Development of a Variable-Spectra LED Array for Optimized Plant Development

by

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A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Environmental Science

Guelph, Ontario, Canada

ABSTRACT

DEVELOPMENT OF A VARIABLE-SPECTRA LED ARRAY FOR OPTIMIZED PLANT DEVELOPMENT

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Plant development can be dramatically influenced by the quality of light present in its environment. Conventional plant lighting systems do not allow for fine control of light quality, which may not be optimal for all species. To address this problem, a novel lighting system was developed using narrow-bandwidth, multi-coloured LEDs. This system was used to test the effects of different light qualities on germination rate, photosynthesis during vegetative growth, and the photosynthetically adaptive nature of several species. It was found that far red light and blue light can significantly inhibit germination in some species. Red and deep red light was generally most effective at driving photosynthesis during vegetative growth. Plants were shown to have an impaired photosynthetic capacity when abruptly switched to a new light quality. Optimizing germination and photosynthesis are the first steps in designing optimal light "recipes" for plant production.

ACKNOWLEDGEMENTS

Thank you to my advisor Dr. Mike Dixon for the opportunity to work on this project, who provided guidance when necessary but allowed me the freedom to explore many ideas independently. Thank you to Dr. Michael Stasiak for his patience in training me in various lab methods for this project and for always being available to bounce ideas off of. Thank you to Jamie Lawson for your hours of wiring and re-wiring LED arrays over the last two years, and for teaching me a few things about electronics in the process. Thank you to my advisory committee Dr. Bernard Grodzinski, Dr. Barry Micallef, and Dr. Alan Scott for keeping me and my project focused throughout its duration, and for their time spent in speaking with me and reviewing my work. Thank you to Per Aage Lyssa for lending your technical knowledge and experience with LEDs to the creation of the LED arrays. Thank you to Lynn Tian for always having plants of all ages ready to go for me every week. Thank you to Polina Bam and Jessica Gal for lending a hand from time to time in experiment preparation and saving me a few slow hours of work. This work was supported by the Natural Sciences and Engineering Research Council (NSERC), Canadian Space Agency (CSA) and Industry partner COM DEV Ltd.

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LIST OF ABBREVIATIONS

A	Amber
BL	Blue
CESRF	Controlled environment systems research facility
CY	Cyan
DR	Deep red
ETR	Electron transport rate
FR	Far red
GN	Green
GP	Germinating pod
HPS	High pressure sodium
LED	Light emitting diode
LPS	Low pressure sodium
MH	Metal halide
NCER	Net carbon exchange rate
RB	Royal blue
RD	Red

CHAPTER 1: INTRODUCTION

Many factors, such as lighting conditions, nutrient availability, humidity, temperature, and many others regulate plant growth and development. When designing an enclosed system for plant growth, whether it be a commercial greenhouse, or a system situated in more challenging environments such as Canada's northern settlements, underground, or on the international space station, it is vital that the system be optimized to the requirements of whatever plant species is being produced. Optimization of this type of growth chamber would demand the implementation of an environment control system capable of specifically manipulating whatever aspect of crop development is desired, whether that be dry matter production, synthesis of desirable compounds, or triggering specific physiological changes. Among the most important environment variables is light. An ideal lighting system would achieve these goals as efficiently as possible, while minimizing cost, energy demand, and user input and training, as compared to conventional lighting systems.

1.1: PLANT-LIGHT INTERACTION

1.1.1: Plant Light Requirements: photoperiod, intensity, and quality

Considering only the presence or absence of light is not sufficient in optimizing plant development. The number of consecutive hours of light in a day a plant receives, the intensity of that light, and the spectral makeup of that light all contribute to the design of ideal lighting conditions. Extensive research has been conducted on photoperiod and light intensity, but due to technological limitations of light sources, studies of the effects of light quality are few.

1.1.2: Photoreceptors and Light-Regulated Processes

It is well established that plants utilize certain wavelengths of light better than others (McCree 1972). These wavelengths are absorbed by specific pigments and photoreceptors, including chlorophylls, cryptochromes, phytochromes, and others, that initiate a variety of photochemical responses when stimulated.

Chlorophyll and b are pigments that most effectively absorb red and blue light (Purves, Sadava et al. 2004). Chlorophyll is a key component of photosynthesis; it is shown in several cases that growing plants under only red and blue light is sufficient to effectively drive photosynthesis and fully develop a plant (Purves, Sadava et al. 2004).

Cryptochrome is a blue-light photoreceptor and is implicated in several responses including phototropism, hypocotyl elongation, cotyledon expansion, petiole elongation, leaf expansion, regulation of the phenylpropanoid pathway, plasma membrane depolarization, and circadian rhythm, and others (Jackson and Jenkins 1995, Ahmad, Jarillo et al. 1998, Guo, Yang et al. 1998, Parks, Cho et al. 1998, Somers, Devlin et al. 1998, Hogewoning, Trouwborst et al. 2010).

Phytochrome exists in both a red-absorbing conformation and a far-red absorbing conformation. It plays many regulatory roles including, but not limited to, involvement in seed germination, cell elongation, activity of nitrate reductase, and flowering (Flint 1934, Walker and Bailey 1968, Shinomura 1997, Batschauer 1998, Devlin, Patel et al. 1998, Lillo and Appenroth 2001, Halliday, Salter et al. 2003).

While chlorophyll, cryptochrome, and phytochome are the dominant, and currently best defined photoreceptors in plants, several others play roles in development. Phototropin regulates

phototrophic response in plants (Briggs and Olney 2001). UVR8 photoreceptor is sensitive to UV-B light, and triggers UV defense mechanisms (Christie, Arvai et al. 2012).

It is not surprising that the majority of research into the effect of light quality on plants has focused on red and/or blue light, given the influence that these wavelengths have on a plant. Recently, experiments using narrow-band red and blue LEDs have been completed to determine physiological responses of some plants to specific wavelengths in the red and blue regions. Goins and Yorio (Goins and Yorio 2000) reported that 690 nm red light and 470 nm blue light yielded increased biomass yield in spinach relative to fluorescent white light. Lefsrud *et al.* (Lefsrud, Kopsell et al. 2008) found that 440 nm light caused a greater accumulation of β-carotene than 400 nm light, and maximum accumulation of chlorophyll a, chlorophyll b and carotenoids beneficial to human health occurred with 640 nm red light, compared to far-red, green, blue, or UVA light. It was found by Ménard *et al.* (Ménard, Dorais et al. 2005) that supplementing high-pressure sodium (HPS) lamps with 455 nm light increased plant biomass and fruit yield significantly, compared to plants grown under HPS lamps alone.

Plants have evolved in an environment where the photon flux in the range of photosynthetically active radiation (PAR) is evenly distributed, similar to white light (Gueymard 2004, Hogewoning, Douwstra et al. 2010). Despite this, it has been shown for some plants that red and blue light alone is sufficient for the plant to complete its life cycle with no significant effects to physiological traits, compared to plants grown under white light (Yorio, Goins et al. 2001). For other plants, it is clear that there are other wavelengths outside the red and blue regions that significantly benefit the plant, but the wavelengths that are most beneficial and complementary are still unclear (Yorio, Goins et al. 2001).

A lighting system more closely representing natural light has been shown to improve plant growth compared to high-pressure sodium lamps, which typically favour red to orange light (Brown, Schuerger et al. 1995, Hogewoning, Douwstra et al. 2010). This suggests that supplementing red and blue lighting systems with other wavelengths can improve various qualities of plants. Britz *et al.* (Britz, Mirecki et al. 2009) showed that supplemental UV-B radiation can increase the accumulation of phenolic compounds that act as natural preservatives and are beneficial to human health. The photosynthetic enhancement that occurs when combining red and far-red light has been labeled the "Emerson Enhancement Effect" (Govindjee 1964). Lettuce supplemented with red, green and blue light versus only red and blue light yielded greater fresh and dry weights as well as increased leaf area (Kim, Goins et al. 2004). The addition of green light to the standard red and blue has also been shown to increase electron transport rate (ETR) and quantum yield of photosystem II in spinach (Liu, Chang et al. 2009). Klein (Klein 1992) found that green light is effectively transmitted through foliage, providing light to lower tissues. These findings illustrate that plants do benefit from wavelengths of light other than red and blue light alone.

1.2: CURRENT PLANT LIGHTING SYSTEMS

There are several different light sources that are used in conventional greenhouse lighting. Some of the more common light sources used include natural sunlight, high-pressure sodium lamps, metal-halide lamps, fluorescent lamps, magnetic induction lamps, and light-emitting diode (LED) systems.

1.2.1: High-Pressure Sodium Lamps

High-pressure sodium (HPS) and metal halide (MH) lamps are high-intensity discharge (HID) lamps, two of the most common sources of artificial light in commercial greenhouses, world-wide. HPS lamps were first manufactured by General Electric in 1961. The technology was built upon previously existing low-pressure sodium (LPS) lamps, with the goal of creating a more neutral-coloured light, as opposed to the distinctly yellow-coloured LPS lamps. HPS lamps are now widely used in a variety of applications including street lighting and greenhouse lighting.

In greenhouse lighting, HPS lamps are desirable for their relatively high efficacy of up to 160 Lm/W, depending on the brand and model of lamp (Philips 2013). Similarly, HPS lamps are known to have a long lifespan, in some cases exceeding 30,000 hours of operation (Sylvania 2013). The quality of light emitted from HPS lamps generally is biased with major peaks in the red-orange region of the spectrum (550 nm -650 nm) and minor peaks in the blue region of the spectrum (440 nm – 500 nm). A sample spectrum of a typical modern HPS lamp is illustrated in **figure 1.1.** This emission spectra is not dissimilar to the action spectra of chlorophyll, and in fact is quite effective in driving photosynthesis. As discussed in section 1.1.2, it is also known that these biases towards longer wavelengths can have a potentially desirable influence on plant physiology.

1.2.2: Metal Halide Lamps

Just as HPS lamps were based upon an earlier technology, MH lamps were built upon the pre-existing mercury-vapor lamp, which had a peculiar blue-green colour-cast. The addition of metal halide salts to mercury-vapor lamps broadened the emission spectra of the lamps, giving a more natural appearance of white light (AmericanHistory 2013). The first MH lamp to go into production was designed by Gilbert Reiling, of General Electric, after receiving a patent on his

design in 1966 (Reiling 1966). MH lamps are now widely used in applications requiring a high-intensity light with accurate colour-rendering, such as lighting for sports stadiums, street lighting, car headlights, and greenhouse lighting.

Metal halide lamps have a similar efficacy and lifespan of HPS lamps (Philips 2013, Sylvania 2013), and are desirable for the same reasons. Although light from these lamps generally appears white to our eyes, its true emission spectra is biased toward the blue regions of the visible spectrum. This is illustrated in **figure 1.2**. Emphasis on this part of the spectrum can be beneficial to plants, but, as discussed in section 1.1.2, will benefit plants in different ways than the red-light-bias of HPS lamps.

Both HPS and MH technologies are effective means of lighting plants, but suffer some limitations. Both produce a tremendous amount of heat, with bulb temperatures exceeding 400°C (Philips 2013). This requires that the bulb be physically separated by a relatively large distance from the plants it may be lighting, as compared to other technologies, so as to not raise the plant's temperature, or the temperature of whatever media it is growing in.

1.2.3: Fluorescent Lamps

Early fluorescent bulb development spanned several years of iterative developments before a commercial product was available. The first publically available fluorescent bulb was released by General Electric in 1938 (Reich 1992). This bulb had a relatively warm colour temperate of 3500K, a lifespan of 1500 hours, and an efficacy of 34 lm/W. The initial success of these bulbs was based on their ability to emit a much broader spectrum than incandescent bulbs and operated with greater electrical efficiency (Withrow and Withrow 1947, Murdoch 1985).

Modern fluorescent tubes are available in a variety of colour temperatures. Different colours are achieved with the use of different phosphors in the fluorescent tube (Sager, Edwards et al. 1982, Wheeler 2008). Fluorescent lamps currently available can have a lifespan up to 42,000 hours, and an efficacy of 97 lm/W (Philips 2013, Sylvania 2013).

1.2.4: Light-Emitting Diodes

Light-emitting diodes (LEDs) are a technology highly dissimilar to those previously discussed. Unlike HPS or MH lamps, which produce light by heating an element, or fluorescent lamps, where light is the product of excited gasses, LEDs release photons by filling electron "holes" in semiconductive materials. At the atomic level, holes are created in energy bands of the semiconductor when an electron is excited from the valence band to the conduction band. That element becomes positively charged, leaving it relatively receptive to accepting incoming electrons. The addition of foreign elements to a semiconductor, a process known as "doping," creates these electron holes in a controlled manner (Woodyard 1950). A single semiconductor crystal can be doped in such a way that it will have two distinct behavioral types, depending on the doping species. Between these two sections resides the "p-n junction," which allows electrical current to flow in only one direction. When current is applied, and electrons pass through this junction, holes in the doped semiconductor are filled, emitting photons (Biard and Pittman 1966).

Biard and Pittman (Biard and Pittman 1966) inadvertently developed the first LED in 1961, using a zinc-doped gallium arsenide semiconductor, while trying to develop a laser diode. Their LED emitted infrared light, and they were awarded a patent on their design in 1966. Shortly after Biard and Pittman's infrared LED, Nick Holonyak (Holonyak and Bevacqua 1962) developed the first LED to emit light in the visible spectrum. He used gallium arsenide

phosphide to produce his red-light LED. George Craford invented the first yellow LED, as well as red and orange LEDs of greater intensity than Holonyak's, in 1972. Like Holonyak, he started with gallium arsenide phosphide as his substrate, but then doped it with nitrogen, a process that other researchers at the time had told him was not feasible (Perry 1995). In 1994, Shuji Nakamura developed the first blue LED. This was achieved using zinc-doped gallium-nitride semiconductors, rather than the gallium arsenide semiconductors described previously (Nakamura, Mukai et al. 1994, Nakamura, Pearton et al. 2000). Since his blue LED, Nakamura has continued work in this field and has produced green and yellow LEDs of far greater intensity than what had previously existed (Nakamura, Senoh et al. 1995).

Since these early key developments, LEDs have become available in a wide array of colours, with far greater intensities than what was achieved with early LEDs. How these colours and intensities are achieved by manufacturers, however, is not publically shared information (Philips 2013). High-intensity LEDs of very specific monochromatic light qualities offer distinct advantages over any other lighting technology available. The combination of such LEDs into a full-spectrum array allows for the engineering of very specific light "recipes," or emission spectra, which can easily be adapted to drive any targeted light-sensitive process in plants. These processes could include, and are not limited to, driving photosynthesis for greater accumulation of dry matter, potentially leading to greater food production, triggering certain metabolic pathways for synthesis of certain compounds, or for managing the uptake and use of specific nutrients. In addition to the specificity of LED light quality, LEDs are also robust, as they are solid-state electronics, are extremely energy efficient, produce relatively little heat, and are physically very small, compared to other lighting systems. These are all highly desirable traits in the engineering of a next-generation lighting system.

1.3: OBJECTIVES AND HYPOTHESIS

The main technical objective was to design and test a light emitting diode array as the sole source of photosynthetic energy for plant production and with the capability to elicit a range of physiological responses. Thereafter, it was proposed to apply this technology in assessing the responses of various plants to different light spectral qualities. It was hypothesized that plants would vary in their response to different spectral qualities at different physiological stages of growth. It was proposed to investigate germination and vegetative growth stages.

CHAPTER 2: DEVELOPMENT OF HIGH INTENSITY, VARIABLE SPECTRA LED ARRAYS

2.1: DESIGN AND PROTOTYPING

2.1.1: LED Selection

As discussed previously, the unique qualities desired in LED selection were that they be of the highest light intensity available, and be monochromatic with a narrow bandwidth available in a range of colours. Other favourable qualities of LEDs such as long operating life, dim-ability, robustness, electrical efficiency, and relatively cool operating temperatures were reasonable assumptions for most modern LEDs available, given the nature of the technology, so were not major factors in selecting the best LEDs for development of this lighting system. Manufacturers considered when seeking the desired LEDs included Philips (Philips 2013), Cree (Cree 2013), General Electric (Electric 2013), Osram Sylvania (Sylvania 2013), and LED Engin (Engin 2013). Philips and LED Engin were both found to have products meeting the requirements of high intensity and narrow-bandwidth, coloured LEDs, in the form of the "LUXEON Rebel Color" and "LZ," respectively.

Philips' Luxeon Rebel Color LEDs are available in Deep red (DR), red (RD), amber (A), green (GN), cyan (CY), blue (BL), and royal-blue (RB), with reported peak wavelengths of 655 nm, 627 nm, 591 nm, 530 nm, 505 nm, 470 nm, and 440 nm, respectively. All, with the exception of amber, have a spectral half-width of 30 nm or less, indicating a very specific light quality, compared to other lighting technologies. Amber has a spectral half-width of 80 nm, which could act as a "background" light quality to supplement more specific light recipes designed with the other colours (Philips 2013).

LZ LEDs from LED Engin are available in a wider selection of colours relevant to plant development, including UV-A1 (365 nm) UV-A2 (400 nm), blue (460 nm), green (523 nm), amber (590 nm), red (623 nm), deep-red (660 nm), and far red (FR) (740 nm). LED Engin's LEDs also provide a greater intensity of light than those provided by Philips (Engin 2013).

After identification of suitable LEDs available from both manufacturers, it was concluded that the most effective means of ultimately building several LED arrays for eliciting various plant responses would be to work with the more affordable Philips LEDs for colours in the visible spectrum, and to obtain the UV and far red LEDs from LED Engin. This strategy allowed for the production of more LED arrays on our budget, while still achieving a very high intensity of light and wide range of colours.

2.1.2: Array Design, assembly, and control

There were several design challenges to overcome when determining how to most effectively deliver light from the selected LEDs to plants in the growth chambers already present at CESRF. The physical size of the array, cooling, channels for wiring, the spatial arrangement and number of the LEDs per array, the addition of optics to the LEDs to mix the light in the chamber, and means of moving the array and creating an effective seal between the array and the growth chamber needed to be addressed. Upon completion of array assembly, cooling and light control systems needed to be developed.

2.1.2.1: Heat Sink and array body

First, the physical size of the array and its housing was considered. The arrays were to be initially mounted on top of, or suspended above the single plant chambers present at CESRF.

These are cylindrical in shape, and have an internal diameter of 0.46 m. It was anticipated that in the future, the arrays would likely be migrated to larger growth chambers at CESRF that have 0.5 m by 1.5 m rectangular windows for lighting on their tops. To accommodate both chambers, a 0.49 m by 0.49 m by 0.02 m block of aluminum was machined by Norwegian industry partner, Intravision, then sent to CESRF in Guelph. The aluminum block consisted of 1 cm thick plates sandwiched together, with coolant channels cut into each on the complimentary faces. Six holes 0.015 m in diameter were evenly spaced radially from the centre of the plate that would act was wiring channels. A rubber gasket was added between the two aluminum plates to prevent any coolant from leaking out of the sides or wiring channels of the heat sink. The aluminum heat sink is illustrated in **figure 2.1** and **figure 2.2**.

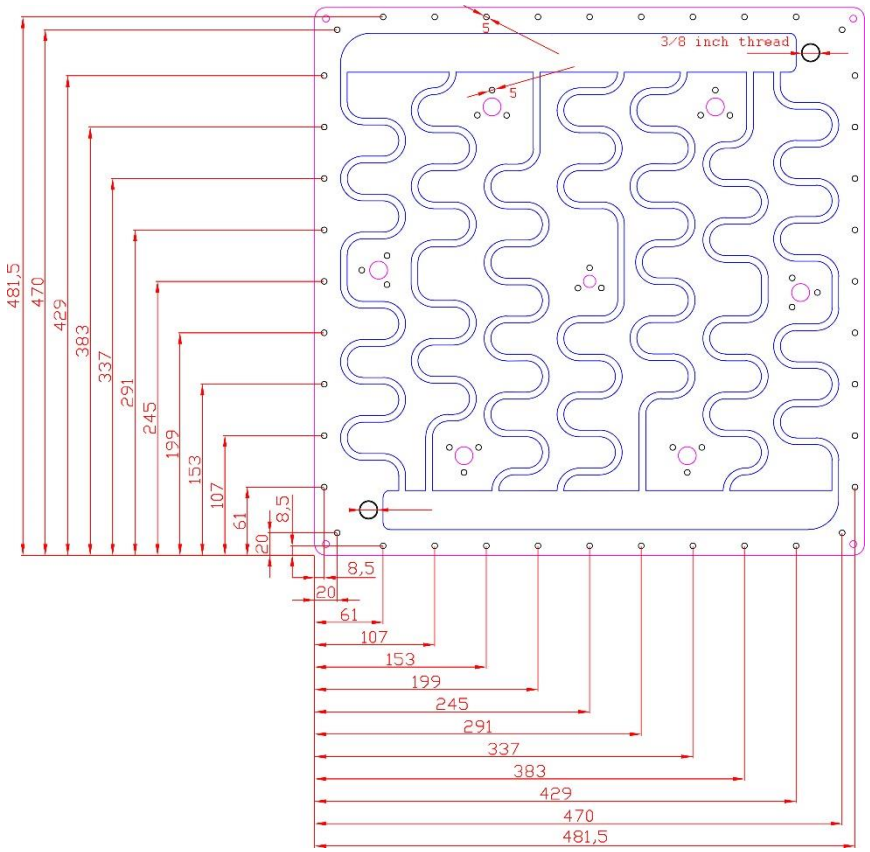


Figure 2.1: Technical drawing of the top half of the LED array cooling plate. Coolant channels are indicated in blue. Pink circles indicate wiring channels. Large black circles are coolant inlet and outlets. Unless otherwise indicated, all values are in millimetres.

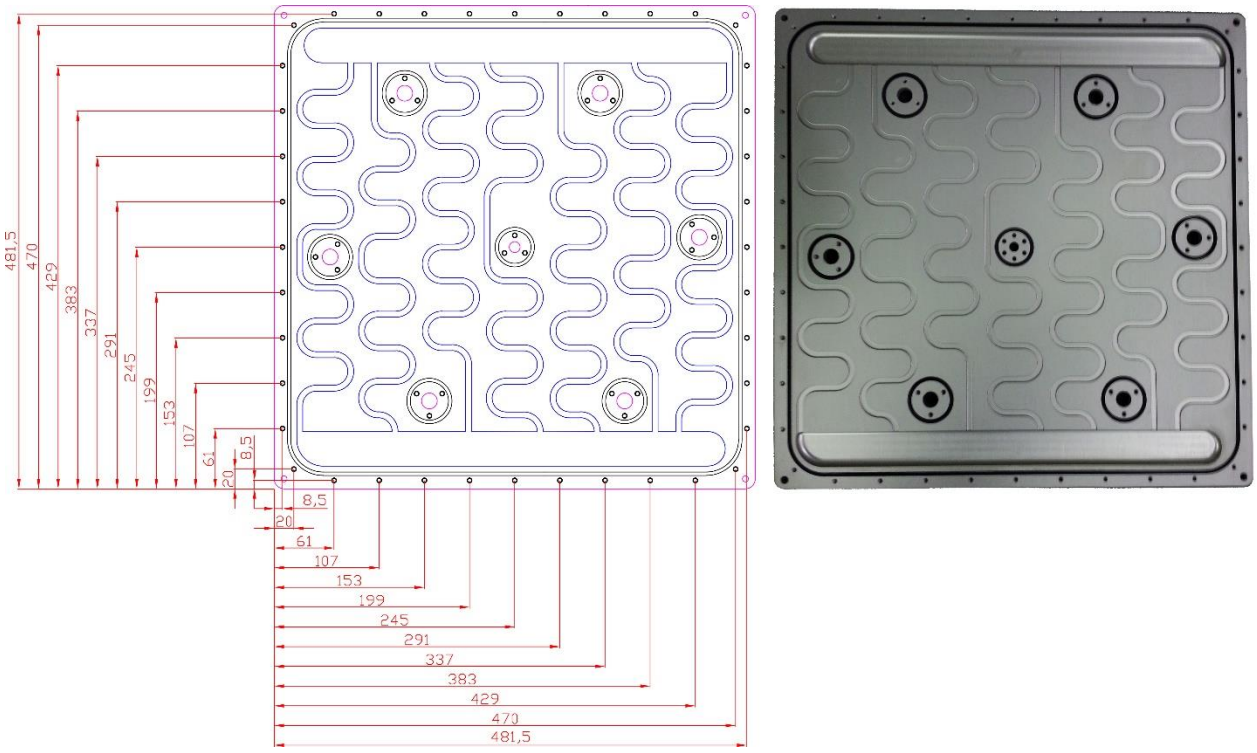


Figure 2.2: Technical drawing and photo of the bottom half the LED array cooling plate. All values are in millimetres.

2.1.2.2: LED array configuration

When determining the physical arrangement of LEDs in the array, the primary objective was to have the colours as evenly distributed as possible. Of the ten total different LED colours chosen, only the seven colours that were of the visible light spectrum were directly incorporated into the initial design of the array. In anticipation of adding the two types of UV LEDs and the far red LEDs at a later date, the arrangement of the initial seven-colour array took into consideration the space, distribution, and wiring requirements of those LEDs.

It was found that a hexagonal arrangement, as shown in **figure 2.3**, allowed for tightest arrangement of seven LEDs. Each cluster of seven LEDs contained all of one colour. Seven different monochromatic LED clusters, each containing a different colour of LED, then came

together to complete one "star" (**figure 2.3**). Each star contained a total of 49 LEDs and emitted seven different colours in a range of combinations and intensity. Given the physical dimensions of the growth chambers at CESRF, and the consequent size restrictions of the array, it was possible to fit up to twelve LED stars into a single array. These were positioned in a "snowflake" configuration (**figure 2.4**) which optimized light distribution and left room for addition of UV LEDs at its centre, and far red LEDs peripherally.

Figure 2.3: LED "star". Each star contained seven differently coloured monochromatic LEDs on a printed circuit board (PCB). Each colour was in a cluster of seven of the same coloured LED, outlined in red. It was originally intended that each cluster of seven LEDs would have one of each of seven colours used, but the circuitry required to allow control of each colour in that scheme was unfeasible. The arrangement exercised was found to still allow for effective light mixing, despite each colour of LED being in a discrete cluster.

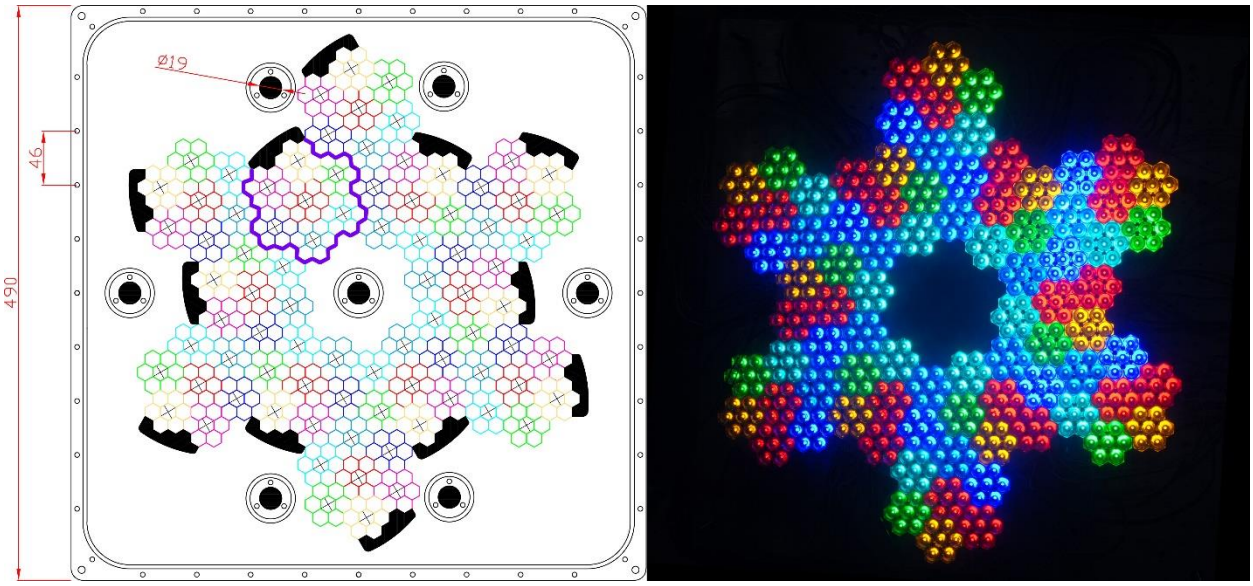


Figure 2.4: Snowflake arrangement of LED stars. Left: one star is outlined in purple to illustrate the interlocking of the stars. Right: the completed LED array.

2.1.2.3: Optics for focusing light

The LEDs are manufactured by Philips with a small lens over each with an exit angle of 120°. To focus the light to a spread of 50°, secondary lenses were added to the LEDs. This angle allowed for homogeneous light mixing 30 cm away from the final array, while focusing as much light as possible onto the plants below. The lenses were equipped with plastic pegs that passed through holes in the PCBs and then melted onto the back of the PCB, securing the lenses in place (**figure 2.5**).

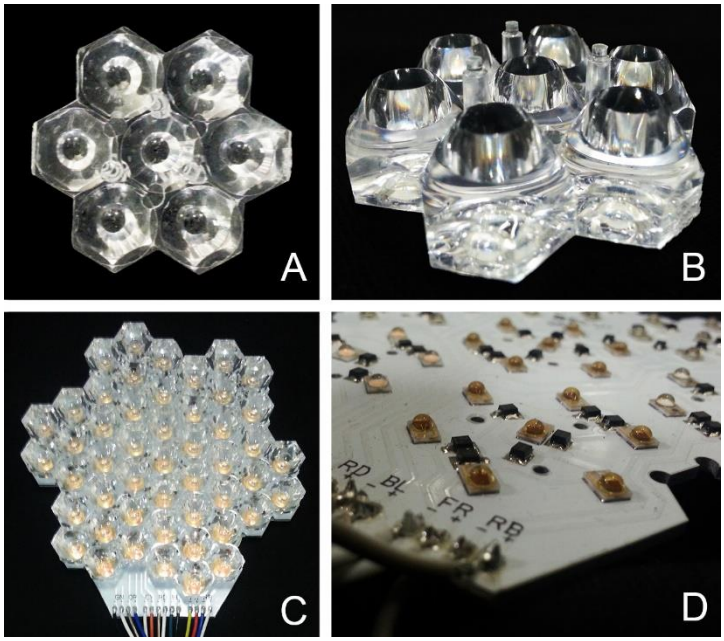


Figure 2.5: Lenses were added to reduce the light spread of every LED from 120° to 50°. ***(A)*** *Lenses were made in the same cluster formation as LED clusters on the PCB.* ***(B)*** *The bottom of each lens cluster was equipped with 3 legs that would extend through holes in the PCB, then would be melted onto the back of the PCB to hold the lenses in place.* ***(C)*** *Each lens cluster interlocks with those around it to form a seamless unit.* ***(D)*** *Three holes in the PCB in every LED cluster to accommodate the lens pegs.*

To complete the installation, a 0.5 cm thick window was fixed over the illuminating face of the array. Several types of glass and plastic for use as material for the window were tested for their light transmittance (**figure 2.6**). Transparent acrylic was chosen for the window material for its minimal light attenuation, and relatively light weight and low cost. The window helped to further secure the lenses in place, as well as provided a flat surface that would be in contact with the top of the growth chambers, allowing for an effective seal. Quarter-inch (6 mm) aircraft cable was added to the back of the array to act as a suspension system when hanging or moving the array was necessary.

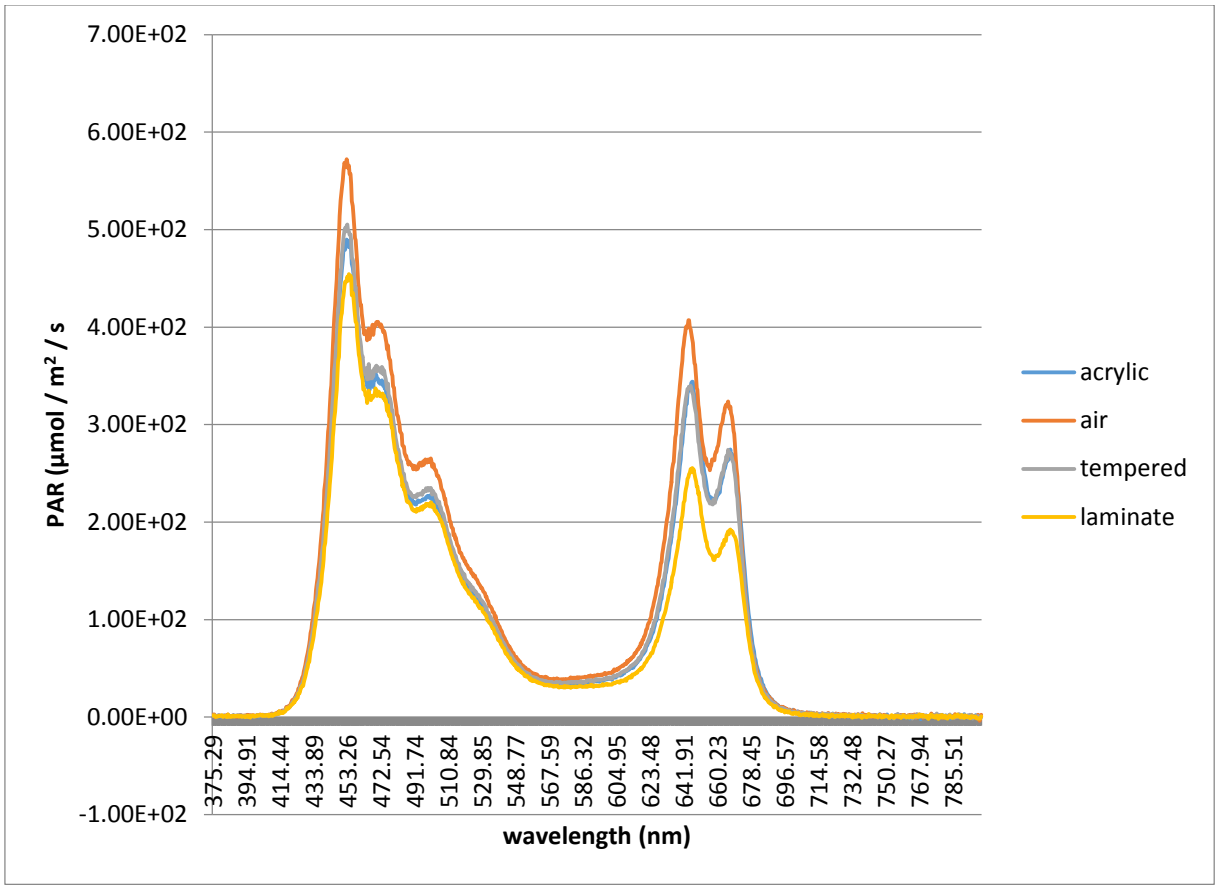


Figure 2.6: Transmitted irradiance of PAR from LED array through air, transparent acrylic, tempered glass, and laminated glass. It was found that none of the tested materials attenuated the light quality in any considerable way. Acrylic and tempered glass both transmitted light well, relative to laminate. For this application, acrylic was chosen over tempered glass for its light weight and low cost. It should be noted that, at the time of this testing, the array was not equipped with UV or far red LEDs. The transmittance of these wavelengths is addressed separately.

2.1.2.5: Powering the array

To maintain control of intensity and operating time of each colour independently, a separate ballast and power supply was required for each. The ballasts used were designed to maintain a constant current of 700 mA, which is the recommended DC forward current for these LEDs (Philips 2013). Light output was manipulated by regulating voltages of each ballast. The required forward voltage (V) varied with each colour, with RB, BL, CY, GN and A each requiring 2.55 V, and red and deep red each requiring 1.80 V. The 365 nm UV-A, 400 nm UV-

A, and 740 nm UV-A LEDs that would later be added to the array require 41.28 V, 41.28 V, and 9.0 V, respectively.

Each ballast contained four operating channels. As there were 12 LED stars, and consequently, 12 LED clusters of each colour on each array, each channel on the ballast would drive three LED clusters. The stars were wired in series; this implied that, for example, if one channel on a ballast were to drive three clusters of RB LEDs at the lowest possible power, it would be outputting

(2.55V x (3 clusters of 7 LEDS)) ➔ 53.55 V at 700 mA == 37.49 W of power.

Typically, all four channels would be active, powering all of the stars, so the total power output of the ballast, in this example, would be

37.49 W x 4 channels == 149.96 W.

2.1.2.6: Cooling and LED control systems

Although LEDs operate at relatively cool temperatures compared to other plant lighting systems, the number and intensity of these LEDs produced enough heat to be problematic if not carefully managed. If left unchecked, increasing temperatures in the array could have resulted in attenuation of the light quality, followed by LED failure.

Two thermistors were fixed to the heat sink of the array. Temperature was constantly monitored and managed by the ARGUS® control system in place at CESRF (Argus 2013). The system was set to maintain each array at 22°C, which is in the range of recommended LED

operating temperature by both Philips and LED Engin. With any increase in temperature, the control system would open a valve that regulated the flow of coolant through the heat sink.

To control the light quality, intensity, and photoperiod of each array, an open-source microcontroller Arduino® Due (Arduino 2013) was connected to every ballast. The microcontroller provided user-programmable instructions to the ballasts regarding how many volts should be applied at a given time, and for what duration. An example of the code for this operation is given in **appendix I**.

2.2: CHARACTERIZATION OF LIGHT QUALITY AND INTENSITY IN SINGLE-PLANT CHAMBERS AT CESRF

2.2.1: Emission Spectra and light intensity

Upon acquisition of the LED stars, it was necessary to verify the spectral output of each type of LED. All spectral graphs in this report were generated using an Ocean Optics USB2000+ miniature fiber optic spectrometer and Ocean Optics SpectraSuite software. The emission spectra of each LED is illustrated in **figure 2.7**.

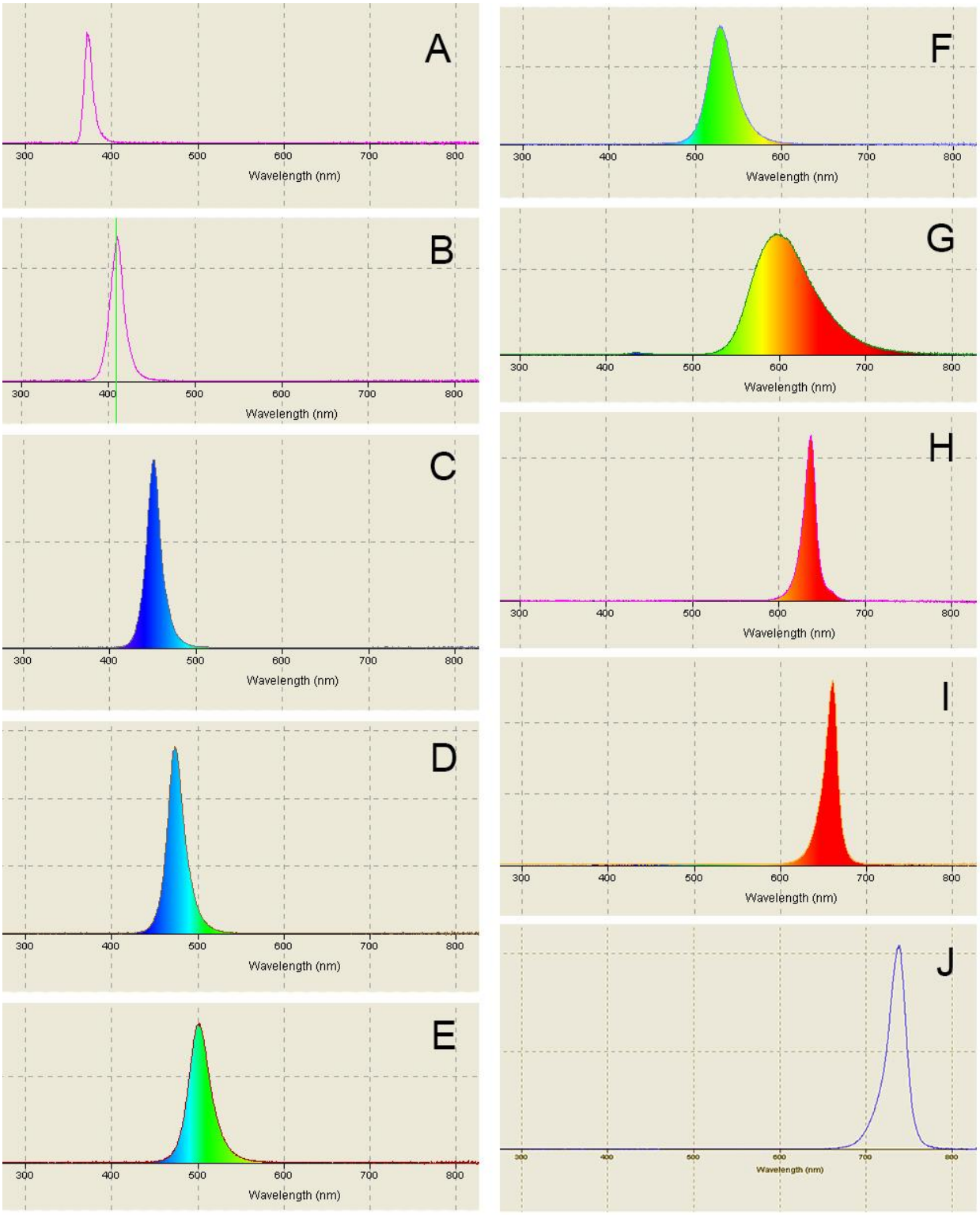


Figure 2.7: Emission spectra of each LED to be included in the array. (A) UV-A1; (B) UV-A2; (C) RB (D) BL (E) CY (F) GN (G) A (H) RD (I) DR (J) FR. These graphs are intended to illustrate the peak wavelengths of each LED. Intensity relative to each other is not portrayed to scale.

Light intensity is typically reported by manufacturers as "efficacy," measured in lumens/W. This is a measurement of how bright a light appears to human eyes, and so is not relevant when discussing light intensities for plants. Regarding plant development, a more relevant measurement of light intensity is the areal flux of photosynthetically active radiation (PAR), measured in µmol of photons/m^2/s. Due to the spatial arrangement of colours in the array and internal light reflection in the growth chambers, it was anticipated that the intensity of any given colour would vary within the growth chamber. To quantify the intensity of each colour at any position within the growth chamber, a 3-dimensional grid was established for measuring PAR. X and Y positions were mapped onto a support plate within the growth chamber (**figure 2.8**). PAR was measured for every colour individually at every intersection on the grid. This was repeated at 40 cm, 60 cm, and 100 cm away from the array. The results of these measurements are summarized in **table 2.1**. From these data, an equation can be derived for each colour at each point to determine the intensity of that colour at a given point and height.

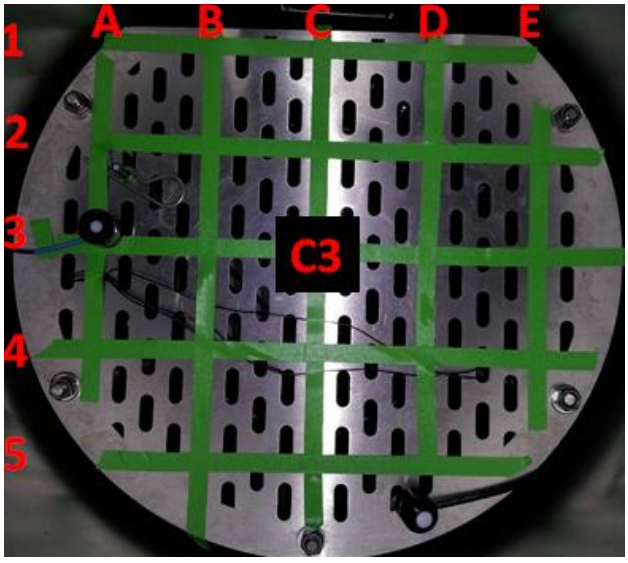
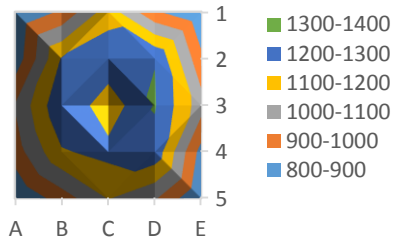
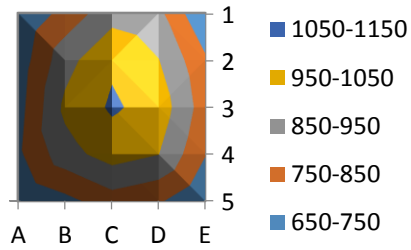
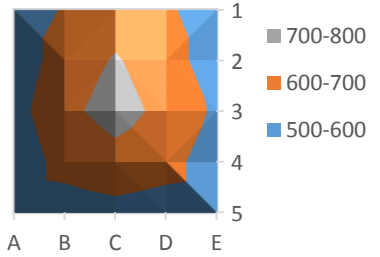
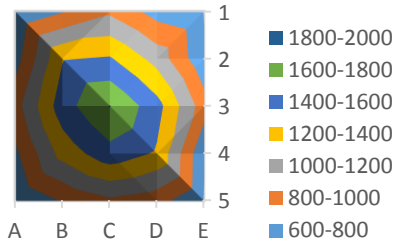
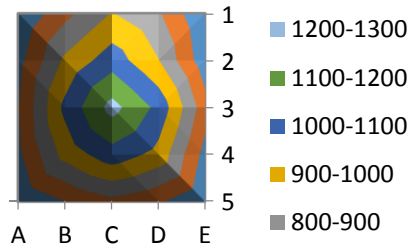
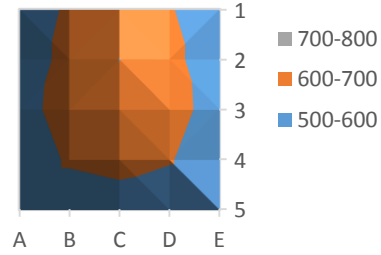
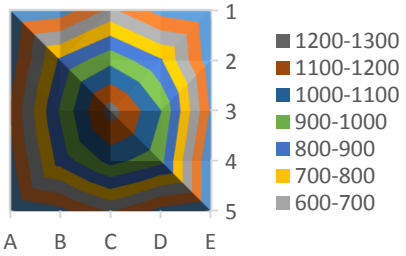
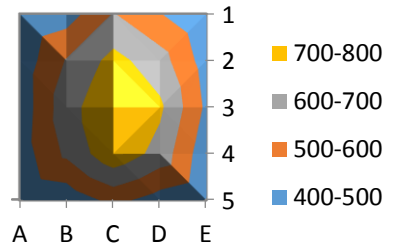
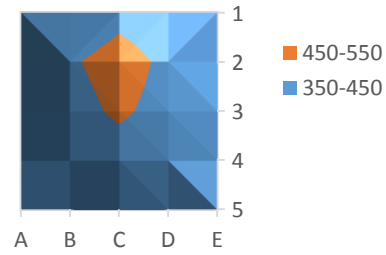
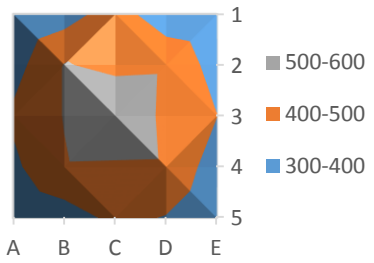
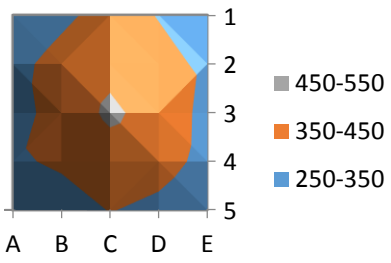
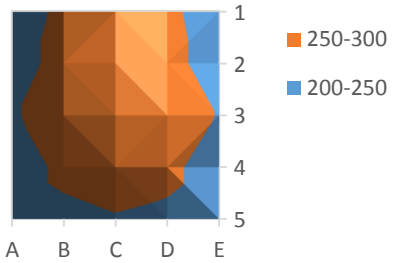
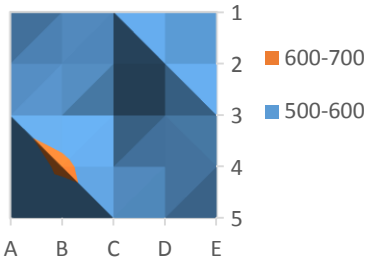
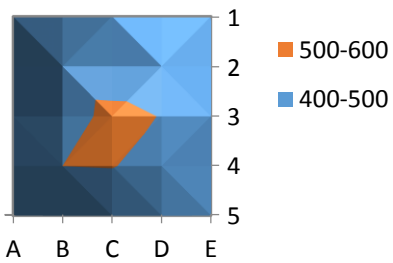
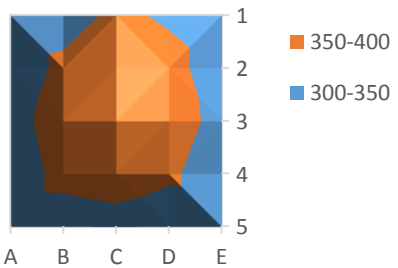
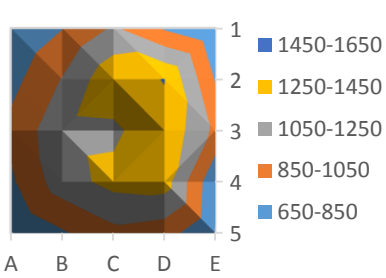
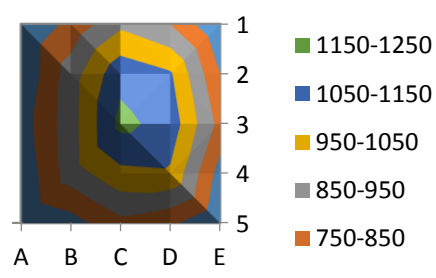
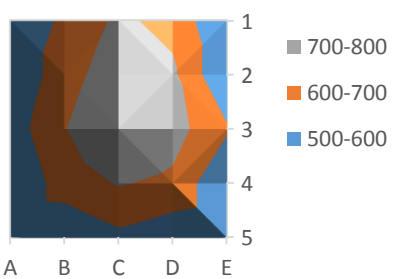
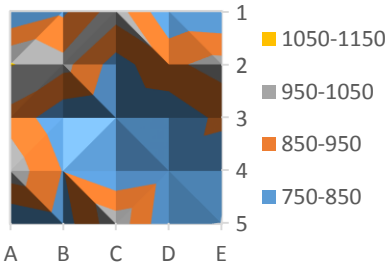
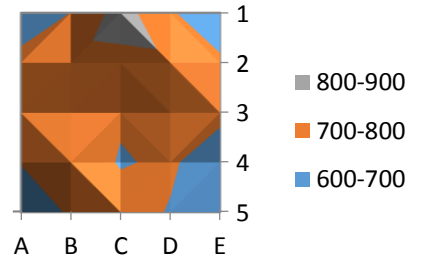
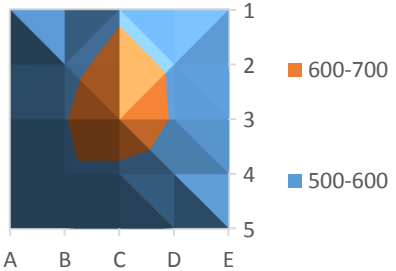


Figure 2.8: X and Y grid on growth chamber support plate. The intensity of light in PAR was measured at each point of intersection. This was repeated at 40 cm, 60 cm, and 100 cm away from the light source.

Table 2.1: Surface diagrams of light intensity in PAR (µmol/m²/s) of seven different colours. Intensity was measured at 21 positions evenly distributed over a horizontal plane below the LED array. These measurements were repeated at 40 cm, 60 cm, and 100 cm below the LED within the single-plant chambers at CESRF.

Colour	40 cm	60 cm	100 cm
RB			
BL			
CY			

GN	 1 2 3 4 5 A B C D E 500-600 400-500 300-400	 1 2 3 4 5 A B C D E 450-550 350-450 250-350	 1 2 3 4 5 A B C D E 250-300 200-250
A	 1 2 3 4 5 A B C D E 600-700 500-600	 1 2 3 4 5 A B C D E 500-600 400-500	 1 2 3 4 5 A B C D E 350-400 300-350
RD	 1 2 3 4 5 A B C D E 1450-1650 1250-1450 1050-1250 850-1050 650-850	 1 2 3 4 5 A B C D E 1150-1250 1050-1150 950-1050 850-950 750-850	 1 2 3 4 5 A B C D E 700-800 600-700 500-600
DR	 1 2 3 4 5 A B C D E 1050-1150 950-1050 850-950 750-850	 1 2 3 4 5 A B C D E 800-900 700-800 600-700	 1 2 3 4 5 A B C D E 600-700 500-600

2.2.2: Intensity calibration

The intensity of PAR among different colours of these LEDs is dramatically different, given the same electrical inputs. This is due in part to the electrical efficiency of the different types of LEDs; for example, measured from 15 cm from the light source, the RB LEDs have a radiometric flux of approximately 165 W/m^2, while the GN LEDs have a radiometric flux of approximately 72 W/m^2. Plants also have a much greater sensitivity to some colours than others, which further confounds an accurate representation of PAR values from multiple monochromatic sources.

To ensure that experiments using the LED array were providing plants only with different light qualities, and not inadvertently providing varied light intensities, all LEDs were normalized to the colour with the weakest PAR. GN emitted the weakest PAR, due to its relatively low efficiency and the relative insensitivity of plants to wavelengths in the green region. Power output of the GN LED ballasts was set at 255, or full power, which yielded a PAR of 280 µmol/m^2/s within the chamber, measured 60 cm from the array.

PAR for each colour was plotted in relation to electrical input[1], and an equation was derived for each colour that described its relationship between PAR and electrical input. An example of this type of plot is illustrated in **figure 2.9** with the CY LEDs. Equations describing the PAR output of each colour in relation to input are summarized in **table 2.2**. Normalizing each colour to a PAR of 280 µmol/m^2/s at 60 cm using these equations determined that RB, BL, CY,

[1] Input was scaled as unit-less values of 0-255, as interpreted by the ballasts. Steps between 0 and 255 are directly proportional to voltage.

GN, A, RD, and DR should have inputs of 74, 82, 141, 255, 220, 126, and 102, respectively. The ratio of each colour would remain approximately the same regardless of measuring height; thus, it was not necessary to repeat this calibration at various heights in the chamber. These levels would serve as a baseline for coming experiments when designing light qualities. To maintain a total PAR of 280 µmol/m^2/s, these values would be divided by however many colours were to be included in equal parts in a light quality "recipe". For example, pure RB light would be coded as "74"; CY and GN together would be coded as "(141/2) and (255/2)"; and the full spectrum would be coded as "(74/7), (82/7), (141/7), (255/7), (220/7), (126/7), and (102/7).

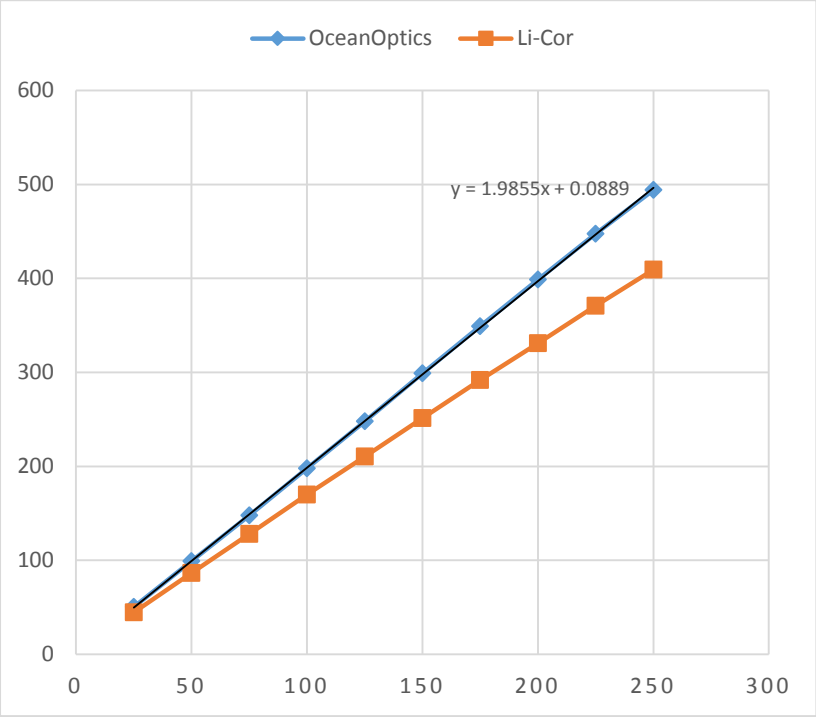


Figure 2.9: PAR output of CY light in relation to power input, scaled 0-255. PAR increased linearly with input, yielding the equation y=1.9855x + 0.0899 for CY PAR. The average PAR at 60 cm from the light source at a given level of input can be calculated from this. Two types of common PAR sensors (Li-Cor LI-190 broadband sensor, and Ocean Optics USB 2000+ spectrometer) were used to verify the linear relationship, however actual values of PAR were determined from the Ocean Optics spectrometer, as it was the greatest range in sensitivity and had most recently been calibrated.

Table 2.2: Equations describing PAR output of each colour in relation to input. Inputs were normalized to a PAR of 280 µmol/m²/s, as this was the maximum output for green light.

COLOUR	EQUATION	IF Y=280
ROYAL BLUE	$y = 3.7617x$	$x=74$
BLUE	$y = 3.4266x$	$x=82$
CYAN	$y = 1.986x$	$x=141$
GREEN	$y = 1.097x$	$x=255$
AMBER	$y = 1.2759x$	$x=220$
RED	$y = -0.0021x^2 + 2.6618x$	$x=116$
DEEP RED	$y = 2.7477x$	$x=102$

CHAPTER 3: GERMINATION AND VEGETATIVE GROWTH OF SEVERAL SPECIES UNDER VARIOUS LIGHT QUALITIES

3.1: INTRODUCTION

3.1.1: Germination under varied monochromatic light qualities

Conditions most suitable for germination in terms of relative humidity, temperature, moisture, nutrient availability, and light quality vary from species to species. A light response at time of germination is termed a "photoblastic" response, and can be positive, yielding faster germination, higher germination rate, hardier seedlings, etc., or negative, consequently inhibiting germination. Photoblastic response in lettuce has been related to gibberellin synthesis and activation and abscisic acid catabolism in response to red light (Toyomasu, Kawaide et al. 1998, Nakaminami, Sawada et al. 2003, Sawada, Aoki et al. 2008). The photoblastic effects of varied light qualities, however, have not been thoroughly explored in most other crop species. In general, seeds are less likely to be exposed to shorter wavelengths in nature, as they are typically underground, or at least on the ground below plant canopies, where short-band light is less likely to reach. Further, as phytochrome has been shown to regulate germination in lettuce, it could be speculated that this is similar in other crop species, and stimulating cryptochrome with shorter wavelengths may override any positive photoblastic activity undergone by phytochrome.

This experiment examined the potential photoblastic effects of nine different light qualities on eight crop species. It was hypothesized that, relative to germination in darkness, relatively long wavelengths in the range of 440 nm to 740 nm may elicit a positive response, while shorter wavelengths in the same range may produce a negative response.

3.1.2: Photosynthesis rates of lettuce, tomato and pepper under several light spectral "recipes"

In commercial greenhouses, the "best" light quality would usually be defined by whatever light quality yielded the most biomass from the plants grown. An effective means of indirectly measuring biomass accumulation in response to light quality is to measure the rate of carbon assimilation, or photosynthesis, under different light qualities. As described in chapter 1, the light-harvesting pigments involved in the light reactions of photosynthesis are most sensitive to red and blue light, however evidence suggests that other light qualities may be more effective in driving photosynthesis than blue and red alone. This experiment measured the photosynthetic rates per unit leaf area of lettuce, tomato, and pepper plants under several different light qualities.

3.1.3: Short and mid-term light adaptation

It is possible that the effectiveness of certain light qualities to drive photosynthesis may be altered by the physiology of the plants adapting to a light quality to which it was previously exposed. This could decrease efficiency in a greenhouse setting, for example, where plants may be seeded and undergo early seedling establishment, then moved to a new environment with more space for vegetative and reproductive development. While previous research has begun to define the mode of adaptation to changing light intensities (Leong and Anderson 1984, Pearcy and Sims 1994, Bellafiore, Barneche et al. 2005), little work has been done exploring the effects of adaptation to, and recovery from a variety of light qualities. This experiment included measurements of rates of photosynthesis after short term and midterm adaption to different light qualities.

3.2: MATERIALS AND METHODS

3.2.1: Germination

Eight crop species, including wheat, barley, radish, lettuce, soybean, tomato, kale, and green pepper were selected for this experiment. These species were selected due to their favourable suitability for future space flight crops as part of a biological life support system, as well as being a suitable size for the growth chambers at CESRF (Wheeler and Strayer 1997). Seed stock for radish (*Raphanus sativus* cv. 'Cherry Bomb II Hybrid') was obtained from Burpee Seeds (http://www.burpee.com). Stocks for tomato (*Solanum lycopersicum* cv. 'Tiny Tim'), lettuce (*Lactuca sativa* cv. '"New" red fire'), bell pepper (*Capsicum annuum* cv. 'California Wonder'), and kale (*Brassica oleracea* cv. 'Vates Blue Curled') were obtained from Stokes Seeds (http://www.stokesseeds.com). Barley (*Hordeum vulgare* L., cv. '2R'), Soybean (*Glycine max* L. Merr. cv. 'OT9814') and durum wheat (*Triticum durum* spp. cv. 'Commander') were both available at CESRF from a previous experiment.

Each species was plated in a separate germinating pod (GP). Germination pods (GP's) were comprised of filter paper placed on a coiled wick, which extended into a water reservoir (**Figure 3.1**). The filter paper and coiled wick were fitted to a petri dish. In separate GP's, 14 seeds of each species were placed on the moist filter paper, and then the lid of the petri dish was placed on top to maintain a humid environment for the seeds.

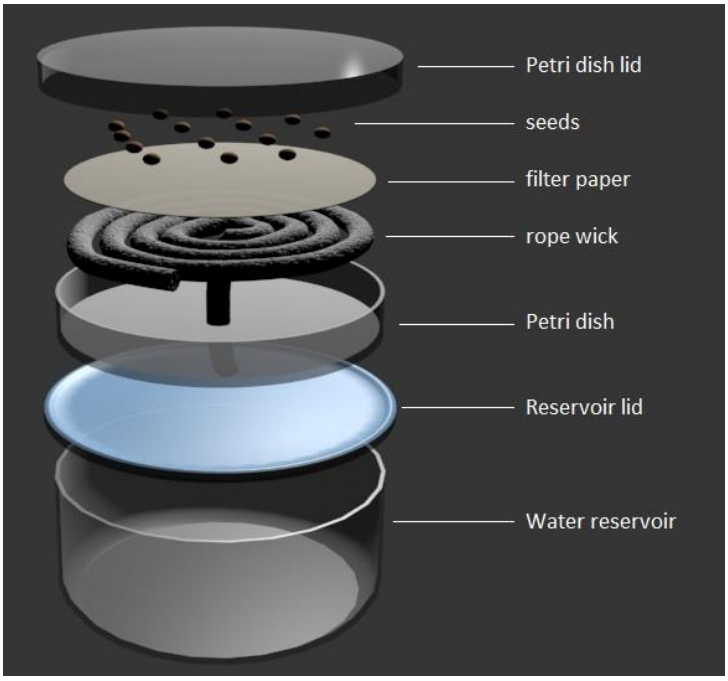


Figure 3.1: Exploded view of germinating pod. The pod consisted of seeds plated onto filter paper, which was kept moist by a wick that extended into a water reservoir. The rope, filter paper, and seeds were contained in a petri dish to maintain relatively high humidity.

One GP of each species was placed in each of seven cylindrical growth chambers. The growth chambers had an internal diameter of 0.46 m, internal height of 1.2 m, and internal volume of 0.2 m^3. Relative humidity, vapour pressure, and temperature were maintained at 54%, 12 mb, and 22°C, respectively. Each growth chamber was equipped with a different quality of light. The light source was positioned 60 cm above the GPs, and exposed the seeds to approximately 280 µmol/m^2/s of photons. Seeds were subject to a 16 hour photoperiod.

The influence of eight different light qualities on seed germination rate were compared. The qualities tested were monochromatic and had peak emissions of 440 nm, 480 nm, 500 nm, 520 nm, 590 nm, 640 nm, 660 nm, and 740 nm. A broad spectrum high-pressure sodium (HPS) lamp (Philips Ceramalux® 1000W), commonly used in greenhouse lighting, as well as a dark treatment were also included as controls. Refer to section 2.2 for details on the emission spectra

of the monochromatic light sources used. The emission spectra of the Sylvania Lumalux HPS bulb is illustrated in **figure 3.2**. Each treatment was repeated three times.

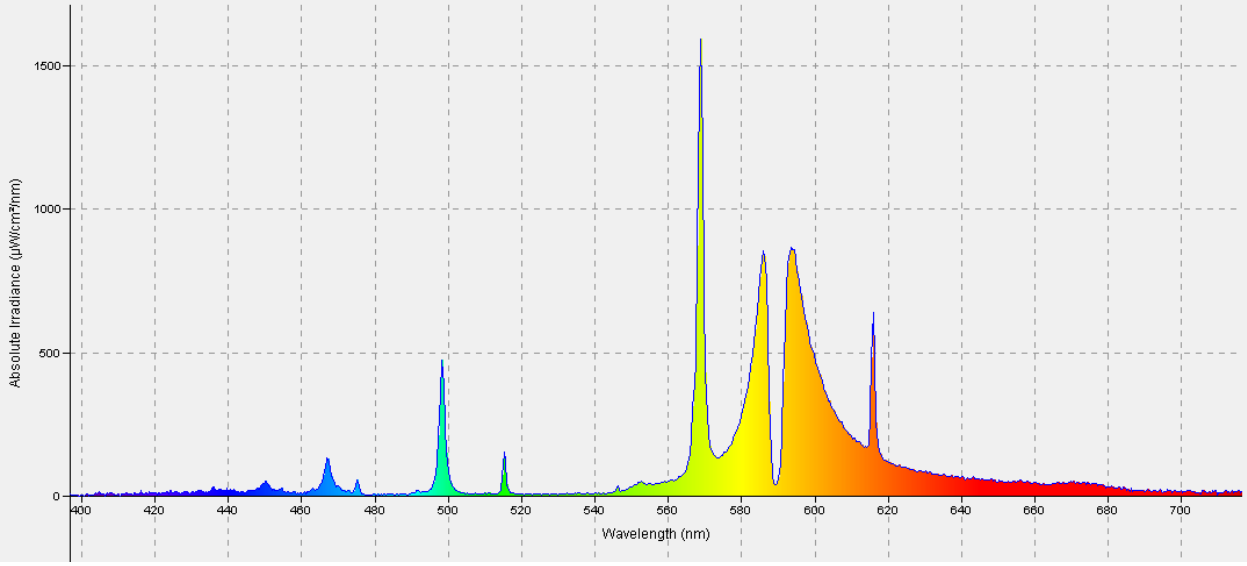


Figure 3.2: Emission spectrum of Sylvania LumaLux HPS bulbs. Bulbs similar to this are commonly used in conventional greenhouse lighting, and were used as a positive control the in conducted experiments.

3.2.2: Vegetative growth

Lettuce (*Lactuca sativa* cv. ‘”New” red fire’), tomato (*Solanum lycopersicum* cv. ‘Sub-Arctic Maxi’), and bell pepper (*Capsicum annuum* cv. ‘California Wonder’) were selected for this experiment for their relatively fast rate of growth. The ‘tiny Tim’ cultivar of tomato used in the germination experiment was substituted for ‘sub-arctic Maxi’, a cultivar with a greater amount of vegetative tissue, allowing for higher-resolution photosynthesis measurements.

Plants were grown from seed to vegetative maturity in a growth room maintained at 22°C under Sylvania cool white fluorescent light, with an intensity of approximately 300 µmol/m^2/s. The spectrum of the fluorescent light is shown in **figure 3.3**. Lettuce was deemed to be at the

appropriate development stage 51 days after seeding; tomato, 37 days; and pepper, 54 days. New plants were seeded each week so that every consecutive week would have plants of the same age.

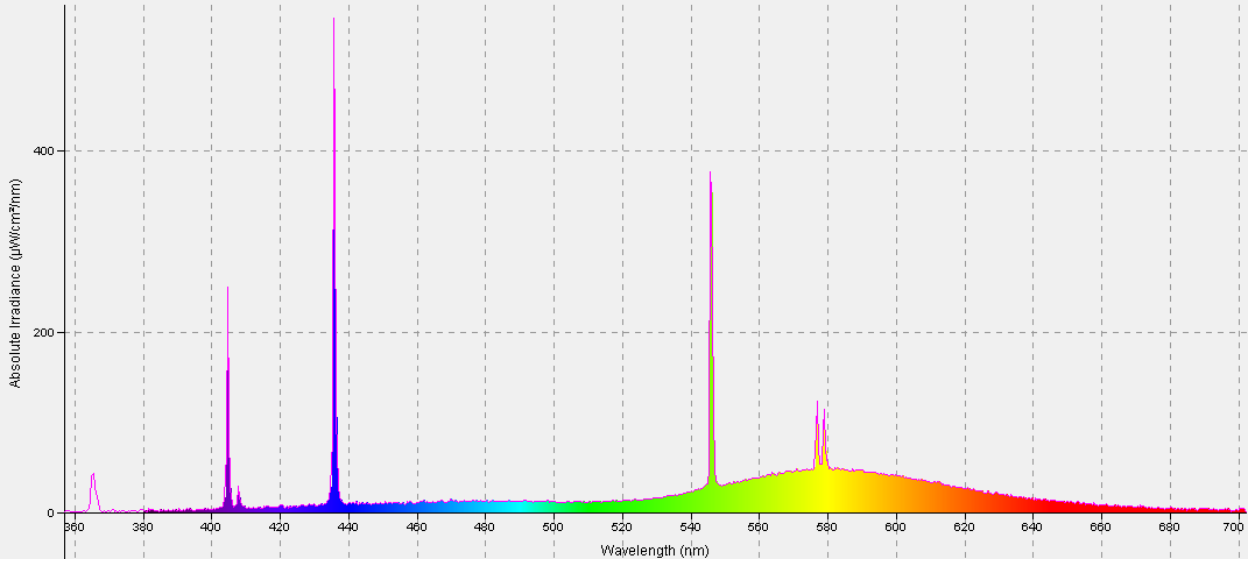


Figure 3.3: Emission spectrum of Sylvania cool white fluorescent bulbs. These bulbs were used in raising lettuce, tomato, and pepper plants to vegetative maturity in a growth room, with a light intensity of approximately 300 µmol/m²/s.

Mature plants were transferred to the single plant growth chambers described in section 3.2.1, with three plants of a single species and of the same age in one chamber, acting as one experimental unit. The use of three plants per chamber was intended to cause a higher, and more rapid consumption of CO_2, which would be easier to measure than single plants when comparing rates of CO_2 fixation. The environmental conditions in the chambers were the same as described in section 3.2.1, changing only the light qualities.

A total of 40 different light qualities, summarized in **Appendix II**, were tested with each species. Each light quality had a total PAR of approximately 280 µmol/m^2/s. The 40 light qualities tested were divided into "codes", of which each contained between six and nine different treatments. One code was run per week, for each species. On a given day, the plants would be subject to a total of 16 hours of near-continuous light (only brief interruption). The first

three hours of each light period was full spectrum "white" light, intended to provide a baseline level of photosynthesis for the plants in the chamber, and to consume the extraneous CO_2 that had accumulated in the chamber from plant respiration during the previous night. The array would then turn off for two minutes, providing a "gap" in the collected data that would indicate a change in light treatment to the researcher. After this gap, the rest of the light treatments in a code would be cycled throughout the day for 45 minutes each, and with each followed by the two-minute gap period. After cycling through all of the light treatments in the code, the array would return to "white" light for the remainder of the 16 hour photoperiod, and then turn off.

CO_2 levels were constantly monitored and maintained with the ARGUS control system. Every time the concentration of CO_2 in the chambers dropped below 400 ppm as a result of photosynthetic activity from the plants, ARGUS would inject additional CO_2 to a concentration of 500 ppm. The rate at which the plants consumed CO_2 under each lighting treatment was recorded. At the end of each code, the leaves from the plants were harvested, and their surface areas measured, using a Li-Cor Li-3100 area scanner. From this, rates of photosynthesis under different light qualities could be interpreted as moles of CO_2 assimilated per unit leaf area. Each treatment was repeated in triplicate simultaneously.

3.2.3: Short- and mid-term adaption to other light qualities

To measure the effects on carbon assimilation rate of short-term adaptation to varying light qualities, 51-day old lettuce plants were alternatingly exposed to photosynthetically unfavourable and favourable light qualities, as determined in the previous experiment. The unfavourable light quality used consisted of CY/GN wavelengths, while the favourable light quality consisted of DR wavelengths. Both qualities had an intensity of 280 $\mu mol/m^2/s$, and were on for four hours at a time, starting with the CY/GN light. This was cycled twice, for a total 16

hour photoperiod. The experiment was executed in the same single-plant growth chambers and environmental conditions as described in section 3.2.1. Carbon fixation rates and leaf area were measured and analyzed with the same methodology as described in section 3.2.2.

Mid-term effects of light adaptation on photosynthetic rates were estimated by measuring photosynthesis in lettuce under several monochromatic light qualities on day one (initial), adapting the plants to a single light quality for three days, then measuring photosynthesis again (final) on day five under the initial monochromatic light qualities. At the end of the fifth day, leaf area of the plants was measured. Photosynthesis per unit leaf area was analyzed as previously described. The light qualities and colour intervals for day one and five were as described when measuring photosynthesis under monochromatic CODE 1 lighting in section 3.2.2. On days two to four, inclusive, plants were adapted to RB light with an intensity of 280 µmol/m^2/s. Two additional experiments were carried out in this manner; one adapted the plants to full spectrum white light, and the other adapted the plants to amber light.

Experimental units consisting of three vegetative lettuce plants per experimental unit were raised and obtained in the same manner as described in section 3.2.2. Results from mid-term adaptation experiments only provide an estimation of the effects that adaption may have on photosynthesis rates. This is because the plants would have grown during the adaptation phase, increasing leaf area, and potentially artificially inflating photosynthesis rates on the final day of measurement.

All adaptation experiments were repeated in triplicate.

3.3: RESULTS

3.3.1: Germination of eight species

Wheat seeds experienced the highest germination rate in darkness, FR, RD, or RD, while RB, GN, and BL significantly inhibited germination. Germination rates of barley were significantly higher when seeds were exposed to FR light or darkness, compared to other treatments. Radish seeds reached 100% germination after 48 hours when exposed to FR light, and while other treatments required at least another 24 hours to achieve the same, FR response was significantly higher than RB response only. Tomato had three significantly different photoblastic responses to light. Dark, DR, RD, GN, HPS, and CY treatments resulted in relatively high germination rates. BL and RB treatments reduced germination rates. FR light completely inhibited germination. Similarly, most light qualities did not influence germination rates of lettuce seeds, with the exceptions of BL and FR light, both of which significantly reduced germination rates. Light quality did not significantly influence the rate of germination of soybean. Germination rate of pepper seeds under GN light was significantly higher than under HPS, BL, or FR light, each of which resulted in significantly reduced germination rates compared to the one before. Germination rate of kale was highest when exposed to dark, RD, CY, HPS, DR, or GN light, while RB, BL, and FR light significantly reduced germination rate. In each experiment, all means were compared using Student's t-test at a significance level of 0.05. Germination rates are summarized in **figure 3.4**. Significant differences between light treatments is summarized in **table 3.1**.

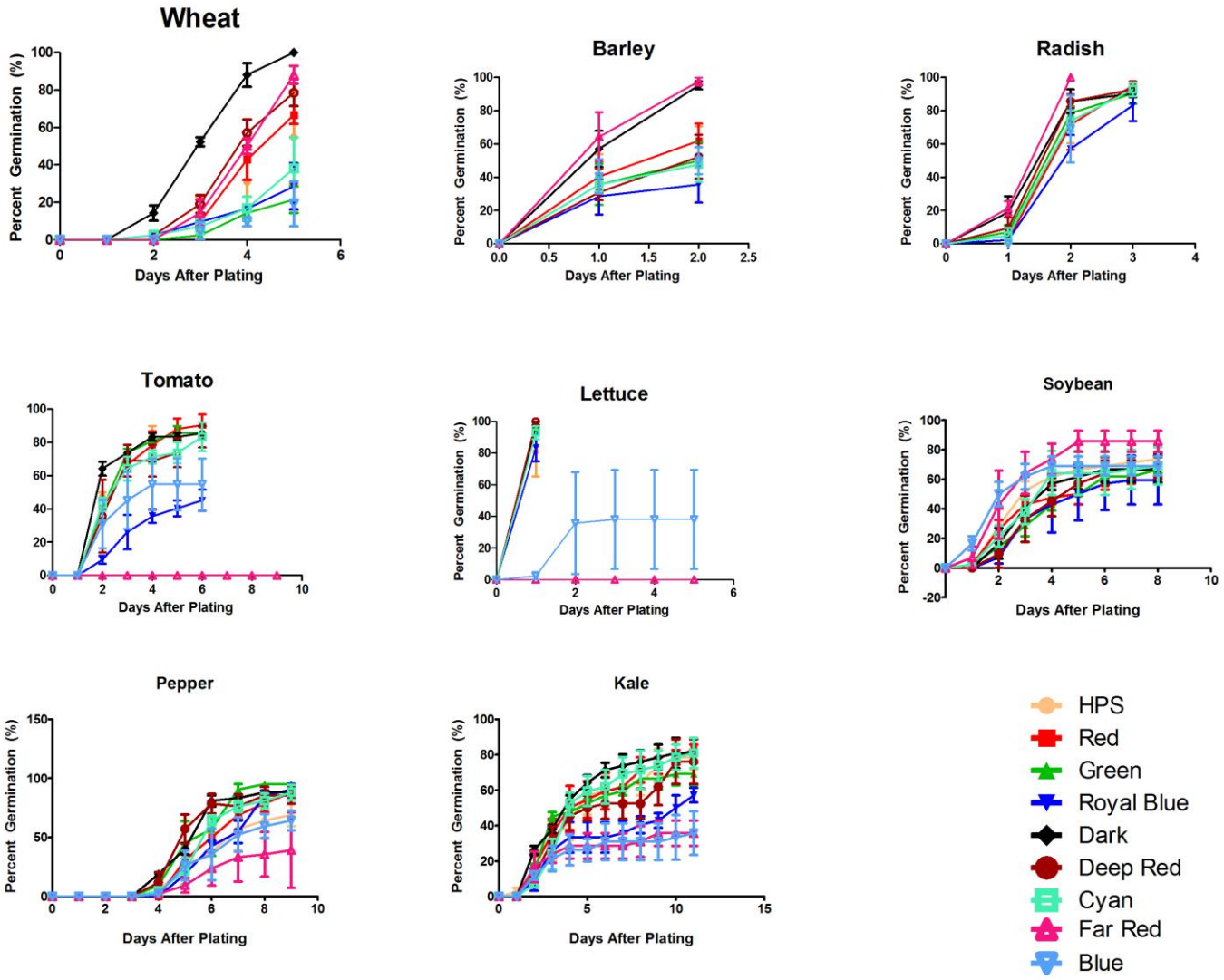


Figure 3.4: Germination rates of wheat, barley, radish, tomato, lettuce, soybean, pepper, and kale, under various light qualities. Fourteen seeds of each species were germinated under each light quality. Seeds were subject to an average PAR of 280 µmol/m²/s, with a 16 h photoperiod. Three replicates were completed, with rearrangement of treatments on each replicate to account for any influence the growth chambers may have had.

Table 3.1: Connected Letters table of significance. Differing letters indicate a significantly different response at α = 0.05.

Wheat					Barley			Radish			Tomato			Lettuce			Soybean		Pepper				Kale			
Dark	a				FR	a		FR	a		RD	a		DR	a		FR	a	GN	a			RD	a		
FR	a	b			Dark	a		DR	a	b	Dark	a		Dark	a	b	HPS	a	RB	a	b		CY	a	b	
DR	a	b			HPS		b	Dark	a	b	GN	a		RD	a	b	BL	a	CY	a	b	c	Dark	a	b	
RD	a	b	c		RD		b	GN	a	b	HPS	a		CY	a	b	DR	a	Dark	a	b	c	HPS	a	b	
HPS		b	c	d	DR		b	CY	a	b	CY	a		GN	a	b	CY	a	RD	a	b	c	DR	a	b	
CY			c	d	BL		b	HPS	a	b	DR	a		RB	a	b	Dark	a	DR	a	b	c	GN	a	b	
RB				d	GN		b	RD	a	b	BL		b	HPS		b	GN	a	HPS		b	c	RB		b	c
GN				d	CY		b	BL	a	b	RB		b	BL		c	RD	a	BL			c	BL			c
BL				d	RB		b	RB		b	FR		c	FR		c	RB	a	FR			d	FR			c

3.3.2: Photosynthesis rates of vegetative lettuce, tomato, and pepper

3.3.2.1: Lettuce

Code one examined the effects of monochromatic light on photosynthesis rates. Increased rates were achieved in code one with longer wavelengths including amber, red, and deep red. When exposed to monochromatic green light, respiration rates exceeded photosynthetic rates. Subtracting only DR light from the full spectrum was sufficient to significantly reduce carbon assimilation rates. Code four subtracted two similar colours at a time from the total spectrum. Subtracting both red and deep red from the total spectrum significantly reduced photosynthetic rates. Photosynthetic rates in response to light qualities in code five were significantly reduced when lighting lettuce plants with only RB, BL, CY, and GN light. Substituting CN with DR light in that combination significantly increased photosynthetic rates, however rates were still below those when lit with a light quality including both RD and DR light and any other two colours. Lighting the plants with only three colours in code six, photosynthetic rates steadily increased as the light quality shifted towards mixing longer wavelengths. A light quality of A, RD, and DR wavelengths yielded significantly greater photosynthetic rates than any other combination in code six. Code seven exposed plants to only two colours at a time. RB/DR, DR/RD, and RD/A light qualities were most effective in driving photosynthesis, achieving rates significantly greater than light qualities made exclusively of mid or short wavelengths. No two colours tested combined to elicit an Emerson-style "enhancement" effect in any of the three species.

Comparing the light qualities that achieved the greatest photosynthetic rates from each previous code, code 8-L found DR alone, or a combination of RB, A, RD, and DR light to be

most effective in driving photosynthesis in lettuce. Both of these light qualities exhibited carbon fixation rates exceeding that of full spectrum "white" light. Light consisting of only RD and DR wavelengths was found to be significantly less effective in driving photosynthesis than full-spectrum light. The relative photosynthetic rates of each treatment are summarized in **figure 3.5**.

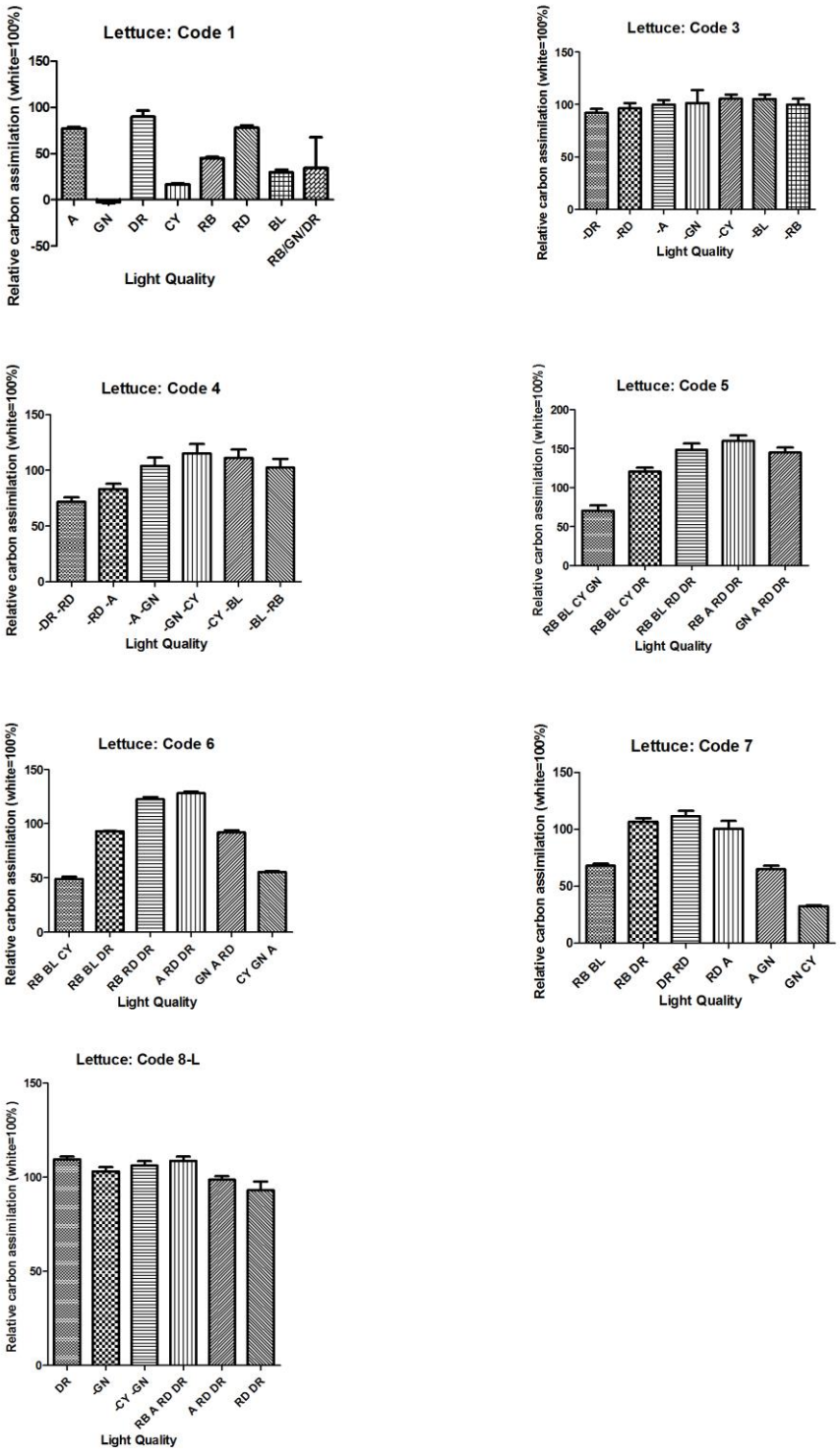


Figure 3.5: Photosynthetic rates in lettuce under varying light qualities. Light intensity was maintained at approximately 280 µmol/m^2/s for each treatment, with a 16 hour photoperiod. Results cannot be directly compared between codes due to variability in plant health between codes. The light qualities in each code yielding the greatest photosynthetic rates were compared on a file set of plants in code 8-L.

3.3.2.2: Tomato

Photosynthetic rates were significantly reduced compared to white light or longer wavelengths when using monochromatic RB, BL, or CY treatments. Code three, subtracting one colour at a time from the full spectrum, resulted in a significant reduction in carbon fixation in every case, compared to full spectrum light. There was no significant change in photosynthetic rates when subtracting two similar colours at a time from the full spectrum. Subtracting three similar colours, as in code five, photosynthetic rates were reduced significantly when removing A, RD, and DR simultaneously. The subtraction of mid-range wavelengths CY, GN, and A achieved similar carbon fixation rates as white light, RB/A/RD/DR light, and GN/A/DR/DR light. Using only three colours in the array, RB/RD/DR light and A/RD/DR light significantly increased carbon fixation compared to white light. Carbon fixation was significantly reduced when using RB/BL/CY light. Using only two colours, RD/DR and RD/A treatments both significantly increased carbon assimilation compared to white light, while RB/BL and GN/CY qualities resulted in significantly decreased carbon assimilation. Comparing the treatments most effective in increasing photosynthesis from previous codes, code 8-T revealed no light qualities to be superior to full spectrum light in driving photosynthesis. However, amber light alone was as effective as full spectrum light in driving photosynthesis. Full spectrum light deficient in BL and CY light, or using only RD/DR light yielded significantly lower carbon fixation rates. Relative photosynthetic rates of each treatment are summarized in **figure 3.6**.

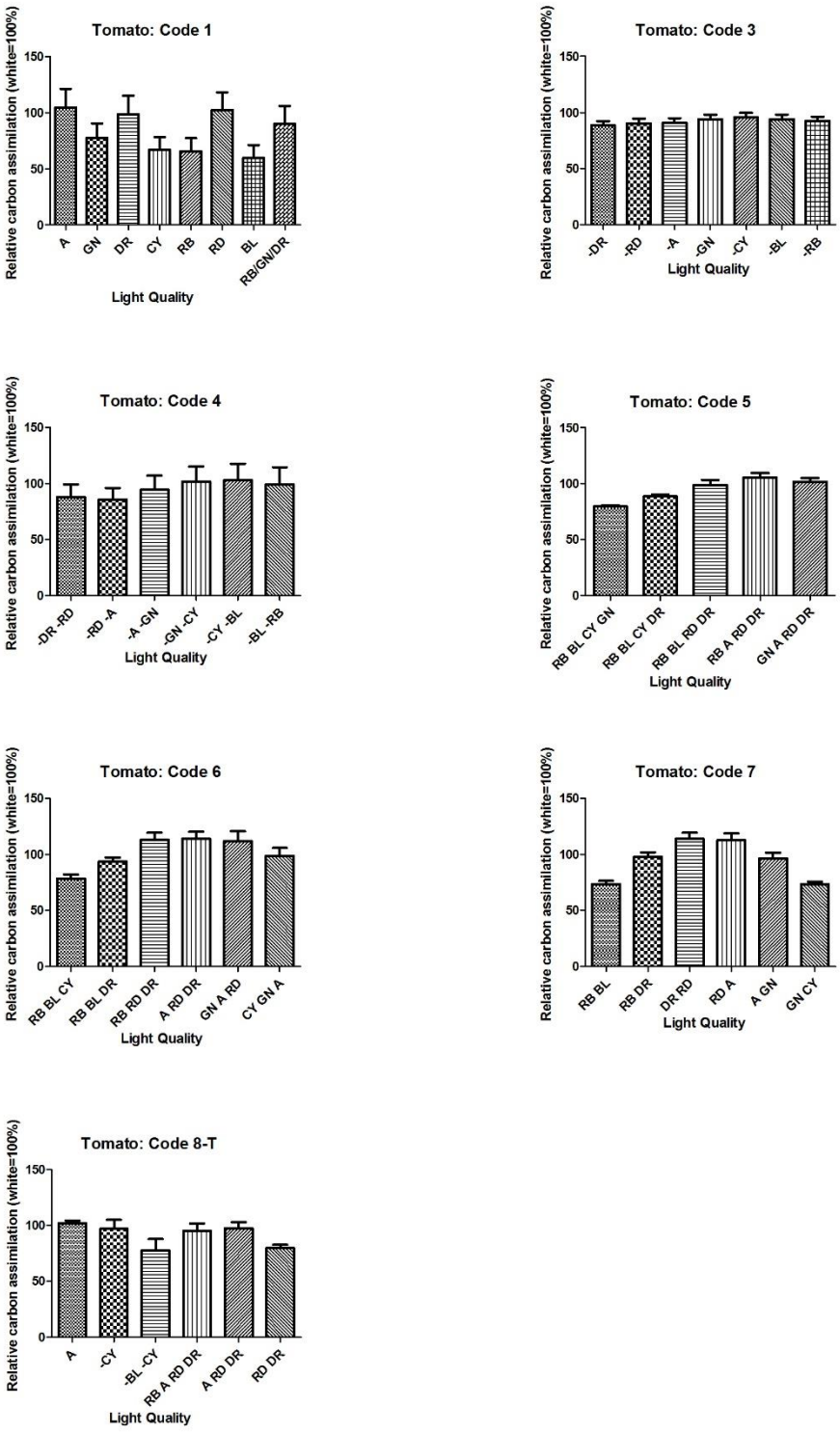


Figure 3.6: Photosynthetic rates in tomato under varying light qualities. Light intensity was maintained at approximately 280 µmol/m²/s for each treatment, with a 16 hour photoperiod. Results cannot be directly compared between codes due to variability in plant health between codes. The light qualities in each code yielding the greatest photosynthetic rates were compared on a file set of plants in code 8-T.

3.3.2.3: Pepper

All monochromatic light qualities significantly reduced photosynthetic rates compared to white light. Most multi-coloured light treatments did not significantly influence photosynthetic rates in pepper plants, and those that did reduced photosynthetic rates compared to white light. Relative photosynthetic rates of each treatment are summarized in **figure 3.7**.

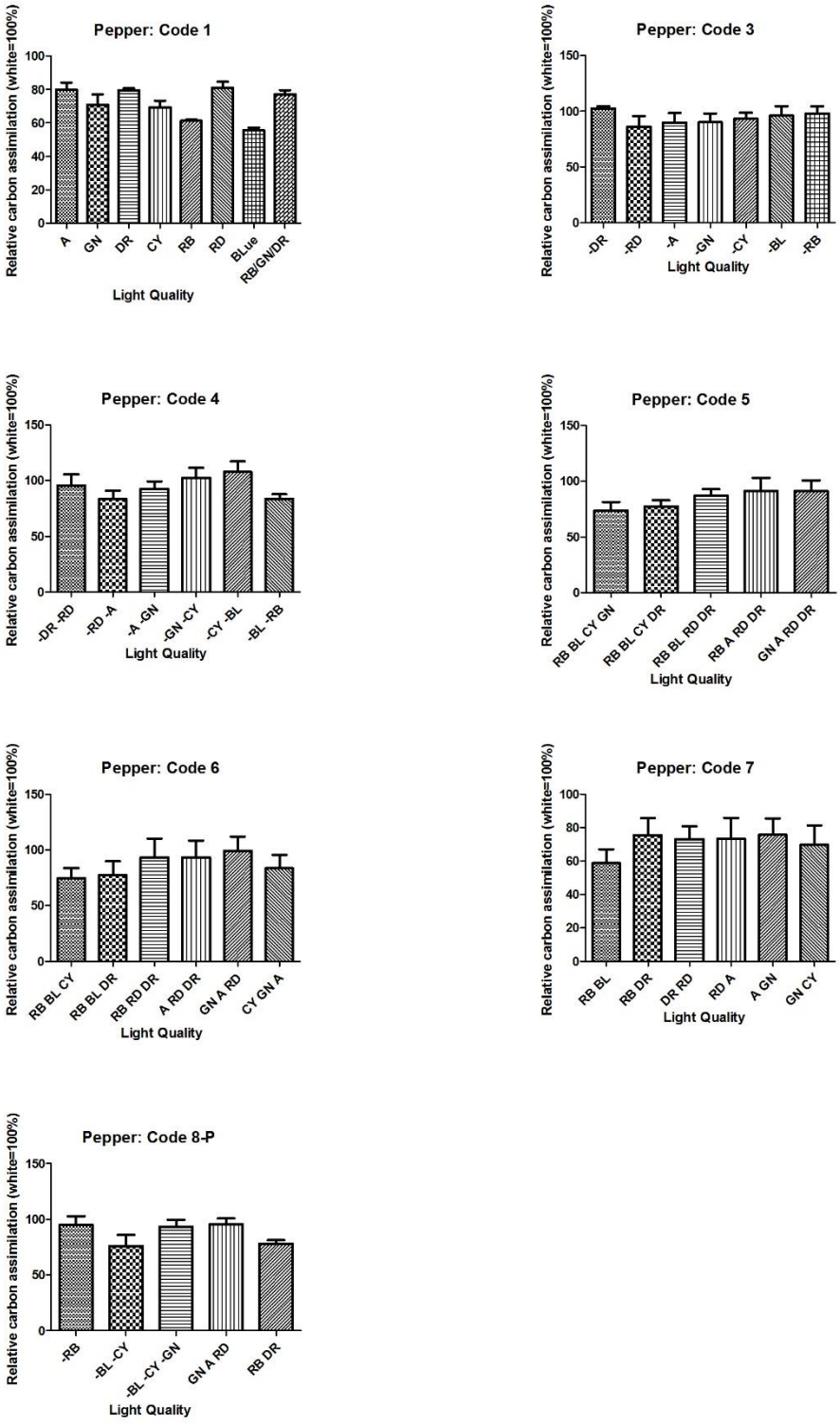


Figure 3.7: Photosynthetic rates in pepper under varying light qualities. Light intensity was maintained at approximately 280 µmol/m²/s for each treatment, with a 16 hour photoperiod. Results cannot be directly compared between codes due to variability in plant health between codes. The light qualities in each code yielding the greatest photosynthetic rates were compared on a file set of plants in code 8-P.

3.3.3: Short- and mid-term adaptation

Short-term exposure to alternate light qualities did not significantly influence photosynthesis rates in three replicates. The collected data suggest that a decrease in carbon assimilation rate may be found on further replication (**figure 3.8**).

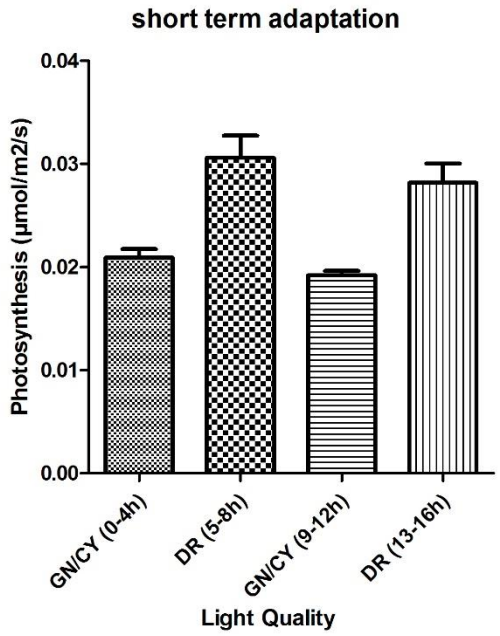


Figure 3.8: Photosynthesis after four hour adaptation of differing light qualities. Plants were exposed to GN/CY and DR light of equal intensities for 4 hours each. This was cycled twice, for a total 16 hour photoperiod. Photosynthesis was not significantly reduced after the plants had adapted to the previous light quality for four hours.

Mid-term adaption to white, amber, and royal blue each significantly reduced photosynthesis under each type of monochromatic light after four days of adaptation (**figure 3.9**).

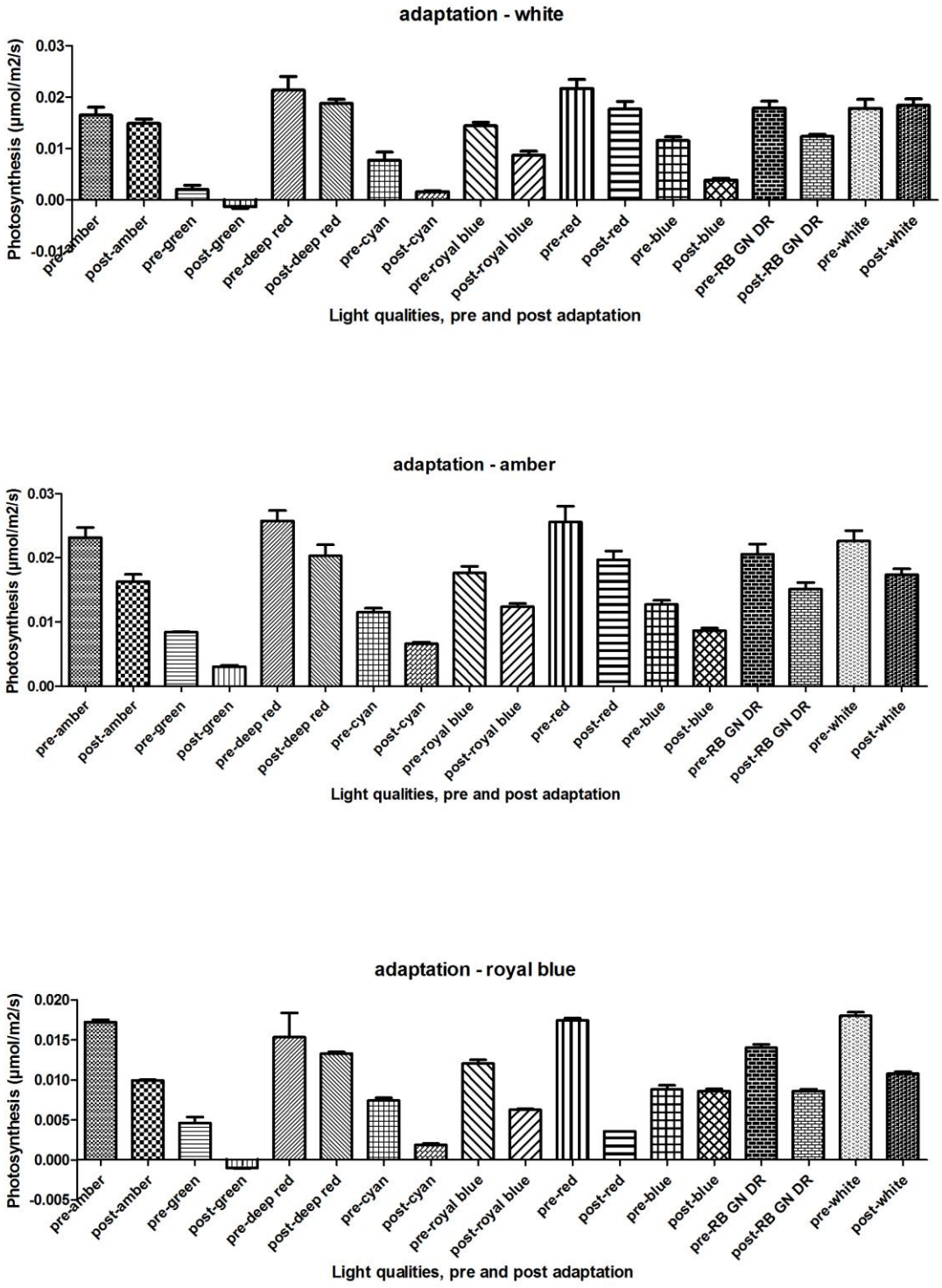


Figure 3.9: Photosynthesis rates under monochromatic light pre- and post- being separately adapted to white, amber, and royal blue light for three days. Each light quality was approximately equal in intensity. Each adaptation significantly reduced photosynthesis under most light qualities compared to pre-adaptation.

3.4: DISCUSSION

3.4.1: Germination rates of eight species

Photoblastic response was very different among the species tested. Thus the original hypothesis that longer wavelengths would cause a positive photoblastic response, and shorter wavelengths would cause a negative response, was not supported, so such a generalization cannot be made. While there was a trend of royal blue light producing the greatest inhibitory response in most species, supporting the original hypothesis, the total inhibition of germination in tomato seeds with far red light was unexpected. As discussed in section 3.1.1, previous work has shown that an increase in red light stimulates germination in lettuce. The negative photoblastic response of lettuce to far red light was not surprising then, as this light would cause a conformational change in phytochome, disabling its red-light mode of action. It may be that tomato germination was inhibited in a similar way. Further, that FR resulted in significantly reduced germination rates in half the species tested, and in the other half yielded some of the highest germination rates, it may be there are at least two distinct phytochrome-mediated pathways that trigger germination. The negative photoblastic effects of RB and BL light qualities in all species may indicate that cryptochrome could also play an inhibitory role in germination of seeds. Future experiments with different ratios of inhibitory RB or BL light and potentially favorable RD light could determine which photoreceptor in tomato is dominant in regulating germination.

Most other light qualities did not significantly differ in their influence on germination rate. From the perspective of commercial greenhouse lighting, it would be most economical, then, to germinate seeds in darkness.

3.4.2: Photosynthesis rates of vegetative lettuce, tomato, and pepper

3.4.2.1: Lettuce

It was anticipated that monochromatic green light would likely be ineffective in driving photosynthetic processes. Very little green light is absorbed by plants, and chlorophyll a and b are relatively insensitive to wavelengths in this region. Conversely, chlorophyll a and b are highly sensitive to red light. This was validated by the effective stimulation of photosynthetic processes by the relatively long wavelengths used in code one. It was demonstrated in code one that RB/GN/DR light had a negative impact on photosynthetic rates, in contradiction to previous work (Kim, Goins et al. 2004). The RGB light used by Kim et al. was composed of different ratios of each colour than what was used in this study, and had a weaker overall intensity. It may be that their red:green:blue were better optimized to drive photosynthesis, or the benefits of adding green to red and blue are only evident at lower overall light intensities. drive Although code three did not yield significant results, the collected data suggested that removing these same long wavelengths from full-spectrum lighting was detrimental to photosynthetic rates. Further replicates and improved experimental design described in chapter 4 may support this theory. Code four supported the conclusion that subtracting wavelengths longer than 600 nm is detrimental to photosynthesis. Further, the data suggested that photosynthesis may be most effectively driven by redirecting energy from mid-range wavelengths in the array to the shorter blue colours and longer red colours. Code six illustrated that photosynthetic rates were nearly identical when lighting plants with RB, BL, and DR light in comparison to GN, A, and RD light.

Although previous codes had illustrated that a combination of RD and DR is highly effective in driving photosynthesis, code 8-L revealed that this combination was significantly less effective than DR alone or several other combinations of colours. This suggested that any energy misplaced from DR is not effectively used for photosynthesis. Further, as DR was the longest wavelength tested, the evidence implied that longer wavelengths than what were available at the time of testing may further enhance photosynthetic rates in this cultivar of red-leaf lettuce.

3.4.2.2: Tomato

Similar to lettuce, it was generally found that the absence of longer wavelengths in PAR were detrimental to carbon fixation rates. It is noteworthy that, in code five, photosynthesis was not significantly different using RB/BL/RD/DR light compared to full spectrum light. These LEDs have a greater electrical efficiency than the rid-range coloured LEDs, which suggests that using this light quality would give equivalent dry-matter yields as white light while consuming less power. Alternatively, a higher intensity of light could be achieved using this light quality instead of white light for the same amount of power, likely increasing yield.

3.4.2.3: Pepper

Like lettuce and tomato, the light qualities that were most detrimental to photosynthesis rates were those lacking in the longer wavelengths of amber and red light. The data obtained when removing only one colour at a time from the full spectrum in code 3 suggested that, in pepper plants, every colour may be utilized in driving photosynthesis. Further replication may support this conclusion. In contrast to lettuce and tomato, pepper's code 4 results illustrated that removing the shorter BL and RB wavelengths from the spectrum was significantly detrimental to

carbon fixation. Lighting pepper with only RB and BL as in code seven, however, caused even greater detriment to photosynthesis.

While this experiment effectively determined which colours were most effective in driving carbon assimilation for dry matter accumulation in lettuce, tomato, and pepper, it is important to note that favourable light qualities in this situation may not necessarily yield an optimal commercial product. Qualitative observations found plants grown strictly under longer wavelengths to generally appear chlorotic, lacking in pigmentation and therefore any nutritional value associated with certain pigments in plants. This would suggest further that concentrations of other compounds within the plants may be changing under differing light qualities as well, which may be favourable or not in other applications such as pharmaceutical production. This could be addressed in future experiments, as described in chapter four.

3.4.3: Short- and mid-term adaptation

In and mid-term adaptation experiments, the data suggested that the rate of photosynthesis would be reduced when measured shortly after switching to a light quality to which the plant has not adapted. The data collected during the short term adaptation experiment, while not statistically significant, suggests a similar trend that may be validated by further replication. It was unexpected that photosynthesis would decrease in all cases. Because the mid-term adaptation experiments required the plants to remain in the growth chambers for a total of five days, during which time they would have increased their leaf area, it was anticipated that the final photosynthesis measurement would have been greater than the initial. That photosynthesis was decreased in every case supports the conclusion that a change (virtually any change) in a

plant's light environment is detrimental to photosynthesis, despite possibly increased mass. It is essential in future work to determine how long the adaptation period takes before photosynthetic capacity is restored. Further, it would be beneficial to determine if there is a temporal threshold during which a plant becomes so adjusted to one light quality that it cannot recover photosynthetic capacity when exposed to what would otherwise be a more favourable quality of light.

CHAPTER 4: CONCLUSIONS AND FUTURE DIRECTIONS

4.1: CONCLUSIONS

Six high-intensity, multi-colour, narrow-bandwidth LED arrays have been assembled at the Controlled Environment Systems Research Facility (CESRF) at the University of Guelph. The arrays each consist of eight different colours of LEDs that are individually dimmable to create light spectra of different qualities. The LEDs were fixed on a water-cooled aluminum block, sized to form the seal on the single-plant growth chambers at CESRF. Optics were fitted over each LED to focus the light on the plants below, while having a sufficient exit-angle to allow effective light-mixing 0.3 m away from the array. An ABS plastic window was mounted on the front of the array to secure the optics and to provide a flat surface that contacted the growth chamber, creating an effective seal. With these arrays, it was possible to more specifically elicit plant responses to different wavelengths of light. This would potentially provide the ability to enhance photosynthetic capacity, manipulate physiological development, regulate production of desirable compounds, and control nutrient use. Further, the arrays were built on robust, solid-state LED technology which is relatively compact in design and very efficient. Such a design could be effectively deployed in harsh environments such as growing systems in northern Canada, underground, "urban agriculture" vertical growth systems, or on future space shuttle craft or the international space station.

Following the design, assembly and testing of the arrays, three experiments using the LED arrays were completed to illustrate the potential of such a lighting system, and to begin the process of designing optimal lighting strategies for several different crop species.

Germination rates were compared in wheat, barley, radish, soybean, kale, lettuce, tomato, and sweet pepper when exposed to eight different monochromatic light sources of equal intensity. The colours of light ranged from 440 nm royal blue light to 740 nm far red light. It was found that far red light completely inhibited germination of lettuce and tomato, and significantly inhibited germination of pepper and kale. Shorter wavelengths in the blue region of the spectrum were also significantly inhibitory to germination in all species. From this experiment, it was concluded that the photoblastic response differs significantly under different light qualities and between species. In some cases, it may be that red light is beneficial to stimulating germination, and there may be competition between red- and blue-light photoreceptors in regulating germination. No treatments were more effective in germinating seeds than darkness, and so from the perspective of a commercial producer, germination in darkness would be most economical.

Rates of carbon assimilation under 40 different light qualities of equal intensity were evaluated in red-leaf lettuce, tomato, and sweet green pepper. It was found that photosynthesis in lettuce was significantly reduced under green light alone, to the point that respiration exceeded photosynthesis. For both lettuce and tomato, photosynthesis was significantly reduced under various light qualities deficient in relatively long wavelengths such as amber, red, and deep red. The greatest carbon assimilation rates were achieved with deep red or a combination of red and deep red light for lettuce, and amber, or royal blue/amber/red/deep red, or amber/red/deep red light in tomato. While these light qualities all yielded the same level of photosynthesis in tomato, it may be that one is more favourable than the others when other elements of plant development are considered in future work. Pepper showed a similar trend, however also had photosynthesis rates inhibited by an absence of royal blue light. The highest carbon assimilation rate in pepper was achieved when using full spectrum white light.

The influence on photosynthetic rates of short term and mid-term adaptation to various qualities was evaluated using lettuce. Short term adaptation cycled photosynthetically favourable and unfavourable light qualities every four hours for a total of 16 hours on lettuce plants. While carbon assimilation rates were not significantly influenced by this, the data suggested that further study may reveal that this short adaptation period to different light qualities was detrimental to photosynthesis. Mid-term adaptation compared photosynthetic rates under various monochromatic light qualities before and after three days of adaptation to a different light quality. In all cases, it was found that photosynthesis was significantly reduced after the adaptation period.

4.1.1: Meaningful developments from this work

- A novel LED-based plant lighting system was developed which can be used to create light "recipes" designed to elicit specific physiological responses from plants.
- Although no colours were more effective than darkness at stimulating germination in the species studied, there was evidence suggesting they can have a significant influence, which indicated the importance of evaluating all species and cultivars for their specific response to germination conditions.
- In general, photosynthetic rates during the vegetative growth stages were highest when exposed to longer wavelengths such as those in the red and deep red region, however some light qualities that include shorter wavelengths were equally effective in some cases.
- Adaptation to light quality conditions may confound the interpretation of photosynthetic responses, especially if the changes to the light regime are made quickly. Otherwise

favourable light conditions were shown to be less effective at driving photosynthesis if the change in light quality was abrupt.

4.2: FUTURE DIRECTIONS

4.2.1: Hardware

The design of the original array initially included the seven LEDs with colours in the visible spectrum from Philips. Later, far red, 365 nm UV and 395 UV LEDs were acquired from LED Engin, and were added to the array fixture. This proceeded without issue using the far red LEDs, however it was found that the ABS plastic window on the front of each array did not pass UV light. A 10 cm window of borosilicate glass, known to be able to pass wavelengths longer than 350 nm was acquired. The next step in developing the array would be to retrofit this glass into a hole cut in the center of the ABS window currently on the front the array, below which the two types of UV LEDs could be mounted. The UV LEDs are equipped with wide angle optics that should sufficiently spread the UV light throughout the growth chamber below, despite their localization to the centre of the array.

4.2.2: Improved Experimental Design

There are several elements in the design of the photosynthesis and adaptation experiments that could be improved. All plants that were raised in a growth room to vegetative maturity were first seeded in a germinating tray, then transplanted to pots a week later. There was potentially sufficient damage to the roots during transplanting in some cases that could yield significantly different plants of the same age. The seeding was done in a separate tray to save space within the growth room. In future, it may be more effective to acquire more space and seed plants directly into pots. The potted plants in the growth room were watered every other day, or

as deemed necessary by the researchers and technicians. To reduce variability in water status of each plant, it may be more fitting to instead use an automated irrigation system that more evenly distributes water amongst the plants. Similarly, once the plants were transferred to the single-plant growth chambers, they were watered as needed. A hydroponics system is being put in place to grow plants in the chambers in future. In addition to maintaining a consistent water status among the plants, this will also allow for the monitoring and manipulation of nutrients in the solution.

Pests in the growth room greatly increased the variability of plant quality from week to week. Earlier implementation of insecticides or bio-controls would have better maintained a pest-free environment, resulting in healthier plants.

Photosynthesis rates measured under different light codes from week to week could not be directly compared because of inconsistent plant health. To compare all light qualities simultaneously, many more growth chambers would be required. Were so many chambers available, however, this would greatly reduce variability in the initial quality of each experimental unit under different treatments.

4.2.3: Experiments

The experiments conducted in this report examined two developmental stages – germination, and vegetative growth – of several species. Resource constraints restricted the vegetative aspect of that work to only three species, compared to the eight species used in the germination experiments. Future work can expand this to, and beyond the full eight species used during germination. Further, there are many more light qualities, especially incorporating far red and UV wavelengths, which can be utilized to manipulate photosynthesis in the future.

Conducting similar experiments, not just on germination and vegetative growth, but also at time of early seedling establishment, and later during reproductive growth, will provide a more complete analysis of ideal light qualities for plants through the full life cycle.

This work would provide a very good starting point for designing a light "recipe" that could be applied to developing a plant from seed to seed. As was seen in the completed adaptation experiments, however, changing light qualities throughout a plant's development may have negative consequences. To account for this, experiments similar to the recommended photosynthetic experiments could be executed, but adapted such that measurements taken at each later phase of development are completed on plants that are subsampled from those that were monitored under different light qualities during previous developmental stages. For example, many lettuce seeds could be germinated under one quality of light. Those seedlings could then be divided to groups under different light qualities. Those groups, after early seedling development, could be further divided to different light qualities for vegetative growth, etc, for the entire life cycle of the species.

Because midterm adaptation experiments showed a decrease in photosynthetic capacity after adaptation to another light quality, it would be beneficial to monitor photosynthesis over several days after being removed from the "adapted" quality to observe any potential recovery of photosynthetic capacity. This could be expanded to determine if there are any light qualities that cause a more permanent alteration in photosynthesis rates than others.

Varying light intensity may reveal more about tolerances to different wavelengths of some species, which could simplify the design of light qualities in the future. For example, it may be that, while far red light inhibits germination in lettuce and tomato, it may be photosynthetically favourable during vegetative growth. In this case, it could simplify array

programming if it was known that, at a certain lower intensity, far red light did not impair germination, but was still present in sufficient quantities to significantly improve photosynthetic rates of vegetative growth. This would allow for coding far red light only once, rather than coding the intensity of far red to dynamically change over the plant's life.

The experiments presented only examined the influence of light quality on photosynthesis. It is very likely that many of the light qualities tested are very influential in the management and uptake of nutrients. Future work will be monitoring nutrient uptake from a hydroponic solution under differing light qualities. Chemical analysis of plant tissues will also be undertaken to determine the effects of light quality on the production of desirable compounds within plants. This information could be utilized to enhance the nutritional or pharmaceutical properties of crop plants. In combination with light qualities known to be favourable in total dry matter accumulation, ideal light recipes unique to each species could be designed that indeed can deliver the "best" possible plants for production commercially, or as an essential element of survival in extraterrestrial habitats.

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APPENDIX I – SAMPLE ARDUINO CODE

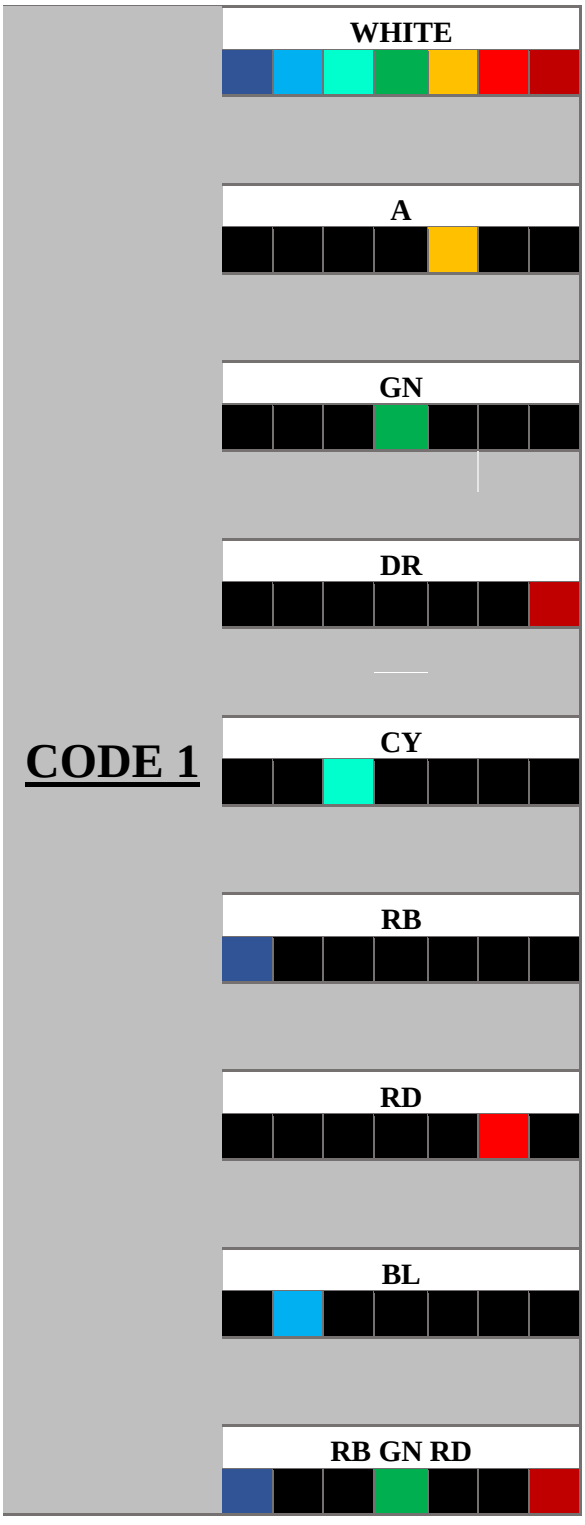
The LED arrays were programmed using an Arduino DUE microcontroller. The following is a sample program, in which all seven colours on the array are active in equal intensities of PAR.

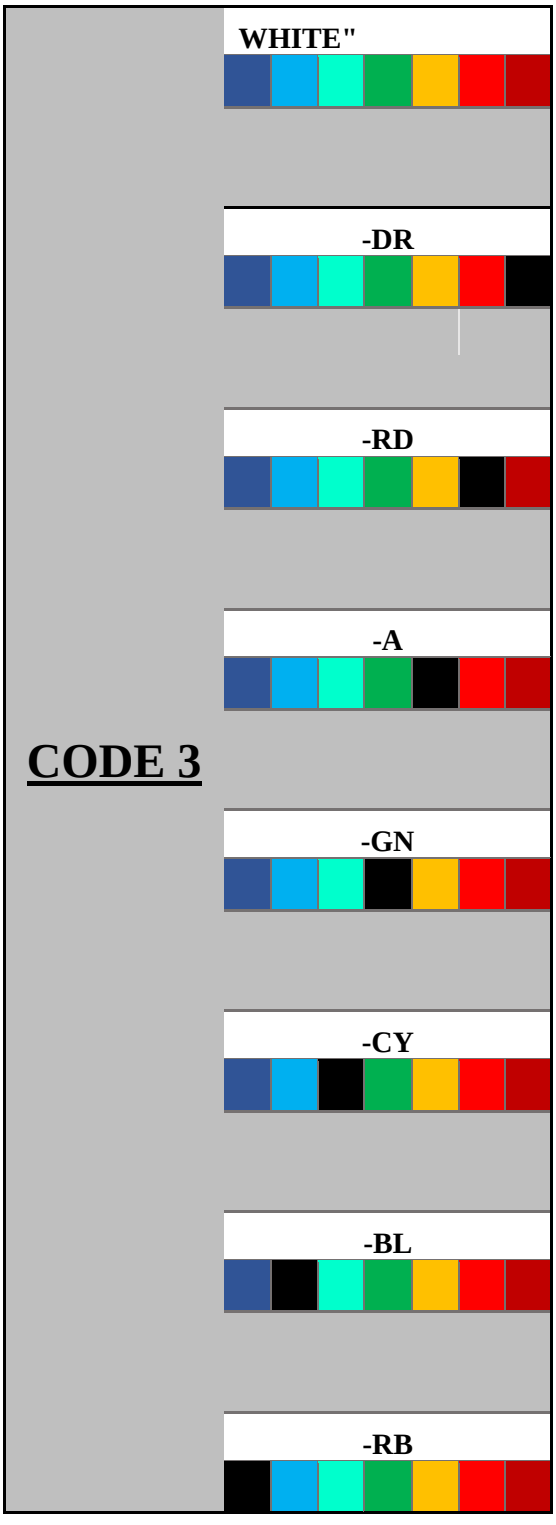
```
// LED array photosynthsis testing with single and combination
wavelengths
/*

Green: 255
Orange: 220
Cyan: 141
Red: 126
Blue: 82
Deep Red: 102
Royal Blue: 74

*/
// set intensities for each wavelength here - use 0-255 PWM values
int RoyalBlue = 74;
int Blue = 82;
int Cyan = 141;
int Green = 255;
int Amber = 220;
int Red =  126;
int DeepRed = 102;

// set the desired interval for each step in milliseconds
int interval = 2400000;  // 3600000 = 60 minutes, 1800000 = 30 minutes
int offgap = 60000;

int RB= 2;    // LED connected to digital pin 2
int B = 3;    // LED connected to digital pin 3
int C = 4;    // LED connected to digital pin 4
int G = 5;    // LED connected to digital pin 5
int A = 6;    // LED connected to digital pin 6
int R = 7;    // LED connected to digital pin 7
int DR = 8;    // LED connected to digital pin 8

void setup() {

  Serial.begin(9600);             // initialize the serial port output
  Serial.println();
  Serial.print("Initializing...");

}
```

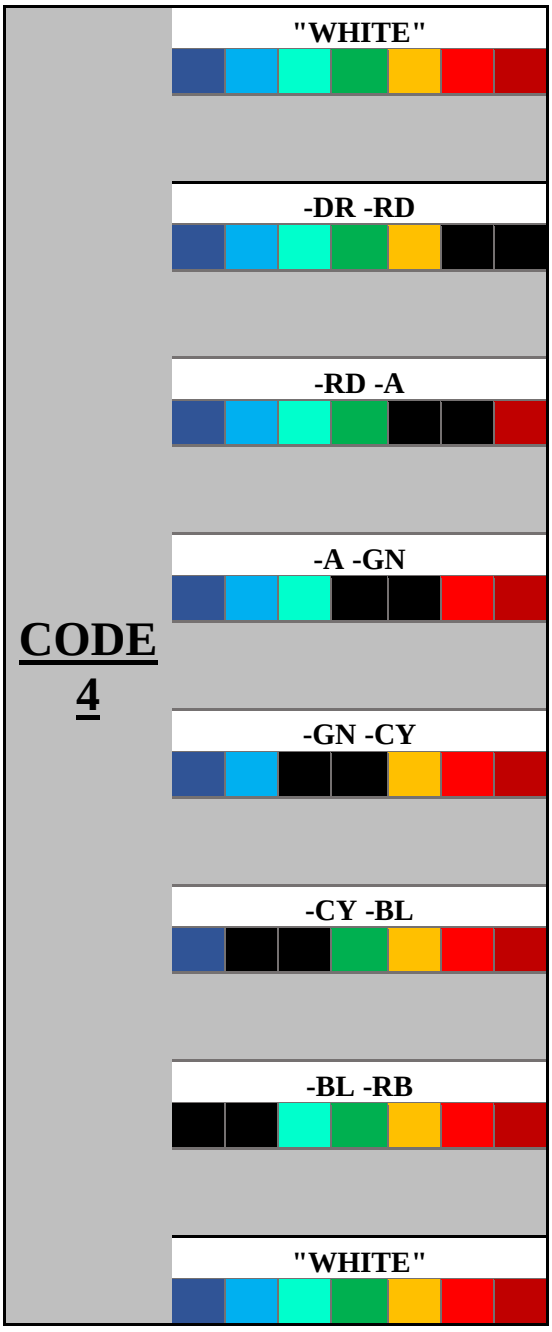
```
void lightsout() {  // turn off all the LEDs - reset and mark
intervals

  analogWrite(RB, 0);
  analogWrite(B, 0);
  analogWrite(C, 0);
  analogWrite(G, 0);
  analogWrite(A, 0);
  analogWrite(R, 0);
  analogWrite(DR, 0);
  delay(offgap);  // oneminute delay to register a change in PAR to
show the point between LEDs
}

void loop() {

/* ALL ON    */
                analogWrite(RB, RoyalBlue/7);
                analogWrite(B, Blue/7);
                analogWrite(C, Cyan/7);
                analogWrite(G, Green/7);
                analogWrite(A, Amber/7);
                analogWrite(R, Red/7);
                analogWrite(DR, DeepRed/7);
                Serial.println("ALL ON /7");
                delay(interval);
                delay(interval);

} //end loop
```

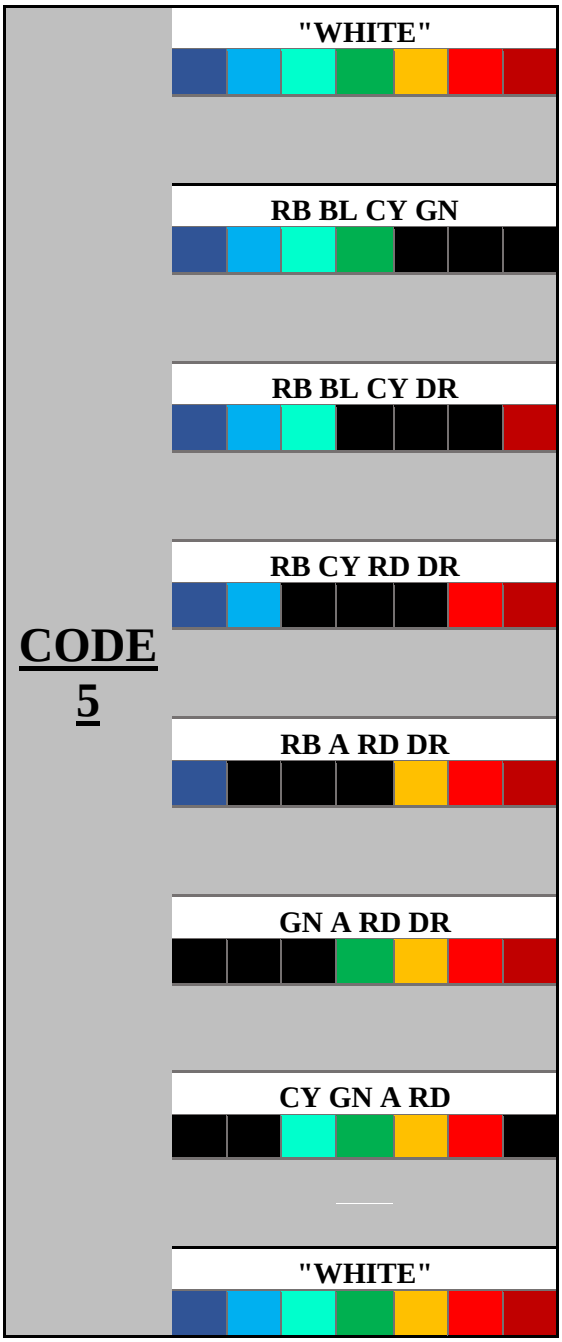
APPENDIX II – LIGHT QUALITIES FOR VEGETATIVE GROWTH

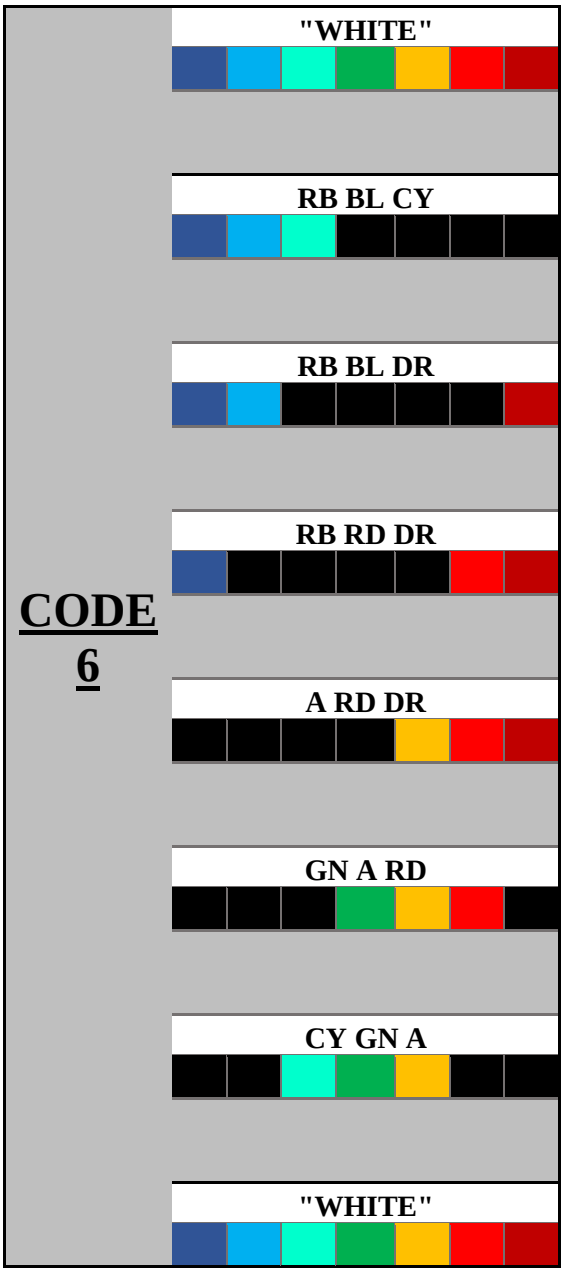
The effect on photosynthesis rates of forty different light qualities were tested on lettuce, tomato, and pepper. Black boxes indicate a colour which was off in a given quality. The non-black colours indicate that that colour was present in a given treatment. All active colours in a given treatment were on in equal parts, with a total PAR in each treatment of approximately 280 µmol/m^2/s. A different code was used every week, with plants the same age throughout the experiment. Due to the variable quality of the plants from week to week, photosynthesis rates from code to code could not be directly compared. The light qualities from each code which yielded the greatest photosynthetic response for each species were compared to each other in a final "code 8" unique to each species, and designated for lettuce, tomato, and pepper as "8-L," "8-T," and "8-P," respectively.

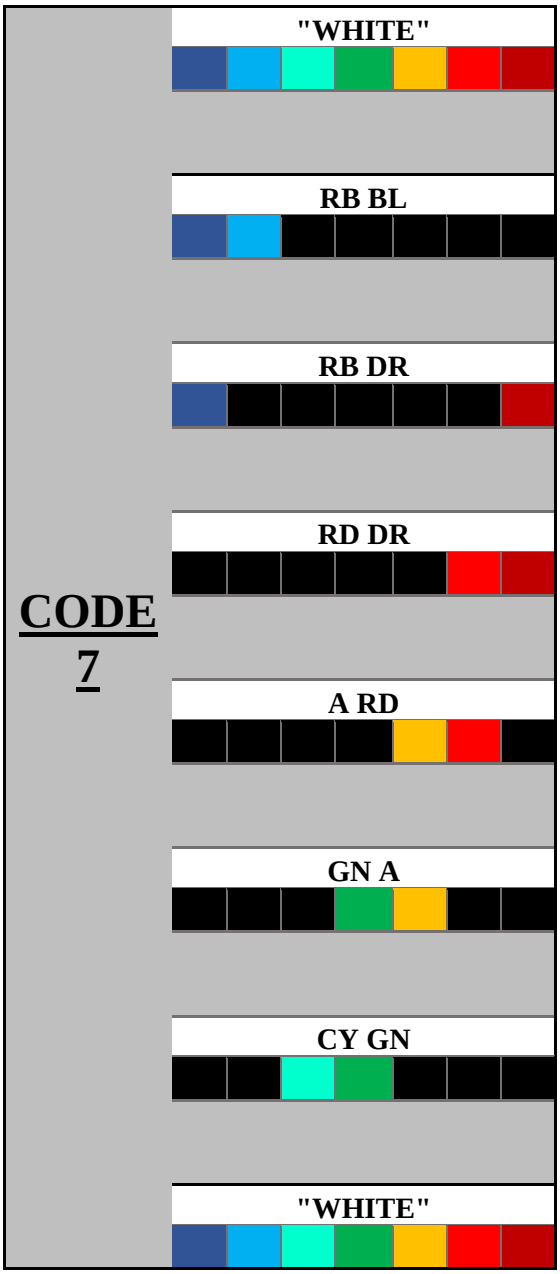

CODE 1
WHITE
A
GN
DR
CY
RB
RD
BL
RB GN RD


CODE 3
WHITE"
-DR
-RD
-A
-GN
-CY
-BL
-RB

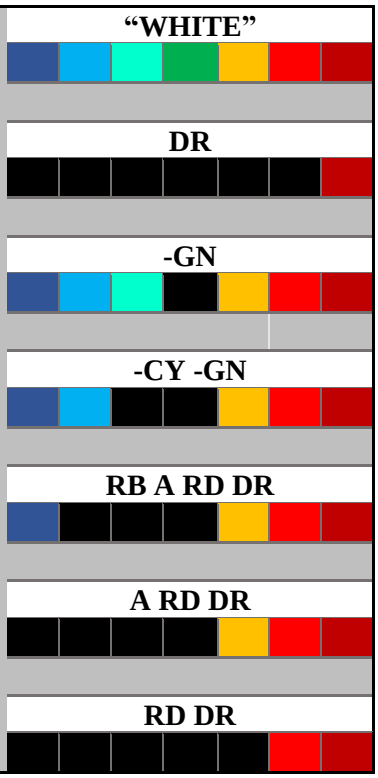

CODE
4
"WHITE"
-DR -RD
-RD -A
-A -GN
-GN -CY
-CY -BL
-BL -RB
"WHITE"


CODE
5
"WHITE"
RB BL CY GN
RB BL CY DR
RB CY RD DR
RB A RD DR
GN A RD DR
CY GN A RD
"WHITE"

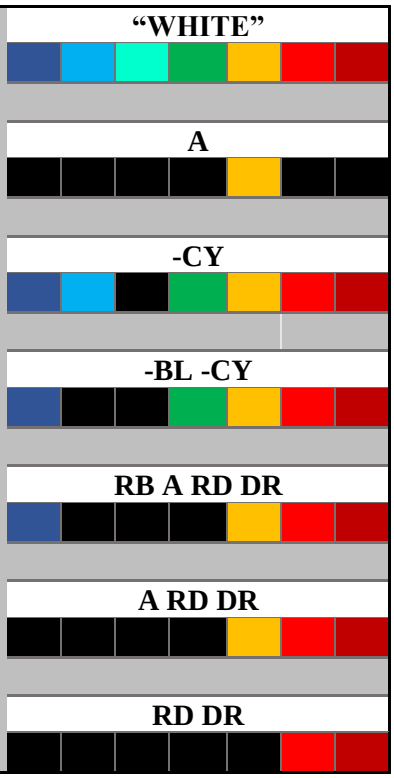

CODE 6
"WHITE"
RB BL CY
RB BL DR
RB RD DR
A RD DR
GN A RD
CY GN A
"WHITE"


CODE 7
"WHITE"
RB BL
RB DR
RD DR
A RD
GN A
CY GN
"WHITE"

CODE 8-L



CODE 8-T



CODE 8-P