therefore insensitive to photoperiod, it may be possible to expose plants to longer periods or even constant periods of light without inducing flowering, allowing more rapid accumulation of biomass. Growers could more accurately match supply to demand, reducing waste and increasing profit. For example, when the consumer demand is high, growers could complete more harvests in a set amount of time, and when it is low, could slow down production by switching back to growth under shorter photoperiods.

6.4.3 Light recipes tailored to growth stage

In chapters 3 and 4, it was determined that photons in the blue, green, red and far-red regions of the spectrum all contribute towards the successful growth and development of the plant. Multiple waveband light causes the induction of numerous, interacting signalling pathways via both the photosynthetic and non-photosynthetic photoreceptors, which allow plant physiology to best adapt to the light environment. In addition, plants provided with this multiple waveband light likely have the best photonic energy balance throughout the crop due to the differences in the absorption and transmission profiles of the individual wavelengths. Therefore, it was concluded that the optimal light recipe for plant growth would contain light from all four wavebands tested.

However, in the experiments carried out in chapter 4, both light quality and quantity were kept constant over the course of plant growth. Growth in terms of biomass production as well as efficiency of the LED lighting system would be further optimised by the careful timing of application of specific wavelengths of light, tailored to plant developmental/growth stage. For example, to save energy, no light (if the crop has no light requirement for germination) or very low intensity red light (if it does) could be applied after sowing until hypocotyl emergence from the soil. In *C. sativum*, it was found that both blue and far-red light were instrumental in the expansion of the leaves. In the early stages of growth, plants could be grown under relatively low light levels with increased levels of blue and far-red light added to a red-light background in order to induce leaf expansion and maximise leaf surface area for photosynthesis. As blue light was shown to increase leaf photosynthetic capacity, once leaves at

the base of the plant have the wide, low light morphology, far-red light could be removed and the quantity of blue light increased, inducing high-light acclimation in the leaves developing closer to the light. Once the canopy becomes more developed, it may be beneficial to add green light to the recipe in order to better balance the photonic energy distribution through the canopy and drive photosynthesis at the base of the plant (see chapter 3).

6.4.4 'Smart' lighting systems

As LEDs are solid-state devices, they can easily be incorporated into digitally controlled, adjustable plant growth programmes and systems (Bhardwaj *et al.*, 2010). Additionally, LEDs can be instantly switched on, dimmed continuously between maximum output and zero output, and instantly switched off, meaning that complex and even wireless control options are available (Massa *et al.*, 2010; Apse-Apsitis *et al.*, 2012). Custom programmes such as that mentioned above, which change light quality and quantity over time, for optimisation of energy efficiency and growth, could be used.

Furthermore, LED arrays can be arranged on panels, rails or bars, either as planar arrays or vertical arrays which can light the canopy from within (Gomez *et al.*, 2013). Sensors which measure the quality and quantity of light available at certain parts of the canopy can be added into the system, allowing targeted application of light when and where needed. For example, in chapter 5, it was demonstrated that photosynthesis in glasshouse lettuce is rarely saturated, even in the summer months. By incorporating ambient PAR sensors into feedback systems, LED lamps could track natural light changes and 'top up' as

much or as little light as required to saturate photosynthesis with adjustments each second, further increasing yield and energy efficiency of the system.

In chapter 3 it was highlighted both light quantity and quality changes through the vertical canopy profile. Plants with tall canopies such as tomato, or smaller plants stacked in a vertical system (see section 6.5.1) could benefit from the incorporation of PAR sensors and light spectrum sensing photodiodes/spectroradiometers at different canopy levels which feedback to the lamps and allow balancing of the light quantity and quality throughout the canopy. This would promote photosynthesis in the lower layers of the crop whilst also ensuring crop uniformity in a vertical system. Plants in the lower layers would not then develop shade avoidance responses (see chapter 1.20) due to reduced quantity of PAR and increased proportion of green and far-red light (see chapter 3).

Feedback to the lamps may come not only from sensors of ambient light but also from the plants themselves. Sensors which detect when plants have met certain parameters, for example a set amount of leaf/green area expansion, could be used to trigger changes in the light spectral or intensity output of the lamps, optimising output to the needs of the plants for the next stage of growth. This sensing could be achieved either by linking a standard camera to the lamps and incorporating an automated version of the leaf area detection protocol in ImageJ (see chapter 2.1.4.2) or by measuring spectral reflectance to detect normalised difference vegetation index (NDVI), which is an indicator of green area (La *et al.*, 1987).

Chlorophyll fluorescence sensors could also be used to provide direct information on the status of plant photosynthetic capacity/ saturation to the lamps allowing them to independently adjust light intensity to the exact amount of light required for optimal plant growth. This could be achieved by running periodic rapid light response curves such as those used in experimental chapters 4 and 5 and detailed in chapter 2.2.3.2.3. Additionally, the information gained from the light response curves measured in chapter 4 and 5 could help growers make informed decisions about what intensity to set the lights and even provide the basis for programming of a smart lamp system in a glasshouse environment to provide only the light needed to stimulate growth and avoid total light saturation and subsequent photoinhibition.

6.5 Further applications of optimal light recipe research

Information gained from controlled environment growth room research where LED lighting is the sole source of light, such as that presented in chapter 4, is becoming increasingly relevant because of the rising need for light recipes for optimal growth in artificial light environments. Two such environments – Vertical farms and growth of crops in space – are discussed further below.

6.5.1 Vertical farming

As mentioned briefly in chapter 5.1, the rising world population coupled with an increasingly unpredictable and unstable climate is putting growing strain on global food security (Murchie *et al.*, 2009; Pinho *et al.*, 2012; Nelson *et al.*, 2014). According to the Food and Agriculture Organisation of the United Nations (FAO) “Food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets

their dietary needs and food preferences for an active and healthy life.” World population is expected to increase from the current approximately 7.3 billion to 9.7 billion people by the year 2050 (United Nations, 2015), and it is estimated that global food production will have to increase by at least 50% in this time if food security is to be maintained (FAO, 2009; Tilman *et al.*, 2011). The issue is that, due to the growing population’s demand on other resources - such as arable land, fossil fuels and water - there is a growing pressure to produce more food in less space with minimum inputs i.e. less gas, electricity, water and fertilisers (Murchie *et al.*, 2009; Pinho *et al.*, 2012).

One way in which this could be achieved is through ‘vertical farming’, i.e. the growth of crops in closed production systems or ‘plant factories’ where plants are artificially stacked vertically to maximise biomass production per hectare of land used (Kozai *et al.*, 2006; Despommier, 2010, 2013). Such systems allow the year-round production of plants, even in places which would not be suitable for conventional agriculture. For production of horticultural crops grown in closed plant production systems, changes in weather or soil fertility are mitigated by protection from the elements and growth in soil-less hydroponic systems, where precise levels of nutrients can be delivered to the root system for optimal growth. Use of fertiliser can be further diminished by the incorporation of fish into the system, in the process of aquaponics, whereby the waste from the fish acts as fertiliser for the plants (Diver and Rinehart, 2010; Blidariu and Grozea, 2011). Crops grown in controlled environment agriculture are also relatively protected from pests and diseases that increasingly threaten yield and global food security, whilst land use is

minimised by stacking growth systems vertically (Despommier, 2010). For example, one vertical farming system producing lettuce can produce 13.8 times more crop, in terms of fresh-weight yield per m² of occupied growing floor area, than a conventional flat farming system (Touliatos *et al.*, 2016).

There are numerous plant factories already in operation worldwide, at least three of which use entirely artificial light for plant growth (as presented in Despommier, 2011), and the idea of 'urban agriculture', i.e. growing plants in cities, is becoming increasingly popular in both developed and developing countries (Altieri *et al.*, 1999; Zezza and Tasciotti, 2010; Ackerman *et al.*, 2014; Rezai *et al.*, 2016). It has already been shown that the resource use efficiency of water, carbon dioxide and light are all considerably higher in closed plant production systems such as these; however there is still much room for improvement in terms of light and electric energy use efficiencies (Kozai *et al.*, 2006). Therefore, demand for increasingly optimised LED light recipes will grow as vertical farming and urban agriculture becomes an ever more necessary means of producing our food, and will have a crucial role in the maintenance of global food security.

6.5.2 Growth of crops in space

With ever growing advances in aerospace technology, NASA aims to send a crewed mission to Mars by the year 2040 (Poulet *et al.*, 2014). Missions such as this will require the production of fresh food whilst in transit. For example, a mission to Mars would take approximately 1000 days, and it would be impossible to carry enough food to support crew members for a trip of this length (Drysedale *et al.*, 2003). It has already been ascertained that plants can be

successfully grown and propagated for multiple generations in space due to work on the International Space Station (ISS) (Stutte *et al.*, 2005; Sychev *et al.*, 2007). Here, LEDs have become the growth light of choice due to their high energy efficiencies, low heat-emission, compact nature (no ballast required) and high degree of programmability (Poulet *et al.*, 2014). As in the closed vertical farming systems described above, light recipes must be formulated that provide all of the necessary wavelengths for healthy growth and completion of the plant life-cycle under a completely artificial light environment. Recipes which increase WUEi are of particular interest, as due to energy, space and mass limitations aboard spacecraft is only possible to carry or produce a limited water supply (Grigoriev *et al.*, 2011).

6.6 LEDs for the future of horticultural lighting

The two main factors currently dissuading commercial growers from converting to LED lighting installations from their current systems are risk and cost (Nelson and Bugbee, 2014). In terms of risk, the experimental evidence presented in chapters 3-5 provides a compelling case for the movement away from crop cultivation under conventional light sources towards smart LED lighting systems. The summer lettuce glasshouse trial in chapter 5 confirmed that LED lighting systems can produce crops of the same or superior yield and quality to the HPS lighting system many growers already have in place. In addition, monochromatic red LED lighting was more efficient in terms of energy input per gram of biomass accumulation or 'crop per watt'. However, before growers will consider the change it must be determined that pay-off in terms

of energy savings and increased productivity must outweigh the cost of purchasing the new lighting systems, control systems and their installation.

Nelson and Bugbee (2014) found that currently, the cost of installing new LED luminaries in a commercial glasshouse may outweigh any electricity cost savings for at least the first 5 years. However, rapid advances in technology mean that LED efficiency is increasing by approximately 35% and price falling by approximately 20% annually (Apse-Apsitis *et al.*, 2012). Combined with the advances in optimisation of LED light recipes for increased crop resource efficiency and production of yield, and smart lamp technology as outlined above, this pay-off time is likely to greatly decrease in the near future. In fact, the LED growth light market is expected to reach a net worth of \$1.9 billion by 2020, with a compound annual growth rate of 26.93% per year (MarketsandMarkets, 2015).

6.7 Final conclusions

Advances were made in the understanding of the effects of green light in plant growth and development, particularly with regards to acclimation of the photosynthetic stoichiometry and with a new hypothesis being presented on its role in canopy water use efficiency. The physiological reasons behind the unsuitability of monochromatic red light as the sole source of light for plant growth were also determined. It was concluded that LED lighting systems are a viable energy efficient alternative to conventional lighting used in horticulture which afford a higher degree of control over plant growth and development. Monochromatic red light may be a suitable for HPS lamps in a commercial glasshouse environment, whilst lamps which produce photons from multiple

wavebands - i.e. blue, green, red and far red light – are likely the best direct replacement for growth in completely artificial light environments. They can be programmed to adjust light spectral output to induce desirable plant characteristics such as those which lead to increased resource use efficiency and whole leaf photosynthesis and ultimately fresh-weight yield. Furthermore, smart LED lighting systems with optimised spectral output will be an integral part of future horticultural practice and will contribute to continuing global food security.

7 References

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