The influence of spectral quality of light on plant secondary metabolism and photosynthetic acclimation to light quality

by

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ABSTRACT

THE INFLUENCE OF SPECTRAL QUALITY OF LIGHT ON PLANT SECONDARY METABOLISM AND PHOTOSYNTHETIC ACCLIMATION TO LIGHT QUALITY

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Light quality can have a profound effect on many aspects of plant development. In this thesis, the influence of light quality on yield, morphology, and secondary metabolite profiles was evaluated in basil, strawberry, and cannabis, and the acclimation to light quality was quantified in lettuce and strawberry. These experiments were conducted using four fixed light spectra described as the “Red-blue (RB) Blade”, “Red-green-blue (RGB) Blade”, “Far-red (FR) Blade”, and “Red-blue + red-green-blue (RB+RGB) Blade” (“Blade” refers to the industry nomenclature for this lamp configuration), as well as an array of nine variable-spectra LED arrays. Growing basil plants under the RB and RGB Blades showed the two spectra to produce statistically comparable profiles of volatiles in basil leaf extracts. Growing strawberry plants under RB, RGB, RB+RGB, and FR Blades, the FR spectrum produced plants with significantly longer petioles than the RGB spectrum. Berry flavour was unaffected by any of the light spectra, with berry juice being comparable in sugar content, pH, and total acid content. Analysis of volatiles in berry juice was inconclusive, with irregular profiles between replications. Deploying the RB and RGB Blades below cannabis canopies as supplemental light sources resulted in a significant impact on yield and secondary
metabolite profiles with both Blades compared to canopies with no sub-canopy lighting. The RGB Blades made the greatest impact on modifying terpene content, and the RB Blades produced the most homogenous bud cannabinoid and terpene profile throughout the canopy. Exploring the potential photosynthetic acclimation of lettuce and strawberry plants to light qualities over time, both species were grown to vegetative maturity under various fixed spectra to acclimatize the plants to given light environments. After acclimation, plants were rapidly subjected to several light qualities, measuring photosynthesis under each light quality. Plants preconditioned to certain basal light spectra achieved significantly different photosynthetic rates in various post-conditioning light qualities. This thesis concludes that spectral quality does significantly modify plant morphology and secondary metabolism, and spectral acclimation can have significant effects on photosynthesis. The observations regarding plant acclimation are novel and have meaningful consequences in both research and commercial production, warranting further exploration.
ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

$\Delta^9$-THC – delta-9-Tetrahydrocannabinol

$\Delta^9$-THCA – delta-9-tetrahydrocannabinol-9-carboxylic acid

AS – acclimation spectrum

CBD – cannabidiol

CBDA – cannabidiolic acid

CBGA – cannabigerolic acid

CESRF – Controlled Environment Systems Research Facility

DMAPP – dimethylallyl pyrophosphate

DXP – 1-deoxy-D-xylulose-5-phosphate

EEE – Emerson enhancement effect

FR – far-red

GPP – geranyl pyrophosphate

IPP – isopentenyl pyrophosphate

LED – light emitting diode

MVA – mevalonate
NFT – nutrient film technique

OA – olivetolic acid

PAR – photosynthetically active radiation

PFD – photon flux density

PPFD – photosynthetic photon flux density

PSI – photosystem I

PSII – photosystem II

RB – red-blue

RGB – red-green-blue

SCL – sub-canopy lighting

SPME – solid-phase microextraction

THC – delta-9-Tetrahydrocannabinol

THCA – delta-9-tetrahydrocannabinol-9-carboxylic acid
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1 Introduction

This thesis was completed with the overarching objective of experimentally determining the most morphologically and metabolically favourable light qualities for plant production, and to quantify the potential for plants to acclimate to light quality. The design of light environments is often driven by specific production goals. For example, a light environment optimized to maximize yield in one species may not be the same environment to achieve a desired morphology or nutritional profile. Similarly, the light that effectively maximizes yield in one species may not be effective in another (ref?).

The optimal light environment for a given crop is not based solely on the light that best enhances a specific aspect of plant development; practical considerations like cost, hardware availability, and practicality of working in certain light spectra or around types of lighting hardware must also be considered. For instance, a spectrum that appears pink or purple may be very effective in driving photosynthesis but will make it more challenging for workers to detect pest or nutrient issues amongst the plants, and may be visually fatiguing compared to a more natural light spectrum. There is also a question regarding which light spectrum is best for a given physiological stage of growth: will a species always respond the same way to a given light spectrum, or will it acclimate and respond differently throughout its development in a single crop cycle?

In an effort to elucidate appropriate light spectra with considerations of the above challenges, experiments were conducted with basil, strawberry, and cannabis using similar lighting systems and have been evaluated based on yield, morphology, and
secondary metabolite profiles. To quantify the effects of plant acclimation to light quality, experiments were conducted with strawberry (*Fragaria x ananassa* cv. ‘Albion’) and lettuce (*Lactuca sativa* cv. ‘Grand Rapids’) plants in which plants acclimated to different specific light environments were subjected to several new light spectra and evaluated based on gas exchange characteristics (photosynthesis and respiration) under these new conditions. These experiments are presented in the chapters two to five.

All the experiments presented in this document take advantage of the unique strengths of light emitting diode (LED) lighting technology. LEDs can emit relatively narrow-bandwidth light ranging across the visible spectrum and beyond, without the need for any spectral filters. Further, the photon flux density of each LED can be independently controlled. Together this narrow bandwidth and independent control can produce a nearly limitless number of unique spectral qualities. The experiments presented herein take advantage of LED spectral flexibility, utilizing both fixed- and variable-spectra arrays to expose plants to differing light environments, and evaluating the morphological, metabolic, and gas exchange responses to those environments.

1.1 Evaluating light spectra for plant production

1.1.1 Morphology and development

Light quality has a profound effect on plant morphology, and the effects that different qualities have change with developmental stage. Blue and red wavelengths have particularly powerful effects on plant morphology. Blue light is captured by
cryptochrome and triggers a phototropic response in which plants bend towards the light (Ahmad et al., 1998). Blue light is generally associated with the production of more compact plants when it is the predominant light quality in a spectrum, initiating hypocotyl de-etiolation during seedling establishment, and reducing internode lengths and leaf areas throughout vegetative development (Cosgrove, 1981; Mortensen and Strømme, 1987; Barnes and Bugbee, 1992; Parks et al., 1998; Shimizu et al., 2006). Interestingly, blue light alone can elongate plants, having the opposite effect of when it is combined with other colours (Hernández and Kubota, 2016). Blue light and cryptochrome are also implicated in the initiation of floral development in some species, acting in concert with red / far-red light and phytochrome responses (Guo et al., 1998; Yanovsky and Kay, 2002).

Phytochrome exists in two conformations that preferentially absorb red- and far-red-light (Walker and Bailey, 1968; Anderson et al., 1970). The two conformations are implicated in several morphological responses from the time of germination through to flowering. A simple study by Flint (1934) first observed increased germination rates in lettuce when exposing seeds to red light compared to shorter-wavelength colours in the visible spectrum. Expanding on this, Shinomura (1997) described differential responses from two distinct, but related phytochrome molecules in Arabadopsis thaliana that modulate germination rates, partially based on intensity of red light. Beyond germination, far-red light induces shade avoidance syndrome in plants, resulting in elongated internodes and petioles, and larger, thinner leaves (Mclaren and Smith, 1978; Devlin et al., 1998; Grime, 2006). Smith and Whitelam (2008) have written a thorough
review on phytochrome-induced shade avoidance and readers are directed to that
review for additional detail.

1.1.2 Yield and photosynthesis

In the majority of applications, the dominant consideration for selecting a specific
light spectrum is yield of the marketable component of the plant. This is not the explicit
objective in all production scenarios, as specific goals, such as increasing production of
a certain compound within a plant, can also be met through increased overall yields.

Biomass yield is a product of primary metabolism in plants, driven by
photosynthesis. Photosynthesis, in turn, can be broken into multiple steps, initiated by
the light reactions of photosynthesis. It is in this stage where light quality directly affects
all downstream yield in plants (Buchanan et al., 2000).

The light reactions occur in a series of protein complexes embedded in the
membranes of thylakoids. Thylakoids exist in pancake-like stacks called grana with
appressed and non-appressed regions in the stacks. Grana are found in chloroplasts,
which in turn are in all photosynthetic plant cells (Berg et al., 2002).

Amongst the numerous proteins involved in photosynthetic light reactions are two
important protein complexes: photosystem II (PSII) and photosystem I (PSI), distinct in
what wavelengths of light they absorb. These photosystems differ in their composition,
with PSII being more rich in chlorophyll $b$ and PSI being more rich in chlorophyll $a$
Both chlorophyll molecules absorb light across the visible spectrum, though some wavelengths are more strongly absorbed than others. Purified in an ether solution, chlorophyll $b$ has strong absorption peaks at 453 nm and 642 nm, while chlorophyll $a$ has absorption peaks at 430 nm and 662 nm (Comar and Zscheile, 1942; Gross, 1991). The relative absorption spectra of these two chlorophyll molecules are illustrated in Figure 1.1.

![Relative absorption spectra of purified chlorophylls](image)

**Figure 1.1.** Adapted from Comar and Zscheile (1942). Relative absorption spectra of purified chlorophylls $a$ (red line) and $b$ (blue line) in ether solution, by wavelength (nm).
In addition to chlorophyll molecules in the photosystems, there are a number of accessory pigments that absorb light energy and pass that energy into photosynthetic reactions (Buchanan et al., 2000). These can be categorized into a family of compounds called carotenoids, and absorb a range of wavelengths between approximately 400 nm and 500 nm (Miller et al., 1935; Zur et al., 2000). Absorbance spectra for some major carotenoids $\alpha$-carotene, $\beta$-carotene, and lycopene are illustrated in Figure 1.2.

![Normalized absorption spectra of alpha-carotene (red line) Beta-carotene (orange line), and lycopene (yellow line) by wavelength (nm), purified in ether and ethanol.](image)

**Figure 1.2.** Adapted from Miller et al. (1935). Normalized absorption spectra of alpha-carotene (red line) Beta-carotene (orange line), and lycopene (yellow line) by wavelength (nm), purified in ether and ethanol.
Absorbance spectra of purified compounds only provide a starting point in understanding the mechanisms of light absorption in plants; in practice, light absorption in whole, intact leaves is more balanced across the spectrum than what is measured in purified compounds (Figure 1.3) (Moss and Loomis, 1952).

Figure 1.3. Adapted from Moss and Loomis (1952). Percent absorption by wavelength (nm) in fresh leaf tissue (green line), purified chloroplasts (red line), disintegrated (via boiling) chloroplasts (blue line), and pigment extraction with methanol (orange line).
As will be discussed, not all of this absorbed light is used for solely for photosynthesis, but this serves as a reminder that focusing only on results *in vitro* can be misleading. Also note that absorption spectra only give an approximation of how plants use absorbed light; generalized photosynthetic action spectra, averaged from action spectra measured in 22 species, is illustrated in **Figure 1.4**.
Figure 1.4. Adapted from (McCree, 1972). Average relative quantum yield (A), action spectra (B), and absorptance (C) spectra of 22 species, for field-grown (red lines) and chamber-grown (blue lines) plants. Note in this figure, McCree reported “absorptance” rather than absorption. These two metrics are measured differently but communicate similar information. In this case, absorptance was a calculation of
the difference in radiance of an internal wall of an integrating sphere, with and without a leaf inside the sphere. Absorption is more often describing the amount of light lost when shining a light through a substance.

These generalized interpretations of how plants absorb and utilize photosynthetic light, as summarized in this section, provide insights that inform the design of production light spectra favourable for yield.

1.1.3 Secondary metabolites

Many compounds in plants are produced for the purpose of interacting with the environment. These compounds are generally called “secondary metabolites”, or products of secondary metabolism. Secondary metabolites can help plants manage different types of environmental challenges including light and drought stress. The most relevant classes of secondary metabolites to this thesis include terpenes, carotenoids, and cannabinoids. The following provides a very brief description of these compounds and their biosynthesis.

1.1.3.1 Terpenes

Terpenes are functionally diverse. Terpenes, and more specialized compounds derived from terpenes, can be volatile aromatics, affecting or contributing to the taste and smell of plants (Goff and Klee, 2006), defense compounds against biotic stresses (Martin et al., 2003), hormones that regulate growth (Milborrow, 2001; Sakakibara,
2005; Hedden and Thomas, 2012), or help plants manage light and drought stress, amongst other things (Buchanan et al., 2000). Some terpenes have been shown to have medicinal value when used appropriately (Goff and Klee, 2006).

Chemically, terpenes contain distinctive carbon chains that are assembled from the 5-carbon isoprenes dimethylallyl pyrophosphate (DMAPP), and its isomer isopentenyl pyrophosphate (IPP). These precursors are synthesized via cytosolic mevalonate (MVA) and plastidial non-mevalonate (also known as 1-deoxy-D-xylulose-5-phosphate [DXP] or methylerthritol [MEP]) pathways. Regardless of the pathway, DMAPP and IPP are ultimately both derived from compounds found in primary metabolism: the MVA pathway begins with acetyl-CoA, and the DXP pathway begins with the glycolysis intermediates glyceraldehyde-3-phosphate and pyruvate (Buchanan et al., 2000; Bartram et al., 2006).

The MVA pathway utilizes acetyl-CoA, a product of glycolysis, in cytosolic terpene biosynthesis. The rate-limiting step in this pathway is the level of HMG-CoA reductase activity, located in the endoplasmic reticulum (Bach et al., 1999). Activity of this enzyme is partially regulated by hormonal signals, pathogen stress, and wounding. The enzyme is inactivated by a protein kinase that also acts on sucrose phosphate synthase and nitrate reductase. The MVA pathway is generally associated with the downstream production of monoterpenes (10-carbon chains), diterpenes (20-carbon chains) and carotenoids (specialized 40-carbon tetraterpenes) (McGarvey and Croteau, 1995).
The MEP pathway begins with glycolysis intermediates glyceraldehyde-3-phosphate and pyruvate and ultimately synthesizes DMAPP and IPP in the plastids rather than the cytosol (Rohmer et al., 1996). Comparatively less is known about the MEP pathway; the MVA pathway, by contrast, is highly relevant to human health and as such has been the focus of many studies (Hunter, 2007). The MEP pathway is generally associated with the biosynthesis of sesquiterpenes (15-carbon chains) and triterpenes (30-carbon chains) and occurs in the chloroplasts of plant cells (Rohmer et al., 1996). Each step of the MEP pathway has been detailed by Hunter (2007).

Numerous studies have demonstrated a relationship between terpene biosynthesis and light (Loveys and Wareing, 1971; Gleizes et al., 1980; Yamaura et al., 1991). Some studies of particular interest to this thesis are those of Schnarrenberger and Mohr (1970), and Tanaka et al. (1989), where they respectively observed regulation of carotenoid and monoterpene biosynthesis by the red-light receptor, phytochrome. Terpenes are also linked to the absorption of blue-green light through a specialized set of compounds called carotenoids.

1.1.3.2 Carotenoids

Carotenoids are a specialized type of tetraterpene involved in light absorption for photosynthesis. As described in Section 1.1.2, carotenoids effectively absorb light in the 400 nm – 500 nm range (Miller et al., 1935; Zur et al., 2000) and pass this energy into the reaction centers of photosynthesis (Caffarri et al., 2014). Carotenoids also play an important photoprotective role, mitigating light stress that would otherwise be
damaging chlorophyll molecules; they effectively absorb energy from wavelengths that would otherwise be particularly difficult for chlorophyll to manage (McCollum, 2006; Kirilovsky and Kerfeld, 2013; Ostroumov et al., 2013). If terpenes are considered secondary metabolites, and photosynthesis is considered primary metabolism, then carotenoids are where these definitions are blurred – carotenoids are secondary metabolites but directly contribute to primary metabolism.

As previously noted, carotenoids are a specialized group of tetraterpenes, and as such, are derived via the mevalonate pathway. While the MVA pathway is highly regulated via HMG-reductase activity, evidence suggests that the biosynthesis of carotenoids is more directly regulated, and will at times be synthesized when IPP and DMAPP precursors are not also actively being synthesized (Narita and Gruissem, 1989).

Considering the role of carotenoids in mid-range wavelength light management, this contributes to one of the overriding rationales for this thesis: a light spectrum with comparatively more green light than one with less or no green light should drive plants to produce more carotenoids to manage green wavelengths, and potentially up-regulate other related terpenes in the process. This rationale can be extended to the hypothesis that the putative up-regulation of carotenoids may indirectly result in the up-regulation of carotenoid precursors, IPP and DMAPP. These two precursors are not exclusive to terpenes; they are also shared with a unique class of related compounds called cannabinoids.
1.1.3.3 Cannabinoids

Cannabinoids comprise a rare class of compounds found in cannabis (*Cannabis sativa*); while there are numerous compounds produced in other species that will also bind to cannabinoid receptors in humans, cannabinoids appear to be unique to cannabis (Bauer et al., 2008; Gertsch et al., 2010). *In phyta*, the role of cannabinoids is not well understood. Shoyama et al. (2008) found that cannabis leaves secrete cannabinoids from the glandular trichomes into leaf tissue to induce cell death. Lydon et al. (1987) found increased THC concentrations when cannabis plants were grown under UV-B light, suggesting that cannabinoids may play some role in UV protection. Beyond this, there appears to be few if any other published studies exploring the role of cannabinoids in plants.

Consumption of cannabinoids and putative benefits to human health are elaborated on in Chapter 4.

Note that this document is not designed to inform or comment on any benefits, or lack thereof, of cannabis consumption to humans.

Cannabinoid biosynthesis shares common precursors to terpene biosynthesis. Geranyl pyrophosphate (GPP) is condensed from DMAPP and IPP, and is the basic subunit of all monoterpenes in higher plants (Banthorpe et al., 1972; Croteau and Purkett, 1989). In the synthesis of cannabinoids, GPP and olivetolic acid (OA) are combined via GPP-OA transferase to produce cannabigerolic acid (CBGA) (Fellermeier and Zenk, 1998; Fellermeier et al., 2001). Various synthases then convert CBGA to
derivatives such as delta-9-tetrahydrocannabinol-9-carboxylic acid (THCA) and cannabidiolic acid (CBDA) (Taura et al., 1995; Taura et al., 1996).

1.1.3.4 Flavonoids

Though given less emphasis throughout this document, flavonoids are another class of important secondary metabolites whose synthesis and regulation are sensitive to light quality. Flavonoids are diverse in both structure and function; for a thorough examination of flavonoids, readers are referred to Harborne (2013). Flavonoids are based upon a two-ring, 15-carbon structure and have many distinct classes including, but not limited to anthocyanins, flavones, flavonols, flavanones, and isoflavonoids (Iwashina, 2000). These classes are defined based on the various accessory groups attached to the central 15-carbon skeleton (Iwashina, 2000). Flavonoids fulfill an appropriately broad range of functions, including, but not limited to pollinator and feeding attractants, feeding deterrents, oviposition stimulants, disease resistance, and managing light stress (Hamamura et al., 1962; Ingham, 1972; Honda, 1986; Noh and Spalding, 1998; Nishida, 2005; Goff and Klee, 2006; Arakawa et al.).

Flavonoids are desirable to human health in that they have well documented antioxidant activity, which in turn has implications in inflammatory heart disease, and cancer susceptibility (Havsteen, 1983; Middleton et al., 2000; Pietta, 2000; Havsteen, 2002).

Biochemically, flavonoids are all derived from the common precursor, phenylalanine (Ferreyra et al., 2012). The first committed steps in flavonoid biosynthesis are the conversion of phenylalnine to 4-coumaroyl-CoA, then to common chalcone
scaffolds that are modified to produce the various flavonoids (Bach et al., 1999; Bowles et al., 2005; Martens et al., 2010; Ferreyra et al., 2012).

Total flavonoid concentrations have been shown to be greater in plants grown under certain light qualities, particularly under UV, blue, and far-red light (Fu et al., 2016; Pedroso et al., 2017; Liu et al., 2018). Thus, in designing an optimal light spectrum for production where the aforementioned benefits to human health are prioritized, these beneficial wavelengths are given greater consideration.

1.2 Overview of experiments

Four studies are presented in this document. The first study is on the influence of two different light spectra on basil volatile profiles. In a prior study not conducted as a part of this thesis, the spectra of the RB and RGB Blades were developed specifically for optimal basil production based on yield and morphology, and the plants they produced did not significantly differ in these regards. Expanding on the prior study, this experiment compared volatile profiles of leaf oil extracts from plants grown under the RB or RGB Blades.

The experimental objective was:

- Evaluate the influence of two different light spectra on primary basil leaf oil volatiles.
This experiment also carried multiple technical objectives to develop skills that would be required in a later study on cannabis. The technical objectives of this experiment were:

- Develop a methodology and broader understanding of clonal propagation, with the intent to transfer these skills to upcoming experiments on cannabis.
- Develop an understanding and methodology for oil extraction and sample preparation for volatile analysis, and GC/MS operation and troubleshooting.

The second experiment evaluated the influence of four different light spectra on strawberry production. The objectives of this study were:

- Evaluate the influence of light spectrum on strawberry plant vegetative development, based on petiole lengths.
- Evaluate the influence of light spectrum on berry quality, based on sugar content, pH, total acid content, and volatile profile.

The third experiment evaluated the effect of deploying two different light spectra as supplemental lighting below a cannabis canopy. The objectives of this study were:

- Evaluate cannabis bud yield with and without supplemental sub-canopy lighting, and between spectra.
- Evaluate bud quality with and without supplemental sub-canopy lighting and between spectra, based on cannabinoid and terpene profiles.
• Evaluate secondary metabolite homogeneity throughout the canopy with and without supplemental sub-canopy lighting, and between spectra.

The fourth study evaluated the possible effect of photosynthetic acclimation to light quality over time. The objectives of this study were:

• Identify any “Emerson\(^1\) Enhancement-like” photosynthetic increases achieved when illuminating plants with combined PAR-based spectra and far-red light versus PAR-based spectra alone.

• Determine if any change in photosynthetic gas exchange is disproportionately additive as suggested by the “Emmerson Enhancement” interpretation historically.

• Evaluate the effect of light acclimation on post-acclimation photosynethic responses, and if any Emerson enhancement-like responses are dependent on a prior spectral acclimation.

\(^1\) The Emerson enhancement effect describes the supplementation of far-red light (\(> 680\) nm) with shorter wavelengths in the visible spectrum to achieve photosynthetic rates greater than the sum of what would be achieved with far-red or shorter visible wavelengths alone (Emerson et al., 1957).
The Introduction part should include the existing literature on the effects of different light spectra on plant physiology, growth and second metabolism, especially the ones you studied in this thesis to show why your work was needed.

2 The influence of two light qualities on basil volatile profiles

2.1 Introduction

Basil (*Ocimum basilicum*) is a leafy green plant in the mint family, valued for its culinary, ornamental, and putative medicinal applications (Nguyen et al., 2010; Khair-ul-Bariyah et al., 2012). Evidence has shown that compounds produced in basil and administered through basil consumption can be effective in treating ailments including convulsions and anxiety, as well as being an effective anti-oxidant (Hiltunen and Holm, 1999; Javanmardi et al., 2002; Chanwitheesuk et al., 2005; Freire et al., 2006). There are obvious benefits to better understanding what production parameters significantly modify the profiles of such compounds *in phyta*.

Carvalho et al. (2016) recently observed that basil plants grown under a red-green-blue spectrum contained significantly higher eugenol concentrations than plants grown under only red and blue, and RGB-grown plants contained significantly higher linalool concentrations than greenhouse (unsupplemented sunlight)-grown plants.
Basil is predominantly a greenhouse crop, and there is a strong desire to improve production quality through utilization of modern LED lighting systems to supplement natural daylight (Monsieur Basilic, personal communication). To this end, this study aimed to compare the secondary metabolite profiles produced in basil when grown under two different light spectra. These two spectra were developed in a previous study comparing six light spectra and evaluating basil plants on yield and morphology. Both spectra were found to be comparable in maximizing plant productivity.

Preliminary trials indicated that the largest components of this basil’s profile of volatiles were comprised of eugenol, linalool, and eucalyptol. Eugenol is a phenylpropene, biosynthetically distinct from linalool and eucalyptol, which are both monoterpenes (Croteau et al., 1994; Pichersky et al., 1995; Kapteyn et al., 2007; Rastogi et al., 2013; Rinkel et al., 2016). The biosynthetic pathway of eugenol is comparatively close to that of flavonoids, and the biosynthetic pathways of linalool and eucalyptol are comparatively close to that of carotenoids and xanthophylls. As described in Section 1.1.3, both of these pathways have interactions with blue and green light, respectively. Thus, a spectrum relatively rich in blue light may up-regulate eugenol compared to a spectrum with a lower ratio of blue light, while a spectrum containing a greater green fraction may up-regulate linalool and eucalyptol.

Building on the findings of Carvalho’s team in 2016, this study utilizes similar light spectra and a different cultivar of basil to better define the relationships that have been observed between eugenol, linalool, eucalyptol, and light quality.
The objective of this study was to assess the influence of red-blue and red-green-blue light qualities as produced from two Intravision Blades (Intravision Light Systems, Inc., Toronto, ON, Canada) on the primary constituents of basil volatile profiles. It is hypothesized that significant differences will be detected in eugenol concentrations between the two treatments, but not in linalool or eucalyptol concentrations.

All of the subsequent procedures were repeated in triplicate. The position of the different light treatments was alternated every replication.

2.2 Materials and methods

2.2.1 Mother plant preparation

Basil (Ocimum basilicum cv. ‘Genovese’) seeds were provided by Monsieur Basilic L’tee. (St-Placide, Québec, ON., Canada). Four plants each were grown from seed under two distinct light spectra produced by Intravision red-blue (RB) and red-green-blue (RGB) Blade fixtures (Intravision Light Systems, Inc., Toronto, ON, Canada). Blade dimensions were approximately 244.0 cm long by 7.0 cm wide by 2.0 cm thick. The RB Blades have spectral peaks at 442 nm and 654 nm. The RGB Blades have spectral peaks at 442 nm, 518 nm, and 654 nm. The normalized spectra of both Blades are illustrated in Figure 2.1. Spectral distribution of each Blade spectrum within the grow benches is illustrated in Appendix IV.
Figure 2.1. Normalized spectra for Intravision red-blue (RB) and red-green-blue (RGB) Blades.

Growth parameters are summarized in Table 2.1. Plants were grown for 40 days, at which point there was sufficient biomass to take cuttings for clonal propagation.

Table 2.1. Environmental parameters for basil mother plant seeding and production.

<p>| Location | Controlled Environment Systems Research Facility (CESRF), University of Guelph, ON., Canada |</p>
<table>
<thead>
<tr>
<th><strong>Chamber</strong></th>
<th>Large “BlueBox 2” sealed growth chamber (Dixon et al., 1999) (Appendix I).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature</strong></td>
<td>20.0 ± 0.5 °C day, 18.0 ± 0.5 °C night</td>
</tr>
<tr>
<td><strong>Relative humidity</strong></td>
<td>57.5 ± 1.5%</td>
</tr>
<tr>
<td><strong>Vapour pressure deficit</strong></td>
<td>1.08 ± 0.27 kPa</td>
</tr>
<tr>
<td><strong>[CO₂] Setpoint</strong></td>
<td>800 ppm</td>
</tr>
<tr>
<td><strong>Fertigation method</strong></td>
<td>Hand watered, alternating deionized water and fertilizer solution (Plant Prod 7-11-27 as per label (Plant-Prod 7-11-27, Master Plant-Prod Inc., Brampton, ON, Canada)) as needed (during production of mother plants)</td>
</tr>
<tr>
<td></td>
<td>Nutrient film technique (NFT) hydroponic (during production of experimental units)</td>
</tr>
<tr>
<td><strong>Nutrient solution</strong></td>
<td>Plant Prod 7-11-27 as per label (Plant-Prod 7-11-27, Master Plant-Prod Inc., Brampton, ON, Canada)</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>6.0</td>
</tr>
<tr>
<td><strong>EC</strong></td>
<td>1300 µS</td>
</tr>
<tr>
<td><strong>Light sources</strong></td>
<td>Ecolux Starcoat High Output fluorescent bulbs (F54W-T5-841-ECO, General Electric, Toronto, ON). (mother plant production chamber)</td>
</tr>
<tr>
<td></td>
<td>Intravision RB and RGB Blades (during production of experimental units)</td>
</tr>
<tr>
<td><strong>Canopy level PPFD</strong></td>
<td>300 µmol m^{-2} s^{-1}</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td><strong>Photoperiod</strong></td>
<td>16 h</td>
</tr>
</tbody>
</table>

Of the eight total plants, the best plant, based on qualitatively observed health and shoot biomass, was selected to be the mother plant for the remainder of the experiment. The rest of the plants were discarded.

Cuttings were taken from the mother plant for clonal propagation by cutting between two and three cm from a vegetative growing tip using a sterile razor blade. Cut ends were immediately dipped into 0.1 IBA powdered rooting hormone (Stim-root no. 1, Plant Products Co. Ltd., Brampton, ON, Canada), then inserted 1.5 cm into Sunshine #5 Propagation Mix (SunGro, Agawam, MA, USA) in a 24-cell propagation tray (vented sqplug tray, blk, Myers Lawn and Garden, Middlefield, OH, USA). The tray nested into a white plastic non-draining tray that was filled with deionized water to a height of approximately 1.5 cm (when the propagation tray was nested into it), to maintain rooting substrate moisture. The tray and cuttings were covered loosely with a clear plastic humidity dome.

Trays of cuttings were placed in a Conviron E15 growth chamber (Conviron, Winnipeg, Manitoba) equipped with eight Ecolux Starcoat High Output fluorescent bulbs (F54W-T5-841-ECO, General Electric, Toronto, ON). Clones were grown in this chamber in a 16h photoperiod at 21.0 °C ± 1.0 °C. After cutting establishment, clones were transplanted into black 2.86 L pots (BM.0300, Myers Lawn and Garden, Middlefield, OH, USA) in Sunshine LC1 growing substrate (SunGro, Agawam, MA,
USA). Cutting establishment lasted approximately 20 days and was gauged by lightly pulling on the clones to feel for resistance caused by rooting. Cloned plants were grown in this manner to establish a stock of several mother plants from which cuttings could be taken for the remainder of the experiment. Floral growing tips were pruned off as soon as they were detected.

### 2.2.2 Clone production under RB and RGB Blades

Clones were taken from the stock of mother plants and prepared in the same manner. The trays of clones were placed in the BlueBox 2 growth chamber at CESRF, half under the RB Blades and half under the RGB Blades. The chamber environmental parameters were the same as in initial mother-plant production, summarized in Table 2.1. For the first 3 days after taking cuttings, the lights were turned off to allow early rooting of the clones without any light stress. On day 4, the lights were turned on to their normal photoperiod, and screens were placed on top of the humidity domes on each tray of cuttings to reduce the light intensity to $95.0 \pm 4.0 \, \mu\text{mol m}^{-2} \text{s}^{-1}$ at plant level. On day 11, the screens and domes were removed. On day 15, the established cuttings were transplanted to 2.86 L pots (BM.0300, Myers Lawn and Garden, Middlefield, OH, USA) filled with Sunshine LC1 growing substrate (SunGro, Agawam, MA, USA), and set into NFT hydroponics troughs with fertigation as per in Table 2.1. Plants were grown for an additional 30 days post-transplant, bringing the total growth cycle to 45 days.
2.2.3 Oil extraction

On the 46th day of growth, 5.0 grams of fresh leaves were taken from each of the two treatments. Leaves were taken from several different clones in a treatment, taking from only the first set of newly fully expanded leaves per growing tip. Batched leaves from each treatment were placed into 250 mL glass beakers, and sufficient n-pentane, an organic solvent, was added to fully cover the leaves. Beakers were covered with aluminum foil and placed into an ice bath in a sonicator (Branson 1200, Emerson Electric Co., St. Louis, MO, USA), and sonicated for 30 minutes. A brick was placed on top of the beakers to stabilize them in the bath. After 30 minutes, the beakers were removed from the sonicator, and the leaf tissue was removed and discarded with forceps that had been rinsed with n-pentane. The remaining extraction solutions were concentrated via evaporation down to 1.0 mL each; n-pentane was selected for this extraction due to its rapid volatilization at ambient environmental conditions, making this concentration relatively rapid. The concentrated 1.0 mL extractions were transferred to 1.5 mL GC auto-sampler vials (National Scientific C4000-92W, ThermoFisher, Waltham, MA, USA) and stored in a -86 °C freezer (Model 923, Forma Scientific, Inc., Marietta, OH, USA) until samples were taken from the extractions for analysis.

2.2.4 Sample preparation

Samples were removed from the -86 °C freezer and thawed at room temperature. In five new GC vials, 300 µL micro-inserts (National Scientific C4010-629, Thermo Scientific, Waltham, MA, USA) were added to each to allow for smaller sample volumes.
Three sample vials were prepared in a VHB-4 fume hood (Versa-air, St. Barnabe-Sud, Quebec, Canada). The first vial contained 200 µL of n-pentane. This served as a blank control, to ensure there were no contaminants in the n-pentane stock that would result in misleading GC-MS reports. The second vial contained 200.0 µL of extract from plants grown under the RB Blades. The third vial contained 200.0 µL of extract from plants grown under the RGB Blades.

All three samples were sealed and taken to the Advanced Analysis Centre at the University of Guelph.

2.2.5 GC-MS

All samples were analyzed via GC-MS. The following was provided by the Advanced Analytics Centre:

“[A] GC-MS system (Agilent, Santa Clara, CA, USA) [which] included an HP 7890A GC interfaced with an Agilent 5975C mass selective detector configured in EI mode was used for volatile compound analysis. Chromatography was performed using Bruker BR-SWax column (30 m 0.25 mm 0.5 µm). The oven temperature was held at 40°C for 2min and raised the temperature from 40 to 100°C with 20°C/min, hold for 5min, from 100 to 220°C with 10°C/min, and then hold for 1 min., from 220 to 250°C with 40°C/min, and then hold for another 1 min. The flow through the column was held constant at 1 mL He/min. The injection volume was 1 µL at the splitless mode. The injection temperature
was 230°C and transfer line temperature was 260°C, respectively. The GC column was equilibrated for 5 min prior to each analysis.

The MS was operated in a scan mode (starting after 3 min, mass range 39-300 amu) at 5scan/sec scanning range. The MS source temperature was set on 230°C and the MS quadrupole temperature at 150°C. The data was inquired in electron impact (EI) positive ionization mode using 70eV energy and analysed on Agilent MSD Chemstation (version E02.02.1431).

2.2.6 Experimental design and statistical analysis

The experiment was arranged as a randomized complete block design with two treatments, replicated over time. A single experimental unit comprised of all of the leaf tissue sampled from multiple plants in a single treatment, resulting in two experimental units per replicate. The experiment was replicated three times. Extraction profiles were compared between the RB and RGB spectral treatments using Student’s t test in SAS JMP 13.2.0 (SAS, Cary, North Carolina). “Light quality” and “Chamber Position” were treated as fixed effects, and “Block” was treated as a random effect.

2.3 Results and discussion

Eugenol, linalool, and eucalyptol were the most abundant compounds in the volatile profiles measured, together accounting for nearly 80% of the entire volatile
profile. The relative concentrations of these compounds did not differ significantly between light treatments (Figure 2.2).

![Graph showing relative concentrations of major volatiles in basil oil extractions.]

**Figure 2.2.** Relative concentrations of major volatiles in basil oil extractions. Cloned basil plants were grown under Red-Blue (RB) or Red-Green-Blue (RGB) spectra for 45 days. Within each replicate, oil was extracted from 5.0 g of the youngest fully expanded leaves at the growing tips of several plants within a treatment, which were batched together as a single experimental unit at the time of extraction. Vertical bars indicate standard error. Horizontal connected bars indicate no significant differences between treatments using Student’s t test, n = 3, α = 0.05.

The results observed in this study are not consistent with those found by Carvalho et al. (2016). There are a number of differences in treatments, experimental design and procedures that could explain this discrepancy, though the most likely culprit was that
this study kept PPFD consistent between both light treatments. Both treatments in this study had a PPFD of 300 µmol m\(^{-2}\) s\(^{-1}\), while the study by Carvalho et al. varied between 100 µmol m\(^{-2}\) s\(^{-1}\) for their red-blue light quality and 150 µmol m\(^{-2}\) s\(^{-1}\) for their red-green-blue light quality. Taken together, it suggests that the differences in eugenol concentration Carvalho et al. observed between their treatments may have been more a product of light intensity rather than light quality.

The theory that flavonoids and eugenol may share some regulatory mechanisms is not supported by the results of this study, though this study was not explicitly designed to test that hypothesis, and could have been better designed to address this question directly by quantifying flavonoids in addition to volatiles. If there were a connection between the regulation of phenylpropanes and flavonoids, it would need to be relatively early in the biosynthesis of each. Eugenol is a phenylpropene, derived from a biochemical path that shares some common precursors up to the synthesis of phenylalanine with flavonoid biosynthesis (Gang et al., 2001; Ferreyra et al., 2012). It is well established that flavonoids are up-regulated in response to high energy wavelengths like UV and blue light (Fu et al., 2016; Pedroso et al., 2017; Liu et al., 2018). The current understanding of this flavonoid upregulation is that photostimulated phytochromes, cryptochromes, and phototropins inhibit an inhibitor of positive flavonoid transcription factors, leading to the increase in transcription of structural flavonoid genes (Jang et al., 2010; Jeong et al., 2010; Liu et al., 2011; Lau and Deng, 2012; Li et al., 2012; Zoratti et al., 2014).
With evidence of tight flavonoid synthesis regulation by light, there may exist a yet-to-be discovered additional regulatory mechanisms earlier in the flavonoid biosynthetic pathway, where precursors are also shared with eugenol biosynthesis. In such a case, eugenol concentrations might mirror flavonoid concentrations to some degree, as their metabolism would be partially jointly regulated. This may still be the case; while no difference was observed in eugenol concentration from plants grown under the relatively blue-rich RB Blades compared to the relatively blue-weak RGB Blades, flavonoids were not measured in this experiment, so conclusions cannot be made as to whether or not flavonoids and phenylpropenes were differently regulated. This potential relationship could be better elucidated in future experiments where flavonoids and phenylpropenes are both quantified from plants grown in several light qualities.

As described in Section 1.1.3, the close relationship between terpene biosynthesis and carotenoid biosynthesis suggested that the concentrations of monoterpenes linalool and eucalyptol might differ between light treatments. This was not observed in this study, and may be explained by neither of the light treatments causing enough of a “stress” to the plants to trigger any metabolic change as a protective mechanism. Future studies could expand on this explanation by growing plants in more dramatically different light qualities, or with the same light qualities at a greater intensity that might induce a metabolic stress response in the plants.
2.4 Conclusions

Prior experiments resulted in the spectra developed for the RB and RGB Blades, with both achieving comparable high yielding, morphologically desirable basil plants. This study compared the profiles of volatiles of basil grown under these RB and RGB Blades and found the profiles to also be comparable. It was concluded that the spectra of both the RB and RGB Blades were equally suitable options for basil production. Were these Blades to be deployed in a large-scale production environment, the RGB Blades created a more natural-looking light environment that would be more comfortable for workers, and likely would allow for easier and more accurate visual evaluation of the plants throughout development.
3 Influence of light quality on strawberry vegetative morphology and fruit flavour

3.1 Introduction

Strawberries are a high-value fruit crop in Ontario, with an annual farmgate value of over $34 million as of 2017 (OMAFRA, 2016). In Ontario, approximately 2000 acres of land are dedicated to strawberry production (OMAFRA, 2016); by contrast, strawberry production in the Northern Canadian territories is limited to only three reporting producers, with less than an acre of production area each (Government of Canada, 2017). The majority of fresh strawberries available for purchase in Northern Canada consequently have to be shipped from southern provinces or the US and Mexico. The combined challenges of growing or shipping fresh produce to Northern regions result in far higher produce prices in these locations than compared to national averages (Nunavut Bureau of Statistics, 2017). Controlled environment strawberry production holds promise as a partial solution to the production challenges of the North.

Light quality is an important consideration in the design of a controlled environment strawberry production system; it can have a significant impact on the vegetative morphology of strawberry plants and could potentially significantly alter flavour profiles of the berries.

The morphological impact of light quality on vegetative plant development is well established (Section 1.1.1). Petiole length in particular has been demonstrated in
several species to be highly sensitive to light quality, with demonstrated correlations with blue light and far-red light spectral fractions (Appelgren, 1991; Eskins, 1992; Lee and Amasino, 1995; Stuefer and Huber, 1998; Ohashi-Kaneko et al., 2007). A controlled environment production system would likely be more dependent on manual labour for pollination of flowers and harvest of berries than field or greenhouse grown strawberries that often use bees for pollination (Kakutani et al., 1993; Slaa et al., 2006). To facilitate ease of access to the flowers and berries for workers, strawberry plants with longer petioles, and therefore a less dense leafy canopy that would obscure said tissues, is desirable.

Strawberry flavour perception is influenced by the acidity, sweetness, and the quantity and ratio of specific flavour compounds produced in the berry (Pérez and Sanz, 2001; Azodanlou et al., 2004; Sadler and Murphy, 2010; Schwieterman et al., 2014). The number of flavour compounds identified in strawberry is exhaustive, but a select few have been identified as “character impact compounds” that have the biggest influence on flavour perception (Zabetakis and A Holden, 1997).

Evidence suggests that these flavour characteristics are sensitive to light quality. Kasperbauer et al. (2001) found that growing strawberries over a red mulch that reflected a high ratio of far-red / red light, as opposed to standard black mulch, significantly increased total sugar content by an average 18.2%. This difference in sugar content was described consistently as noticeably sweeter in a blind tasting panel. In the following year, Loughrin and Kasperbauer expanded on their findings and observed increased concentrations of many aromatic volatile compounds when berries had
ripened over the same red mulch compared to the black (Loughrin and Kasperbauer, 2002). Post-harvest light quality additionally appears to affect concentrations and ratios of strawberry volatile organic compounds, with far-red increasing levels of methyl butyrate compared to white or blue light, blue light decreasing concentrations of hexyl butyrate compared to white, red, far-red light, or darkness, and ethyl caproate concentrations varying significantly between white light, blue light, red light, or darkness, while cis-3-hexan-1-ol concentrations were unaffected by light quality (Colquhoun et al., 2013). Further, these apparent photosensitive regulations for each compound do not appear to be conserved between species, with harvested tomato fruits undergoing the same treatments and expressing significantly different trends in volatile expression in response to light quality (Colquhoun et al., 2013).

To evaluate the influence of spectral quality in a controlled environment strawberry production system, this study compared four different production light spectra. The primary objectives of this study were to evaluate strawberry plant vegetative morphology based on petiole length, an indicator of overall canopy density with several production implications, and to evaluate berry quality based on sugar content (˚brix), pH, total acid content (% weight by volume), and volatile profiles.
3.2 Materials and methods

3.2.1 Strawberry production

Strawberry plants (*Fragaria x ananassa* cv. ‘Albion’) were grown from 275 cm³ root stock acquired from Carther Plants (Thamesville, Ontario, Canada) under four distinct light spectra. This root stock volume refers to the cell volume the rootstock was grown in. These included the RB and RGB Blades, defined in Section 2.2 as well as a spectrum produced with the RB and RGB Blades used together, and a far-red (FR) Blade (Intravision Light Systems, Inc., Toronto, Ontario, Canada). Spectral distribution of each Blade spectrum within the grow benches is illustrated in Appendix IV.
Environmental parameters were otherwise kept constant between treatments and are summarized in Table 3.1. The plants were grown in this environment for approximately 100 days, as determined until sufficient berry production was achieved. Root stock was planted in a 50% perlite (#C633, Therm-o-rock, New Eagle, PA, USA), 25% sphagnum peat moss (#3002, Acadian Peat Moss Ltd., Lameque, NB, Canada), and 25% mixed coir (provided by local grower of cut flowers, details not available) blend.
in black 2.86 L pots (BM.0300, Myers Lawn and Garden, Middlefield, OH, USA), modified with the top half cut off so the soil surfaces would be the same height as the hydroponics troughs.

Table 3.1. Strawberry production environmental parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamber</td>
<td>4 m x 3 m x 2.5 m (LxWxH) custom growth chamber (Dixon et al., 1999)</td>
</tr>
<tr>
<td>Temperature</td>
<td>20.0 ± 0.5 °C day, 18.0 ± 0.5 °C night</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>57.5 ± 1.5%</td>
</tr>
<tr>
<td>Vapor pressure deficit</td>
<td>1.08 ± 0.27 kPa</td>
</tr>
<tr>
<td>[CO₂] setpoint</td>
<td>800 ppm</td>
</tr>
<tr>
<td>Rooting substrate</td>
<td>50% perlite (#C633, Therm-o-rock, New Eagle, PA, USA), 25% sphagnum peat moss (#3002, Acadian Peat Moss Ltd., Lameque, NB, Canada), 25% mixed coir (provided by local grower of cut flowers, details not available), by volume</td>
</tr>
<tr>
<td>Fertigation method</td>
<td>Nutrient film technique (NFT)</td>
</tr>
<tr>
<td>Nutrient solution</td>
<td>Plant Prod 6-11-31, as per label. (Plant-Prod 6-11-31, Master Plant-Prod Inc., Brampton, ON, Canada)</td>
</tr>
<tr>
<td>pH</td>
<td>6.0 ± 0.3</td>
</tr>
<tr>
<td>EC</td>
<td>1200 μS – 800 μS</td>
</tr>
<tr>
<td>Lighting</td>
<td></td>
</tr>
<tr>
<td>Light sources</td>
<td>RB, RGB, RB+RGB, and FR spectra produced by Intravision Blades</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>Canopy level PPFD</td>
<td>300 µmol m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>16 h</td>
</tr>
</tbody>
</table>

Runners were pruned from the plants as needed. Flowers were manually pollinated by vibrating on their backsides with an electric toothbrush, or by gently dabbing a soft bristled paint brush onto the reproductive organs of the flower. Berries were harvested with shears on the second day that a berry was fully red in colour. Berries were bagged (Ziploc Sandwich Bags, SC Johnson, Brantford, ON), and stored in a -86 °C freezer (Model 923, Forma Scientific, Inc., Marietta, OH, USA) until analysis.

3.2.2 Vegetative growth analysis

Vegetative development was evaluated on petiole and inflorescence lengths. Petioles and inflorescences were measured after photosynthetic acclimation response testing (see Section 5.2.2.2). Petioles were measured from where they were separated from the crown to their longest points before the leaf blade. Inflorescences were measured from where they were separated from the crown to their longest point at the base of a flower.

3.2.3 Berry flavour analysis

Strawberries were removed from the freezer and thawed at ambient temperature while still in their storage bags. Once thawed, berries were pulverized by hand in their
bags, then squeezed through a small opening in the bag, through two layers of cheese cloth into a 250 mL glass beaker. The pH of this juice was measured using a sympHony B10P benchtop pH meter (VWR, Radnor, PA, USA). Sugar content was measured using a pocket refractometer (Hoskin Scientific, Burlington, ON, Canada).

Total acid content was measured as described by Sadler and Murphy (2010), titrating with 0.095 M NaOH to a pH of 8.1. Total acid content (also referred to as total acidity or titratable acidity) is a better indicator of how a fruit’s acidity will affect flavor than pH, itself (Sadler and Murphy, 2010).

Juice volatile profiles were analyzed via solid-phase micro extraction (SPME) headspace analysis. To prepare samples for analysis, 2.0 mL of juice from each sample was added to 10.0 mL glass vials (#20-1100, Wheaton, Millville, NJ, USA). Sodium chloride salt was added (0.72 g; 36% w/v) to each sample to increase volatility of compounds (Poll and Flink, 1984; Steffen and Pawliszyn, 1996). Vials were sealed with silicone caps (#20-0050MLS, Wheaton, Millville, NJ, USA), and samples were submitted to the Advanced Analytics Centre at the University of Guelph.

3.2.4 SPME-GCMS

The following has been provided by the Advanced Analytics Centre:

“The vial septum was bored and CAR-PDMS fiber was exposed to the headspace. The extraction was performed by placing the vial into an aluminum heating block (4 cm in height by 14 cm in diameter) on a temperature-controlled heating plate
(45°C) with 600 rpm agitation for 45 min. Following extraction and pre-concentration, the fiber was then inserted directly into the GC injector for desorption at 250 °C over 5 min.

GC-MS system (Scion 436GC-TQ, Bruker Ltd. Milton, Canada) included a Scion 436 GC interfaced with a Bruker triple quadrupole MS detector configured in EI mode was used for volatile compound analysis. Chromatography was performed using Bruker BR-SWax column (30 m x 0.25 mm x 0.5 µm). The oven temperature was held at 60°C for 5 min and raised the temperature to 240°C with 3°C/min and then hold for 10 min. The total GC run was 75 min.

The MS was operated in a scan mode form 35m/z to 450m/z at 5scan/sec scanning range. The MS source temperature was set on 220 °C, the transfer line temperature 280 °C and the MS quadrupole temperature at 40 °C. The data was inquired in electron impact (EI) positive ionization mode using 70eV energy at and analysed on Bruker MS Workstation (version 8) data analysing software.”

3.2.5 Experimental design and statistical analysis

The experiment was arranged as a randomized complete block design with four treatments, replicated over time. For berry juice analysis, a single experimental unit comprised of the combined juice collected from multiple berries in a given treatment, resulting in four experimental units per replicate. For the petiole length analysis, a single experimental unit comprised the average petiole length of all petioles measured from four representative plants in a given treatment. The experiment was replicated three
times. Sugar, pH, total acid content, and extraction profiles were compared between the four lighting treatments using least-squares regression and Tukey’s multiple comparison analyses using SAS JMP 13.2.0 (SAS, Cary, North Carolina). “Block” was considered a random effect; “Position” and “Light quality” were considered fixed effects in all analyses.

3.3 Results and discussion

Vegetative morphology properties, including petiole and inflorescence lengths, are illustrated in Figure 3.2.

**Figure 3.2.** Strawberry petiole lengths when grown under four different light spectra. Petioles were measured from where they were separated from the crown to their longest points before a leaf. Within each replicate, all of the petioles for five plants were removed and measured. The average petiole length
was calculated from those measurements. That average was calculated for each treatment, and for each replicate. Those values were then averaged and standard error of the mean was calculated and plotted in this figure. Vertical bars indicate standard error. Disconnected horizontal bars indicate significant differences between treatments using Tukey’s multiple comparisons, $\alpha = 0.05$. $n = 3$.

The plants grown under the FR Blades had significantly elongated petioles compared to RB+RGB-grown plants. While not significant at $\alpha = 0.05$, canopies of the RB and RGB-grown plants anecdotally were perceived to more compact, with greater leaf area in a given volume than FR-grown plants, and additional replication may detect this morphological difference.

The less dense FR canopy was easier to visually evaluate on a day-to-day basis; newly expanded flowers were much easier to identify and hand-pollinate, and ripe strawberries were easier to identify for harvest. A consequence of these elongated petioles is that they were more prone to lodging with loss of turgor than more compact tissues developed in the other light treatments.
Attributes pertaining to berry flavour, including pH, sugar content, and total acid content, are summarized in Figure 3.3. None of these qualities differed significantly between light treatments.

![Figure 3.3](image)

**Figure 3.3.** Total acid content, sugar, and pH of extracted strawberry juice from batched berries in a given treatment and replicate, after producing fruit in four different light spectra. Vertical bars indicate standard error. No significant differences were found when comparing means using Tukey’s multiple comparisons, $\alpha = 0.05$, $n = 3$.

Primary volatile flavour profiles are summarized in Figure 3.4.
Figure 3.4. Relative percentages of primary volatile compounds in strawberry juice, when strawberry plants were grown in four different light spectra. Data from three replicates are presented for each compound, noting that in most cases, a given compound was not detected in all three replicates. The four light spectra (FR, red dots; RB, blue dots; RB+RGB, yellow dots; and RGB, green dots) are indicated on the x-axis, and have additionally been color-coded for clarity. Squares surround data indicate the third replicate. Significant block effects were detected in 2-pentanone and methyl butyrate.

Profiles of volatiles were highly irregular between treatments and replications. Given the irregularity of results, conclusions cannot be made as to the effect of spectral quality on these profiles. A significant block effect was detected in 2-pentanone and
methyl butyrate, with higher concentrations of these compounds in the third replicate than in replicates one and two. The first two replicates of this sample set were submitted to the Advanced Analysis Centre simultaneously, while the third replicate sample set was submitted months later, after an additional crop cycle. There was not an experimental reason for submitting samples in this manner; samples from replicates one and two were being stored in the freezer, and it seemed pertinent to begin this component of the analysis while the third crop cycle was underway. The observed block may be explained by undetected changes to the analytical equipment at the Advanced Analysis Centre over time. It is also possible that profiles of berry volatiles changed with extended storage at -86 °C; reports on the effect of freezing on berry volatile profiles are conflicting, and few if any studies are freezing their samples at such a low temperature (de Ancos et al., 2000; Ouellet and Pedneault, 2016). The rationale for doing so in this study was that volatility of the entire aromatic profile would be extremely low at such temperatures, and losses would be minimal. Were this experiment to be repeated in the future, samples from all replicates would be analyzed simultaneously to eliminate possible variability of the analytical hardware over time. If such an experiment were being replicated over time, however, then there would still be the requirement of storing samples until all required crop cycles were complete. Ideally, this experiment would be repeated with simultaneous replication, eliminating the potentially confounding effects of sample storage or changes to analytical equipment.
3.4 Conclusions

This experiment has revealed that light spectral quality can have a meaningful impact on strawberry production in a controlled environment. The morphological differences induced by varying light quality regimes have a profound effect on day-to-day plant husbandry, while leaving the primary flavour constituents of the berries unchanged. Controlled environment strawberry production, compared to field or greenhouse production, is more likely to rely upon manual labour (as opposed to using bees) for pollination, so plants with more accessible flowers are of value. Plants grown under the FR Blades produced longer petioles, making them easier to manage given the overall less dense canopy. Which spectrum to deploy for optimal production would largely depend on the rest of the growth system. If an irrigation system is in place that ensures all plants will constantly be receiving appropriate irrigation at all times, and there is no reason to think the plants might undergo any major mechanical stress, the FR spectrum would be optimal. This spectrum would produce a less dense canopy that would allow easier flower and berry management, while not significantly modifying berry sweetness or acidity compared to other tested spectra. If a grower was struggling to manage pests that thrive in dense canopies with poor air infiltration, a less dense canopy resulting from a FR-rich spectra could conceivably reduce the pest pressure.

Regarding the RB, RGB and RB+RGB spectra, these produced plants that offered no distinct advantage; the plants were not of poor quality but were morphologically unremarkable. A grower could successfully produce strawberries with any of these
spectra, but given proper management, the FR spectrum is ultimately recommended as the optimal spectra for controlled environment strawberry production of the four spectra tested.
4 Influence of two sub-canopy lighting systems in cannabis on bud quality and yield

4.1 Introduction

The production and consumption of drug-type cannabis (Cannabis sativa L.) has seen increased acceptance and legalization in North America in recent years (ArcView Market Research, 2017). “Drug-type” cannabis, as opposed to “hemp” or “fiber-type”, is characterized by high concentrations of $\Delta^9$-tetrahydrocannabinol-9-carboxylic acid ($\Delta^9$-THCA) and relatively low concentrations of cannabidiolic acid (CBDA) (Vollner et al., 1986; van Bakel et al., 2011). “Drug-type” cannabis will henceforth be referred to in this study more simply as cannabis. Like any other cash crop, producers seek to maximize yield, while optimizing or otherwise standardizing quality.

Floral bud tissue is of primary interest when attempting to maximize cannabis yield, as it is the tissue that produces (in the greatest concentration) the compounds of both medicinal and recreational interest. There are relatively few peer reviewed studies on optimizing environmental parameters for bud yield, and commercial cannabis producers are typically guarded with respect to their production strategies. Nonetheless, one could assume that producers are taking advantage of typical plant responses to the production environment when trying to achieve high yields: more light and more CO$_2$ generally yield more dry matter. The specifics of optimal light qualities and CO$_2$ concentrations are known to vary with species, cultivars, and production strategies.
(Critten, 1991; Nemali and Iersel, 2004; Fu et al., 2012; Ilić et al., 2012; Blom et al., 2016; Li et al., 2017). Given the paucity of scientifically peer-reviewed cannabis production data, it is likely that producers have not yet determined the optimal light (quality and quantity) and CO₂ inputs for their specific cultivars and production methods (e.g., indoor), but are supplying reasonable levels based on historical illegal production information or for what would be optimal in similar species.

Optimizing and standardizing bud quality is considerably more challenging than just increasing yields in cannabis. This is particularly challenging because it is not yet established what “optimal” bud quality is, medicinally. Further, the definition of “optimal” may vary according to the nature of the medical disorder being treated. Clinical studies have yet to determine which specific compound or combination of compounds provide the purported medicinal benefits to users, or the quantities and ratios of these compounds that are optimal in treating various ailments. The currently held theory is that two groups of metabolites together may have medicinal applications: cannabinoids, a class of compounds reserved to only a few plant species, and certain terpenes, common to many plant species (Potter, 2014). There is some evidence to suggest that different compounds in these families can act together in an “entourage effect”, medicinally of greater benefit than the compounds alone (Russo, 2011). Given the novelty of legal commercial cannabis production, relatively few developments have been made through breeding, genetic modifications or production strategies aimed at producing consistent cannabinoid and terpene profiles. Without access to consistent
metabolite profiles, clinical studies have been unable to thoroughly assess the medical applications of cannabis on a broad scale (Dr. Dedi Meiri, personal communication).

The majority of commercial cannabis production occurs in greenhouses or growth chambers with supplemental or sole source electric lighting respectively (Knight et al., 2010; Vanhove et al., 2011; Potter and Duncombe, 2012; Vanhove et al., 2012). Many horticultural lighting companies looking to capitalize on the cannabis boom are now offering lighting systems that claim to optimize cannabis production. Some companies offer data supporting their claims, though these data are rarely replicated, reviewed, or published in a peer-reviewed journal. While the influence of spectral quality on plant development is well documented in the scientific literature (Goins and Yorio, 2000; Loughrin and Kasperbauer, 2001; Yorio et al., 2001; Lefsrud et al., 2008; Beaman et al., 2009; Chang et al., 2009), none yet, to our knowledge, have demonstrated the influence of spectral quality on cannabinoid and/or terpene profiles in cannabis.

To directly investigate the impacts of lighting on cannabis bud yield and quality, supplemental LED Blades of two different spectra were deployed below the cannabis canopy in a commercial production environment. Supplemental sub-canopy lighting (SCL), as opposed to overhead lighting, was used in this case because it required minimal modifications of infrastructure in the production room, did not add any bulky hardware around plants that would make general plant husbandry cumbersome, and has been proven in the past to be a viable strategy for manipulating plant development (Stasiak et al., 1998; Jiang et al., 2017). The objectives of this study were to evaluate bud yield, cannabinoid and terpene contents when plants were grown with no SCL
(control), Red-Blue SCL, or RGB SCL. Two crop cycles are presented; the results of the first crop cycle had variability in metabolome that informed changes to data collection and analysis for the second crop cycle.

4.2 Materials and methods

Most materials and methods were consistent between crop cycles. There were some changes to data collection and analysis in the second crop cycle that are detailed at the end of this section. All experiments were conducted at ABcann Medicinals production site (Napanee, Ontario, Canada).

4.2.1 Supplemental lighting

Plants were exposed to three different supplemental SCL spectra during their reproductive (bloom) stage of development. The RB and RGB spectra are illustrated in Figure 2.1, while the Control treatment had no supplemental SCL. An acknowledged limitation of this experiment is that the Control treated plants were exposed to a reduced total photosynthetic photon flux density (PPFD) compared to the other two treatments. The study has been done in this way to accommodate two somewhat competing objectives: measuring the value of added sub-canopy light, and of measuring changes in metabolome as a product of spectral quality.

4.2.2 Plant preparation: propagation and vegetative growth

Two hundred Cannabis sativa (cv. ‘Wappa’) plants were clonally propagated via cuttings taken from mother plants maintained under ‘mother room’ environmental
conditions detailed in Table 4.1. During propagation of the first crop cycle, cut stems were dipped in EZ Gro Root Gel 0.20% IBA (EZ Gro, Kingston, ON). During propagation of the second crop cycle, cut stems were dipped in EZ Gro Root Liquid 1.00% IBA and 0.50% NAA. Cuttings were established in PRO-MIX RG600 soil (Premier Tech, Rivière-du-Loup, QC) and Jiffy 7 Peat Pellets (Jiffy Products of America Inc., Lorain, OH) in the first and second crop cycles, respectively. Mother plants were originally established from seed obtained from Paradise Seeds (Amsterdam, Netherlands).
Table 4.1. Controlled Environment Chamber schedules for the various production phases of cannabis

<table>
<thead>
<tr>
<th>Controlled environment chamber/stage of production</th>
<th>Mother room</th>
<th>Propagation</th>
<th>Vegetative</th>
<th>Bloom</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature (°C)</strong></td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td><strong>Humidity (%)</strong></td>
<td>56</td>
<td>Days 1-7: 100</td>
<td>Days 1 – 5: 80</td>
<td>Days 1 – 21: 65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Days 8-10: 90</td>
<td>Days 6 – 16: 75</td>
<td>Days 22 – 31: 63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Days 14-18: 80</td>
<td></td>
<td>Days 45 – 56: 55</td>
</tr>
<tr>
<td><strong>Carbon dioxide (ppm)</strong></td>
<td>800</td>
<td>800</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td><strong>PPFD (µmol m⁻² s⁻¹)</strong></td>
<td>400</td>
<td>Days 1 -9: 50</td>
<td>Days 1 – 3: 100</td>
<td>Days 1 – 7: 400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Days 10 -13: 80</td>
<td>Days 4 – 5: 200</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Days 14-18: 100</td>
<td>Days 6 – 10: 300</td>
<td>Days 8 – 56: 500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Days 11 – 20: 400</td>
<td></td>
</tr>
<tr>
<td><strong>Photoperiod (hr/24hr)</strong></td>
<td>12</td>
<td>18</td>
<td>18</td>
<td>12</td>
</tr>
</tbody>
</table>

The cuttings were placed in a Conviron ATC60 Multi-Tier Arabidopsis Growth Chamber (Conviron, Winnipeg, Manitoba), henceforth described as the “propagation
chamber”, for 18-days, during which time the environmental conditions were adjusted according to the schedule outlined in Table 4.1. After establishment, the rooted cuttings were transplanted into 10.2 cm (4.0”) square pots (JKV vcc10us, JVK, St. Catharines, ON) and transferred to a vegetative plant production room for 20 days during which time the environmental conditions were adjusted according to the schedule outlined in Table 4.1. After completing the vegetative production phase, 140 of the original 200 plants were selected for homogeneity and transplanted to 11.4 L (#3 nursery pot) pots (NS C1200, Nursery Supplies, Ancaster, ON) containing a custom organic growth substrate (Premier Tech Promix TD Custom Blend Organic HP-CC MYC BIO Perlite C028733RG586; Premier Tech, Rivière-du-Loup, Quebec). The pots were then transferred to ‘Bloom Room 2’ for 56 days during which time the environmental conditions were adjusted according to the schedule outlined in Table 4.1. Plants were fertigated as needed via drippers with EZ-Gro Organic Grow 4-3-2 during vegetation, and a solution of EZ-Gro Organic Bloom 4-3-2, Organa Add 2-0-0, EZ-Gro Calmag 0-0-0, and Ez-Gro Enzymatic Complex 0-0-0 during bloom (EZ-Gro, Kingston, Ontario).

4.2.3 Layout and production with sub-canopy lighting

In the bloom room, the 140 plants were evenly divided between four benches and arranged in a 5 x 7 grid on each bench. The first, third, and fifth rows of plants on each bench were exposed to one of the three SCL treatments, which were randomized within each replicate bench. The rows with SCL had two upward-facing 244 cm long LED lamps of the Red-Blue or RGB spectra irradiating the plant canopy from below. The lamps were positioned 15 cm to either side of the plant stem and raised 2 cm off
the soil surface (Figure 4.2). Rows two and four acted as “barrier” rows, blocking light contamination between treatments. For most of the time that plants were exposed to SCL treatments (days 8 – 56; Table 4.1), overhead lighting had a PPFD setpoint of 500 µmol m\(^{-2}\) s\(^{-1}\) at the top of the canopy, and the treatments provided 95 ± 5 µmol m\(^{-2}\) s\(^{-1}\) at the bottom of the canopy, measured 20 cm from the SCL. Overhead lighting was supplied using Philips 315-watt Green Power Master Elite Agro ceramic metal halide lamps (Philips Lighting Canada Ltd., Markham, ON., Canada).

![Figure 4.1. Light spectrum of Philips 315-watt Green Power Master Elite Agro ceramic metal halide lamps (www.lighting.philips.com).](image)

All light measurements were taken using an Ocean Optics USB2000+ Spectroradiometer (Ocean Optics, Largo, Florida). Some light leakage between treatments was unavoidable as the barrier row plants did not offer complete light exclusion and installation of solid light barriers would have confounded the environmental conditions by obstructing air flow. The amount of light contamination
between rows was less than 5 μmol m\(^{-2}\) s\(^{-1}\) at the bottom of the canopy, which was less than 1% of the total light to which the treatment rows were exposed, and approximately 5% the PPFD of the treatment itself. Given the operational restrictions and objectives of the study, this level of light leakage was considered acceptable.

**Figure 4.2.** Left: side view of LED lamp spacing on a bench of plants. Red-Blue, RGB, and Control sub-canopy lighting treatments are illustrated respectively by the pink, green, and black rectangles. Right: Top view. Treatment rows are indicated by the coloured circles. Circles marked with an ‘X’ were discarded.

During production in the bloom room in the first crop cycle, plants were gyped as needed. In this case, plants were gyped up to 20.0 cm from soil level, effectively
eliminating any leaves the hung down very close to, or touching the sub-canopy lights. In the second crop cycle, no Gyping was done.

4.2.4 Harvest and analysis

After 56 days, the middle five plants of each treatment row were harvested. During the first production run, each treatment row was batched. In the second production run, the bud tissue was separated into upper (upper two thirds of shoot) and lower (lower third) canopy buds. Plants were harvested by cutting the shoot at soil level. Plant height was measured with a metre stick and the intact shoots were photographed using a Samsung Galaxy S7 smart phone (Samsung Canada, Mississauga, Ontario). The leaves, stems, and buds were separated and fresh weights of each were collected using a Rice Lake 480plus-2A scale (Rice Lake Weighing Systems, Rice Lake, WI). Stems and leaves were completely dried over six weeks in an 18 °C drying room in brown paper bags (#20, Kraft, Montreal, QC). Bud was spread on mesh drying racks and dehydrated over four weeks in an 18 °C drying room to a moisture content of 12.0 ± 2.0 % according to ABcann standard operating procedures. Five grams of dehydrated bud tissue from each experimental unit (batched sample for production run one), or upper and lower canopy for production run two, were sent to RPC Science and Engineering (Fredericton, New Brunswick) for cannabinoid and terpene analysis.

According to the Canadian Access to Cannabis for Medical Purposes Regulations (ACMPR), packaged cannabis to be sold to consumers must indicate the
percent $\Delta^9$-THC, total $\Delta^9$-THC (also described as “potential potency”), CBD, and total CBD on the package label (Government of Canada, 2016).

Total $\Delta^9$-THC provides a clearer representation of THC potency for a given sample and is calculated as:

$$\text{[Total } \Delta^9\text{-THC]} = [\Delta^9\text{-THCA}] \times (0.877) + [\Delta^9\text{-THC}]$$

compensating for the loss of molecular weight as $\Delta^9$-THCA would be decarboxylated to the psychoactive $\Delta^9$-THC. The rational is the same for “Total CBD” and “Total CBG”.

4.2.5 Experimental design and statistical analysis

This study comprised two experiments, divided over two crop cycles. Both were as randomized complete block designs with three treatments. The first experiment was replicated four times simultaneously. The second experiment was replicated three times simultaneously. For analysis of bud yield in both experiments, all of the bud from all of the plants in a single treatment and block was considered a single experimental unit. For analysis of metabolites in the first crop cycle, a random sample was taken from all of the bud from all of the plants in a single treatment and block, and was considered a single experimental unit. For analysis of metabolites in the second crop cycle, experimental units were similar to the first crop cycle, but were taken separately for bud from the upper and lower canopies, resulting in twice as many experimental units. Means comparing sub-canopy lighting treatments were compared using least-squares regression and Tukey’s multiple comparison analyses using SAS JMP 13.2.0 (SAS,
“Crop Cycle” and “Block” were treated as random effects in yield analysis. “Block” was treated as a random effect in analysis of cannabinoid and terpene concentrations and in analysis of canopy position. Means comparing canopy positions were compared using Student’s t test.

4.3 Results and discussion

4.3.1 Yield

Red-Blue and RGB SCL treatments significantly increased dry bud yield in the second crop cycle, while only RGB SCL significantly increased yield in the first crop cycle (Figure 4.3). This was expected due an overall greater amount of light being delivered to the plants in these treatments relative to the Control treatment (Peat, 1970; Stasiak et al., 1998; Ralph and Gademann, 2005). Further, the additional light energy was being delivered to leaves that would have otherwise been shaded by upper canopy leaves. Regardless, it is notable that Red-Blue and RGB SCL treatments increased yield by 19.8 and 24.5% respectively relative to the control in the second crop cycle, which is a disproportional yield enhancement with the RGB treatment given that the SCL only contributed an additional 19% greater PPFD measured at mid-canopy than the control treatment. By contrast, in the first crop cycle the RGB SCL treatment increased yield by only 18.9% relative to the Control treatment. Below the point of light saturation and limitations of water, CO₂, or nutrition, increasing the intensity of light generally increases photosynthesis, and ultimately yield, proportionally (Stasiak et al., 1998). The disproportionate increase in yield via SCL may be explained by improved
light distribution and penetration into the lower canopy than what would be available by simply increasing the overhead PPFD. The difference in yield enhancement between crop cycles one and two may be explained by the second crop cycle having more vegetative tissue in the lower canopy; in the first crop cycle, leaves and branches were pruned from the bottom 20 cm of stem, which is a common practice (gyping) in cannabis production. Gyping was not performed in the second crop cycle. Future studies with lights positioned above and to the sides of the plant canopy are planned to address this further.

Figure 4.3. Total dry bud yields of five batched plants per treatment per crop cycle when grown with no sub-canopy light (control), Red-Blue, or RGB sub-canopy light. Vertical bars indicate standard error.
Horizontal disconnected bars indicate significant differences between treatments using Tukey’s multiple comparisons test, $\alpha = 0.05$, $n = 4$ (crop cycle 1) and $n = 3$ (crop cycle 2).

While the degree of yield *enhancement* with SCL vs the control was greater in the second crop cycle, yield *overall* was less than in the first crop cycle. This was likely a product of the numerous changes to environmental parameters between the two crop cycles: rooting substrate, plant density, irrigation scheduling, and pruning of lower canopy branches (or not) are some examples of differing parameters that likely explain the discrepancy in crop cycle yield. These parameters were accounted for and were considered a random “crop cycle” factor during statistical analysis.

In both crop cycles, both Red-Blue and RGB SCL treatments significantly increased the ratio of bud to non-bud tissue (*Figure 4.4*). This is desirable for production; a canopy less dense with leafy tissue will have better air circulation, and bud will be more accessible for monitoring.
Figure 4.4. Ratio of bud to non-bud (stem and leaf) tissue. Plants were grown with no sub-canopy light (Control), Red-Blue, or RGB sub-canopy light. Vertical bars indicate standard error. Horizontal disconnected bars indicate significant differences between treatments using Tukey’s multiple comparisons test, $\alpha = 0.05$, $n = 4$ (crop cycle 1) and $n = 3$ (crop cycle 2).

4.3.2 Secondary metabolism

Sub-canopy lighting had a local stimulatory effect on $\Delta^9$-THC and select terpenes in bud tissue harvested from the lower canopy (Figure 4.7 and Figure 4.8). In the first crop cycle, no significant differences were found in measured cannabinoid and terpene concentrations from the pooled bud tissue samples (Figure 4.5 and Figure 4.6). Cannabinoid and terpene concentration variability was considerably higher amongst the RB and RGB SCL treatments than the Control SCL treatment (Figure 4.7 and Figure
It was theorized that the observed variability was the result of vertical stratification of secondary metabolite production due to the presence of the SCL. It has been established in other crops that light quality affects secondary metabolism (Lydon et al., 1987; Loughrin and Kasperbauer, 2001; Chang et al., 2009; Chang, 2015) although evidence for such localized effects (within canopy stratification) is not as widely available. To identify potential variability in cannabinoid and terpene concentrations due to canopy position, bud samples from the upper and lower canopy were analyzed separately in the second crop cycle.
Figure 4.5. Cannabinoids. Crop cycle 1. Cannabinoid concentrations when grown with no sub-canopy lighting (triangles), Red-Blue sub-canopy lighting (squares), or RGB sub-canopy lighting (circles). Data produced from 5.0 g of dehydrated bud (12% ± 2% moisture) sampled randomly from each experimental unit. Vertical bars indicate standard error. Horizontal disconnected bars indicate significant differences between treatments using Tukey’s multiple comparisons test, $\alpha = 0.05$, $n = 4$. 
Figure 4.6. Terpenes. Crop cycle 1. Terpene concentrations when grown with no sub-canopy lighting (triangles), Red-Blue sub-canopy lighting (squares), or RGB sub-canopy lighting (circles). Data produced from 5.0 g of dehydrated bud (12% ± 2% moisture) sampled randomly from each experimental unit. Vertical bars indicate standard error. Horizontal disconnected bars indicate significant differences between treatments using Tukey’s multiple comparisons test, $\alpha = 0.05$, $n = 4$.

Cannabinoid concentrations from the lower canopy of crop cycle two are presented in Figure 4.7. Lower canopy concentrations of $\Delta^9$-THC, total $\Delta^9$-THC, and
their biosynthetic precursor $\Delta^9$-THCA were significantly increased under RGB and Red-Blue SCL treatments compared to the control. Concentrations of CBDA, total CBD, CBG, total CBG, and CBGA were not significantly different between treatments.

**Figure 4.7.** Cannabinoids. Crop cycle 2, lower canopy. Cannabinoid concentrations when grown with no sub-canopy lighting (triangles), Red-Blue sub-canopy lighting (squares), or RGB sub-canopy lighting (circles). Data produced from 5.0 g of dehydrated bud (12% ± 2% moisture) sampled from the lower third of shoots from each experimental unit. Letters indicate significant differences between treatments using Tukey’s multiple comparisons test, $\alpha = 0.05$, n = 3.
In the lower canopy, RGB SCL significantly increased concentrations of alpha-pinene and borneol, and both Red-Blue and RGB SCL significantly increased concentrations of cis-nerolidol compared to Control SCL (Figure 4.8). The other measured terpenes did not differ at $\alpha = 0.05$, though the general patterns suggest similar overall tendencies which may be borne out in further studies with tighter between chamber error control and greater replication.

Figure 4.8. Terpenes. Crop cycle 2, lower canopy. Terpene concentrations when grown with no sub-canopy lighting (triangles), Red-Blue sub-canopy lighting (squares), or RGB sub-canopy lighting (circles).
Data produced from 5.0 g of dehydrated bud (12% ± 2% moisture) sampled from the lower third of shoots from each experimental unit. Vertical bars indicate standard error. Horizontal disconnected bars indicate significant differences between treatments using Tukey’s multiple comparisons test, $\alpha = 0.05$, $n = 3$.

In the upper canopy of crop cycle 2, there were no significant differences in cannabinoid concentrations between treatments (Figure 4.9); however, there were detectable differences in terpene profiles (Figure 4.10). Alpha-pinene, limonene, myrcene, and linalool were present at significantly higher concentrations in the RGB SCL treatment than in the Control treatment, while cis-nerolidol concentration was significantly higher in both Red-Blue and RGB SCL than in the Control (Figure 4.10).
Figure 4.9. Cannabinoids. Crop cycle 2, upper canopy. Cannabinoid concentrations when grown with no sub-canopy lighting (triangles), Red-Blue sub-canopy lighting (squares), or RGB sub-canopy lighting (circles). Data produced from 5.0 g of dehydrated bud (12% ± 2% moisture) sampled from the upper two thirds of shoots from each experimental unit. Vertical bars indicate standard error. Horizontal disconnected bars indicate significant differences between treatments using Tukey’s multiple comparisons test, $\alpha = 0.05 \ n = 3$. 
Comparing cannabinoid and terpene concentrations in the upper and lower canopy of crop cycle two, the control and RGB SCL treatments had significantly more CBGA and total CBG in the upper canopy than the lower canopy (Figure 4.11). The
control SCL upper canopy had significantly more trans-ocimene in the upper canopy than the lower canopy (Figure 4.12). The Red-Blue SCL yielded the most consistent cannabinoid and terpene concentrations throughout the upper and lower canopy. (Figure 4.11 and Figure 4.12).

**Figure 4.11.** Cannabinoid concentrations in the upper and lower canopy of plants grown with Control, Red-Blue, and RGB sub-canopy lighting. Filled diamonds indicate lower canopy; empty diamonds indicate upper canopy. Vertical bars indicate standard error. Shaded cells indicate a significant difference between canopy positions using Student’s t test, \( \alpha = 0.05 \), \( n = 3 \).
Figure 4.12. Terpene concentrations in the upper and lower canopy of plants grown with Control, Red-Blue, and RGB sub-canopy lighting. Filled diamonds indicate lower canopy; empty diamonds indicate upper canopy. Vertical bars indicate standard error. Shaded cells indicate a significant difference between canopy positions using Student’s t test, $\alpha = 0.05$, $n = 3$.

### 4.3.3 Cannabinoid and terpene biosynthesis

Careful consideration of the biosynthetic pathways for cannabinoid and terpene biosynthesis offers a possible explanation for the differences observed between SCL treatments. As described in Section 1.1.3.3, terpene and cannabinoid biosynthesis share the common precursors dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) (Figure 4.13).
Figure 4.13. Simplified overview of terpene and cannabinoid biosynthesis. Dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) (shaded blue) are the common precursors to light-stress mitigating terpenes and xanthophylls (shaded green) and to cannabinoids (shaded yellow). Condensation of geranyl pyrophosphate (GPP) with olivetolic acid (OA) yields cannabigerolic acid (CBGA). Various synthases cyclize CBGA to subsequent cannabinoids such as Δ9-tetrahydrocannabinol-9-carboxylic acid (THCA) and cannabidiolic acid (CBDA). THCA and CBDA are decarboxylated to Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), respectively.

Perhaps the additional light, particularly a spectrum relatively rich in green light that is normally absorbed by some terpenes, allowed the plants to up-regulate terpene biosynthesis in response to that environmental condition. In so doing, IPP and DMAPP precursors were also up-regulated to supply the demand for these terpenes. A greater pool of IPP and DMAPP would also theoretically be available for the production of GPP to be condensed with OA to produce CBGA, and Δ9-THCA and CBDA in turn. Conversion from CBGA to Δ9-THCA via THCA synthase may happen with a high enough efficiency that the majority of extra CBGA produced was converted to Δ9-THCA,
accounting for the lack of increase in observed CBGA concentrations. By contrast, CBDA synthase may have an extremely low activity in this cannabis variety, so even in the presence of an increased CBGA pool, there was no increase in the amount of CBDA produced.

4.4 Conclusions

Results suggested that supplemental sub-canopy lighting can increase bud yield and modify cannabinoid and terpene profiles. The increase in bud yield was likely a product of greater PPFD compared to production with overhead lighting alone. Red-Blue SCL yielded a more consistent metabolite profile throughout the canopy, and RGB SCL had the greatest impact on up-regulating metabolites. Future studies could expand on this research by deploying light qualities richer in the green region of the spectrum, modifying the overhead light spectrum, and deploying LED arrays within the canopy, rather than below it. It would be of academic value in future studies to quantify IPP and DMAPP pools to better understand the influence of spectral quality on terpene and cannabinoid biosynthesis.
5 Photosynthetic acclimation to light spectral quality

5.1 Introduction

Light quality is known to have a profound influence on nearly all aspects of plant growth and development (Goins and Yorio, 2000; Loughrin and Kasperbauer, 2001; Yorio et al., 2001; Lefsrud et al., 2008; Beaman et al., 2009; Liu et al., 2009). These phytological responses to spectrum can be harnessed to achieve targeted production goals simply by modifying the light environment.

Advanced light emitting diode (LED) lighting systems employing multiple tunable wavebands can provide a staggering number of spectral compositions. Each of these specific spectral ‘recipes’ has the potential to elicit a range of unique plant responses from subtle through to profound (McCree, 1972; Park and Runkle, 2017). The effect of a given spectral waveband may also vary in response to the plants developmental stage (Miller, 1958; Kasperbauer et al., 1963; Kasperbauer, 1987; Skinner and Simmons, 1993). The spectral history of a plant can further complicate our understanding of spectral responses, as previous light exposure responses can influence future light utilization and response patterns (Xu et al., 2011; Wollaeger and Runkle, 2015).

Sequential, short duration (i.e., 30 - 60 min) spectral comparisons provide snapshots of photosynthetic efficiencies and can indicate the most efficient light spectrum for driving photosynthesis at that particular moment. It cannot be assumed that the same spectrum will remain the most efficient over an extended period, or
between plant developmental stages. Further, there is some evidence to suggest that a plant’s light history can influence how the plant might utilize light in the present and future, though there are very few studies exploring this phenomenon in higher plants (Xu et al., 2011). This becomes of real-world consequence when considering spectral optimization for a plant that may be exposed to several different production environments throughout its development, such as in the cases of commercial kalanchoe or cannabis production (Schneider-Moldrickx, 1983). Light history is rarely a consideration when selecting a spectrum for commercial production; the assumption is made that photosynthetic response will be consistent throughout the production cycle.

Another assumption that is often made when designing light spectra, whether it be for research or commercial applications, is that light outside of the Photosynthetically Active Radiation bandwidth (PAR, 400-700 nm) is of little consequence to photosynthetic rates in plants. Within PAR, plants can utilize some wavelengths for photosynthesis better than others, as illustrated by McCree (1972). Based on these ideas, light spectra for plant growth typically emphasize wavelengths best utilized by plants in the range of 400-700 nm.

Emerson and Lewis began exploring the limitations of the upper region of PAR in 1943. They described a so-called “red-drop” effect in the green algae *Chlorella pyrenoidosa*, wherein the quantum yield of photosynthesis dropped off rapidly beyond 685 nm (Figure 5.1) (Emerson and Lewis, 1943).
Figure 5.1 Adapted from Emerson et al., (1957): “Red-drop” effect. Quantum yield of photosynthesis drops off rapidly in *Chlorella* when irradiated with wavelengths beyond 685 nm. Continuous line indicates no supplementary light, with cell culture at 20 °C. Dashed line indicates far-red plus supplementary light in the range of PAR, with cell culture at 20 °C.

Emerson and his team later expanded on this research and found that by supplementing these relatively long red wavelengths (i.e., > 685 nm in their study) with shorter wavelengths within the range of PAR, the red-drop would occur at longer wavelengths than without supplementary shorter-wave light (fig. 1) (Emerson et al., 1957). This enhancement, now known as the Emerson enhancement effect (EEE), was consistently observed, whether he supplemented far-red with the full spectrum provided
by a mercury-cadmium lamp, or used filters to supplement with only the red, green, or blue parts of the spectrum (Emerson et al., 1957). This strongly supported the theory that the light reactions of photosynthesis have two distinct reaction centers with different absorption peaks. It further suggested that the use of complementary light spectra can drive photosynthesis more efficiently by taking advantage of those distinct peaks (Emerson et al., 1957).

The definition of the EEE is sometimes misconstrued by lighting manufacturers, growers, and researchers alike. In such cases, the EEE is interpreted such that supplementing PAR spectra with far-red light disproportionately enhances overall photosynthesis. In fact, very few studies have explicitly demonstrated this, though a recent report by Zhen and van Iersel (2017) has demonstrated increased photosynthetic rates in lettuce when adding 735 nm far-red light to warm white or red-blue light. While we are unable to conclude from their report if the effect of adding far-red light was photosynthetically additive or disproportionately enhancing without knowing photosynthetic rates achieved in far-red alone, the results are very encouraging and warrant further exploration.

This chapter details two experiments designed to quantify the influence of far-red and UV-A light when separately added to monochromatic light qualities within the PAR spectrum on whole-plant photosynthesis. Further, these studies aim to quantify the effects of photosynthetic acclimation to light quality. It was hypothesized that far-red light does not significantly modify whole-plant photosynthetic rates compared to otherwise PAR-based light spectra. If far-red light does modify photosynthetic rates, it
was hypothesized that this will only occur in plants that have been exposed to far-red light throughout their development, and theoretically have acclimated to a broader range of photosynthetically active radiation than traditional PAR. We hypothesize that in such case, any photosynthetic increases achieved with added far-red light will be additive, rather than producing any disproportional enhancement. We expect similar results with UV-A; few if any studies exist that explore a potential “UV-enhancement effect” as has been done with far-red, but with the rationale that the far-red enhancement effect is a product of plants acclimating to a broader range of PAR over time, the rationale should theoretically hold true on the short-wave boundary of PAR as well.

To test these hypotheses, two experiments were completed where plants were grown to vegetative maturity in various PAR-based light spectra with and without added far-red or UV light (the “acclimation phase”), and then exposed to various monochromatic light spectra with and without added far-red or UV light (the “response phase”). Whole-canopy photosynthetic rates in each light quality during the response phase were compared. Experiments one and two were completed using lettuce and strawberry plants, respectively.
5.2 Materials and methods

5.2.1 Phase 1: Acclimation

5.2.1.1 Lettuce

Lettuce (*Lactuca sativa* cv. ‘Grand Rapids’) plants were grown from seed (Stokes Seeds, packaged September 2016, Thorold, Ontario, CA) in nine identical controlled environment growth chambers (*Appendix II*) under three different acclimation light spectra (AS). Light treatments and replications were arranged as illustrated in Figure 5.2. All other environmental parameters were consistent among growth chambers and are summarized in Table 5.1. Environmental parameters were automatically controlled with an Argus Titan control system (Argus, Surrey, BC). The growth and acclimation phase lasted 35 days.

![Figure 5.2](image)

*Figure 5.2.* Arrangement of light treatments and replications in growth chambers in lettuce acclimation experiment, as viewed from above. R1, R2, and R3 indicate replicates one, two, and three, respectively. $A_{\text{PAR}}$, $A_{\text{FR}}$, and $A_{\text{UV}}$ indicate PAR, PAR+FR, and PAR+UV acclimation treatments, respectively.
Table 5.1. Plant chamber growth parameters. Lettuce plants were grown under these environmental conditions for 35 days in their “acclimation” phase, with varied light qualities between treatments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamber height</td>
<td>1.2 m</td>
</tr>
<tr>
<td>Chamber diameter</td>
<td>0.46 m</td>
</tr>
<tr>
<td>Chamber internal volume</td>
<td>0.20 m³</td>
</tr>
<tr>
<td>Temperature</td>
<td>22.0 ± 0.5 ºC (isothermal)</td>
</tr>
<tr>
<td>Relative humidity setpoint</td>
<td>56%</td>
</tr>
<tr>
<td>Vapor pressure deficit setpoint</td>
<td>1.24 kPa</td>
</tr>
<tr>
<td>[CO₂] setpoint</td>
<td>450 ppm</td>
</tr>
<tr>
<td>Rooting substrate</td>
<td>SunGro Sunshine LC1 substrate, as per label (Sungro Horticulture, Agawam, MA, USA)</td>
</tr>
<tr>
<td>Irrigation</td>
<td>Sub-irrigated manually alternating between deionized water and Plant Prod 6-11-31 media, as per label (Plant-Prod 7-11-27, Master Plant-Prod Inc., Brampton, ON, Canada) as needed</td>
</tr>
<tr>
<td>Light sources</td>
<td>Snowflake 2.0 nine-band custom LED arrays (Intravision Canada Ltd., Toronto, ON, Canada)</td>
</tr>
<tr>
<td>Average canopy level PPFD</td>
<td>200 μmol m⁻² s⁻¹</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>16 h</td>
</tr>
</tbody>
</table>
Multiple seeds were sown in each chamber, and all the plants that developed in a given chamber were treated as a single [canopy] experimental unit. The number of plants comprising each canopy ranged between nine and twelve plants per chamber.

The three acclimation spectra shared a common RGB spectrum, with and without additions of far-red and UV-A. These spectra are henceforth referred to as “AS\textsubscript{PAR}”, “AS\textsubscript{PAR+FR}”, and “AS\textsubscript{PAR+UV}”, and are illustrated in Figure 5.3.
Figure 5.3. Lettuce acclimation spectra. Each spectrum had a total PPFD of 200 µmol m\(^{-2}\) s\(^{-1}\) distributed evenly across red, green, and blue wavelengths. Given that the UV and far-red wavelengths indicated are outside the range of PAR (starred in the inset tables), their photon flux densities are above and beyond the total PPFD of 200 µmol m\(^{-2}\) s\(^{-1}\) achieved with red, green, and blue. The percentages for UV and far-
red are indicative of their relative proportions to the PAR colors. * indicates percentage relative to total light within the range of PAR.

These spectra were produced using nine identical custom LED arrays henceforth called the “Snowflake 2.0” arrays. These arrays are the second generation of custom made high intensity, variable spectrum LED arrays deployed in sealed plant growth chambers (Figure 5.4) at the Controlled Environment Systems Research Facility (CESRF). They each have 2200 W total power, are water-cooled, and have eight independently controlled spectral peaks: UV (368 nm), royal blue (448 nm), blue (470 nm), cyan (500 nm), lime (544 nm), red (628 nm), deep red (656 nm), and far-red (724 nm). Spectrum and intensity of each array was programmed through the Argus interface. The spectrum of each color on the array is illustrated in Figure 5.5. For each colour in the array, spectral distribution 100 cm from the light source within the chamber is illustrated in Appendix V.
Figure 5.4. Growth chambers at the Controlled Environment Systems Research Facility, equipped with variable spectrum LED arrays.
Figure 5.5. Normalized spectra of each individual color represented and independently dimmable on the Snowflake 2.0 arrays.

Each acclimation treatment had a total PPFD of 200 µmol m\(^{-2}\) s\(^{-1}\), which did not include any added photon flux from UV or far-red, where relevant. Any photon flux provided by wavelengths that fell outside the normal range of PAR (400 – 700 nm) had
a flux of 67 $\mu$mol m$^{-2}$ s$^{-1}$, and were in addition to the 200 $\mu$mol m$^{-2}$ s$^{-1}$ provided within the PAR spectra.

5.2.1.2 Strawberry

Strawberry plants were grown as described in section 3.2.1.

5.2.2 Phase 2: Response

5.2.2.1 Lettuce

On days 36 and 37 after planting, acclimated lettuce plants were exposed to 27 different light spectra of equal PPFD. Amber, with or without added far-red or UV, is an exception to this; due to hardware limitations, Amber-based spectra had a PPFD of 129 $\mu$mol m$^{-2}$ s$^{-1}$, while each other spectrum had a PPFD of 200 $\mu$mol m$^{-2}$ s$^{-1}$. Each spectrum lasted 0.5 hours, and all were scheduled within the confines of the original 16-hour photoperiod to which the plants had originally been acclimated. These response-phase measurements occurred in the same growth chambers in which the plants were acclimated. Environmental scenarios were shuffled between replications to account for any “day of growth” or “time of day” effects. Spectra scheduling can be found in Appendix IV.

$\text{CO}_2$ concentrations were measured continuously using a LI-COR Li-820 $\text{CO}_2$ gas analyzer (LI-COR, Lincoln, Nebraska), and logged via Argus. Net carbon exchange rates under each light spectrum were determined as $\text{CO}_2$ fixation over time. All Pn
were normalized on a “per leaf area” basis. Leaf areas were measured on day 38 using a LI-COR LI-3100 area meter (LI-COR, Lincoln, Nebraska).

5.2.2.2 Strawberry

Three mature strawberry plants were transferred, one treatment at a time, to a high fidelity sealed controlled environment chamber hereafter referred as the “Phridge.” The Phridge is a precision research tool developed to enable a better understanding of plant physiological responses to the manipulation of multiple environmental variables such as temperature, humidity, CO₂ and oxygen concentrations, light quality and intensity, nutrient availability, plant water status, insect predation, pathogen application / response, chemical application, etc. The Phridge is a modified Conviron A1000 chamber (Controlled Environments Ltd., Winnipeg, Manitoba) (Figure 5.6). Custom modifications include a new door and frame with multipoint closure and hermetic seal, specular aluminium interior cladding, and a Mettler-Toledo 32 kg 0.1 g balance (Mettler-Toledo Inc., Mississauga, ON) for evapotranspiration measurements. Chamber HVAC is custom with chilled and hot water heat exchangers, and variable speed air flow with bottom up distribution. Environmental parameters are automatically controlled with an Argus Titan Control system (Argus, Surrey, BC). Lighting systems are described in detail below. Carbon dioxide concentrations were measured in real time with a LI-COR LI-820 CO₂ gas analyzer (LI-COR, Lincoln, Nebraska), and logged via Argus. A technical summary is provided in Appendix III.
Figure 5.6. "Phridge" growth chamber front (A) and back (B).

Plants were exposed to 45 sequential environmental scenarios over 48 hours that varied in light quality, intensity, and CO₂ concentration. Each scenario lasted 0.5 hours, and all were scheduled within the confines of the original 13-hour photoperiod that the plants had originally acclimated to. Only data from environmental scenarios relevant to this report are presented. Far-red light intensity was kept at a constant photon flux of 100 µmol m⁻² s⁻¹ between all response spectra where far-red light was present. All presented response spectra had a PPFD of 258 ± 4 µmol m⁻² s⁻¹, plus far-red photon flux where relevant.

Photosynthetic rates in each environmental scenario were determined as CO₂ fixation over time. All photosynthetic rates were normalized on a “per leaf area” basis. Leaf areas were measured on day 38 using a LI-COR LI-3100 area meter (LI-COR,
Lincoln, Nebraska). Petiole and inflorescence lengths were measured as described in Section 3.2.2.

5.2.3 Light sources

5.2.3.1 Lettuce

Both the Acclimation and Response phases of the lettuce experiment used the Snowflake 2.0 arrays described in Section 5.2.1.1. There were two noteworthy complications with the arrays in the context of this experiment. First, far-red LEDs emit wavelengths that extend slightly into the range of PAR. This has been measured to be 13% of the total output from the far-red LEDs. In the context of this experiment, this equates to an extra 8.5 µmol m\(^{-2}\) s\(^{-1}\), or an extra 4.3% more PAR light in each acclimation and response spectra where far-red is used. Second, at the PPFD used in this experiment, the UV LEDs caused photo-excitation in the far-red LEDs on the array, resulting in unavoidable emissions from far-red when only sending power to UV. This can be seen in Figure 5.5 and was calculated to be 10% of the total photon flux density. With UV added at a flux density of 66.0 µmol m\(^{-2}\) s\(^{-1}\) for in relevant treatments, 6.6 µmol m\(^{-2}\) s\(^{-1}\) of far-red is inevitably also added.

5.2.3.2 Strawberry

During the Acclimation Phase, plants were grown under the Intravision RB, RGB, RB+RGB, and FR Blades illustrated in Figure 2.1 and Figure 3.1.
In the response phase, plants were irradiated using a custom LED array integrated into the Phridge. The Phridge was equipped with a 2200 W water-cooled variable spectrum LED array (Intravision Canada, Toronto, ON). The array has seven independent spectral peaks: UV (368 nm), UV (380 nm), blue (448 nm), white (5650 K), green (568 nm), red (655 nm), and far red (735 nm). Spectrum and intensity of the array was programmed through the Argus interface. In this experiment, only the 368 nm UV LEDs are used, of the two types of UV LEDs in the arrays. As with the lettuce lighting systems, the far-red LEDs used emit wavelengths that extended slightly into the range of PAR. This was measured to be 13% of the total output from the far-red LEDs. In the context of this experiment, this equated to an extra 8.5 µmol m\(^{-2}\) s\(^{-1}\), or an extra 4.3% more PAR light in each acclimation and response spectra where far-red was used.

### 5.2.4 Calculated Pn values

Photosynthetic rates in far-red light (Pn\(_{\text{FR}}\)) and in UV light (Pn\(_{\text{UV}}\)) alone were added to photosynthetic rates achieved in each base light quality. These new values, termed “+FR calc.” and “+UV calc.” respectively, provided a measure of what photosynthetic rates would be expected if FR and UV increased photosynthesis additively (as opposed to disproportionately) when added to light spectra containing only PAR. When adding FR and UV, total photon flux densities still fell within the linear range of photosynthetic light responses for both species.
5.2.5 Statistical analysis

5.2.5.1 Lettuce

The experiment was arranged as a Latin square design. Means of measured and calculated photosynthetic rates were compared using least-squares regression and Tukey’s multiple comparison analyses using SAS JMP 13.2.0 (SAS, Cary, North Carolina). “Block” and “Position” were treated as fixed effects. Linear regression analysis was performed to determine the likelihood of a relationship between photosynthetic rates and phytochrome photostationary state.

5.2.5.2 Strawberry

Means of measured and calculated photosynthetic rates were compared using least-squares regression and Tukey’s multiple comparison analyses using SAS JMP 13.2.0 (SAS, Cary, North Carolina). “Block” was treated as a random effect in the mixed model. Linear regression analysis was performed to determine the likelihood of a relationship between photosynthetic rates and phytochrome photostationary state.

5.3 Results and discussion

5.3.1.1 Lettuce

Photosynthesis in each spectral quality by all acclimated plants are illustrated in Figure 5.7. For all acclimation treatments, adding far-red or UV to base response spectra did not significantly alter photosynthesis. This is consistent with calculated
values for photosynthesis for each base colour with added UV or FR. The data suggested that neither far-red nor UV made significant direct contributions to photosynthesis in either an additive or enhancing manner when added to light spectra in the range of PAR. Further, the ability of plants to utilize supplemental UV or FR light for photosynthesis did not appear to be dependent on acclimation to these spectra.

**Figure 5.7.** Lettuce whole-plant photosynthesis (Pn) when exposed to various base monochromatic light qualities, with and without added far-red (FR) or UV light. Plants were acclimated to either PAR-spectrum light (AS\textsubscript{PAR}), PAR with added far-red light (AS\textsubscript{PAR+FR}), or PAR with added UV light (AS\textsubscript{PAR+UV}); these acclimation spectra are indicated on the right y-axis. Dotted line indicates phytochrome photostationary
states for each response spectrum. For each base spectrum, Pn was measured in just the base spectrum indicated on the x-axis, base colour plus far-red, and base colour plus UV. For each base colour, two additional data points were calculated that indicate expected Pn if far-red or UV were contributing to Pn in an additive manner with the base spectrum. Vertical bars indicate standard error; disconnected horizontal bars indicate a significant difference at $\alpha = 0.05$. For cells without horizontal bars, there were no significant differences. Means were compared using Tukey’s multiple comparisons and least-squares analysis; $\alpha = 0.05$, $n = 3$.

Plotting Pn against phytochrome photostationary state (PSS) of each response spectrum, significant relationships between Pn and PSS were found amongst $\text{AS}_{\text{PAR}}$ and $\text{AS}_{\text{PAR+UV}}$ plants, but not amongst $\text{AS}_{\text{PAR+FR}}$ plants (Figure 5.8). This analysis suggested that phytochrome may be involved in overall acclimation, though there was no obvious relationship between PSS of the acclimation spectra ($\text{AS}_{\text{PAR}}$ PSS = 0.87; $\text{AS}_{\text{PAR+FR}}$ PSS = 0.72; $\text{AS}_{\text{PAR+UV}}$ PSS = 0.85) and the strength of the post-acclimation photosynthesis-PSS relationship. The relationship between Pn rates and the PSS of the post-acclimation response spectra illustrated in fig. 5 was likely coincidental.
Figure 5.8. Lettuce whole-plant photosynthesis (Pn) as a factor of phytochrome photostationary state (PSS). Plants were acclimated to either PAR-spectrum light (AS\textsubscript{PAR}), PAR with added far-red light (AS\textsubscript{PAR+FR}), or PAR with added UV light (AS\textsubscript{PAR+UV}); these acclimation spectra are indicated on the right y-axis. Shaded areas indicate 95% confidence interval. Phytochrome photostationary states were calculated from each post-acclimation response spectrum. Significant relationships were found between Pn and PSS in plants that had been acclimated to PAR or PAR+UV light as determined by quadratic bivariate analysis; \( \alpha = 0.05, n = 3 \).
Averaging Pn for all response spectra within each base response colour (i.e., average of base colour, base+FR, and base +UV for each colour) found average Pn within all spectra of a base colour to differ significantly between acclimation treatments (Figure 5.9). The AS_{PAR+UV} plants achieved significantly greater Pn than the other acclimation treatments in blue, red, and deep red light. This would suggest that supplemental UV light can indeed increase Pn, albeit through a different mechanism than hypothesized. Given how well our blue, red and deep red LED spectral peaks aligned with absorbance spectra of photosystems I and II (PSI and PSII) (Figure 5.10), it suggested that UV acclimation modified the photosystems in some way. Another possibility was that the more compact morphology of the AS_{PAR+UV} plants somehow better captured light, though both theories are highly speculative.
Figure 5.9. Average photosynthesis (Pn) of all spectra in a given base colour (e.g., Average Pn of Blue, Blue+FR, and Blue+UV) by acclimation spectrum. Circles: $A_{SPAR}$; squares: $A_{SPAR+FR}$; diamonds: $A_{SPAR+UV}$. Means were compared using least-squares analysis; $\alpha = 0.05$, $n = 3$. Vertical bars indicate standard error, disconnected horizontal bars indicate significant differences.
Figure 5.10. Adapted from Laisk et al., (2014). Absorbance spectra of photosystem II (PSII) and light harvesting complex II (LHClII) and photosystem I (PSI) and light harvesting complex I (LHClI) (wide lines, indicated). LED peaks are overlaid: Blue (B, dotted line); Red (R, empty dashed line); Deep Red (DR, long dashed line). PSII+LHClII has much stronger absorbance in the ranges of these LEDs than PSI+LHClI.

There are very few studies exploring the relationship between light harvesting pigment concentrations and UV light. Salama et al. (2011) reported that enhanced UV light of similar wavelength used in this study severely reduced chlorophyll concentrations in four desert plants, though carotenoid concentrations were largely unchanged. Li and Kubota (2009) found β-carotene and xanthophyll concentrations to be unchanged in lettuce when grown under fluorescent white light with and without added UV-A, though it should be noted that there was still a small amount of UV-A present (0.74% of total photon flux) in their unsupplemented white light treatment. Both
of these studies seem to provide evidence against the explanation of increased pigment concentrations after UV acclimation. Another possibility is that UV acclimation allowed for more efficient electron transport in the thylakoid membrane by increasing protein fluidity. Kirchhoff et al. (2011) found that light-adapted thylakoids from spinach leaves had an expanded internal volume compared to dark-adapted thylakoids, allowing for different spatial arrangements of light harvesting complexes and greater diffusion mobility of plastoquinone.

Further experimentation is required to determine the mechanism of increased photosynthesis under blue, red, and deep red light after acclimation to UV light. Future experimentation should quantify chlorophyll and secondary pigment concentrations; although two previous studies suggested that UV light did not increase pigment concentrations, it cannot be discounted as a possible explanation for the results observed in this study.

5.3.1.2 Strawberry

Net carbon exchange rates achieved in each spectral quality by all acclimated plants are illustrated in Figure 5.11. There were no significant differences in Pn between spectral qualities with or without added FR or UV light, consistent with the general findings in lettuce.
Figure 5.11. Strawberry whole-plant photosynthesis (Pn) when exposed to various base monochromatic light qualities, with and without added far-red (FR) or UV light. Plants were acclimated to either Red-Blue (RB), Red-Green-Blue (RGB), RB+RGB, or Far-Red Blade light spectra; these acclimation spectra are indicated on the right y-axis. For each base spectrum, Pn was measured in just the base spectrum indicated on the x-axis, base colour plus far-red, and base colour plus UV. For each base colour, two additional data points were calculated that indicate expected Pn if far-red or UV were contributing to Pn in an additive manner with the base spectrum. Vertical bars indicate standard error; disconnected horizontal bars indicate significant differences at $\alpha = 0.05$. For cells without horizontal bars, no significant
differences were detected. Means were compared using Tukey’s multiple comparisons and least-squares analysis; $\alpha = 0.05$, $n = 3$.

There are significant relationships between Pn and PSS in plants from all acclimation spectra except that of $AS_{RB}$ (Figure 5.12).
Figure 5.12. Strawberry whole-plant photosynthesis (Pn) as a factor of phytochrome photostationary state (PSS). Plants were acclimated to either Red-Blue (RB), Red-Green-Blue (RGB), RB+RGB, or Far-Red Blade light spectra; these acclimation spectra are indicated on the right y-axis. Shaded regions indicate 95% confidence interval. Phytochrome photostationary states were calculated from each post-acclimation response spectrum. Significant relationships were found between Pn and PSS in plants that
had been acclimated to RB, RB+RGB, or FR light as determined by quadratic bivariate analysis; \( \alpha = 0.05, n = 3 \).

Finally, \( P_n \) was significantly higher for the \( \text{AS}_{RB+RGB} \) acclimated plants than for the \( \text{AS}_{RGB} \) plants when averaging all the response spectra for each base response colour (i.e., Average of base colour, base+FR, and base +UV for each colour) (Figure 5.13).

Figure 5.13. Average photosynthesis (\( P_n \)) of all spectra in a given base colour (ex. Average \( P_n \) of Blue, Blue+FR, and Blue+UV) by acclimation spectrum. Vertical bars indicate standard error; disconnected horizontal bars indicate a significant difference at \( \alpha = 0.05, n = 3 \).
The $\text{AS}_{\text{RB+RGB}}$ spectrum is the most balanced acclimation spectrum in the range of PAR; $\text{AS}_{\text{FR}}$ covers a broader spectral range but has relatively little energy in the blue-region of the spectrum. The results suggested that a more balanced, broad PAR spectrum allowed the plants to develop photosynthetic machinery with greater capacity to utilize blue light. Possible modes of action for this effect were difficult to speculate on. Were this effect mainly because of the added blue light in the RB+RGB spectrum compared to the RGB spectrum, one would expect a stronger response from the RB spectrum, with its relatively high blue content compared to the other spectra.

Similar to the results in lettuce, a possible explanation for these results may come down to the morphology of the plants as influenced by their acclimation spectra. The $\text{AS}_{\text{PAR+UV}}$ lettuce plants were very compact, and as described in Figure 3.2, the $\text{AS}_{\text{RB+RGB}}$ strawberry plants were significantly more compact than the $\text{AS}_{\text{FR}}$ plants, while the other treatments were all statistically similar. Taken together, it implied that perhaps more compact vegetation and/or thicker leaves somehow resulted in better photosynthetic efficiency. If this was the case, one might expect that if a more compact plant were to achieve higher photosynthetic rates under a specific colour, that colour would be green, known for transmitting through dense leafy canopies better than other colours (Klein, 1992; Smith, 1994). In fact, the opposite was observed. Further studies evaluating photosynthesis as a factor of canopy density are required to explain this observation.
5.4 Conclusions

In this study, the addition of far-red or UV light to PAR-based spectra after acclimation did not significantly modify photosynthetic rates.

Acclimating lettuce plants to PAR-based light spectra supplemented with 368 nm UV enabled greater photosynthesis in blue, red, and deep red light spectra after acclimation. Variations on this acclimation, such as duration, wavelength, and intensity required to achieve a similar acclimation response, as well as the biological mechanisms underlying this response will be subjects of further study.

In applying these findings to the design of light spectra for plant production in the future, it may be reasonable to conclude that if a spectrum is going to be relatively rich in red and blue wavelengths, adding UV-A light to the spectrum may achieve greater yields through increased photosynthesis. There was no obvious generalization that could be made to explain the results observed in both lettuce and strawberry. Further research on this topic would benefit from exploring the phenomenon of acclimation at the morphological, cellular, and biochemical levels. Specifically, factors of plant compaction, content and distribution of photosystems I and II, and quantification of specific carotenoids would better explain the results found in this study.
6 Summary and conclusion

In this thesis, the influence of light spectral quality on secondary metabolism has been explored, and novel findings regarding plant acclimation to spectral quality have been demonstrated. The basil, strawberry, and cannabis studies all utilized Intravision RB and RGB Blades, as well as the FR Blades in the case of strawberry. Between the spectra emitted by these Blades, relative volatile concentrations in basil were consistent with each other. In strawberry, sugar content, pH, and total acidity, were consistent between light treatments, while volatile profiles varied considerably between treatments and replications. Morphologically, the FR Blades produced strawberry plants with significantly longer petioles, which may be of benefit to growers in terms of easier visual inspection of the plants, easier access to flowers for pollination, and easier access to berries for harvest, as well as improving airflow through the canopy.

In cannabis, both the RB and RGB blades significantly increased yield and bud to non-bud ratios compared to a control treatment without any supplemental lighting. In the lower plant canopy, concentrations of $\Delta^9$-THC, total $\Delta^9$-THC, and $\Delta^9$-THCA were significantly increased using the RGB and RB Blades compared to the control. Concentrations of alpha-pinine and borneol were found to significantly increase in the lower canopy growing with the RGB blades, and cis-nerolidol concentrations significantly increased when growing with either the RB or RGB Blades, compared to the control. The RB Blades produced the most consistent cannabinoid and terpene
profiles throughout the canopy compared to the control or RGB Blade treatments, and
the RGB Blades had the greatest impact on terpene profiles overall.

Acclimating lettuce plants to PAR-based light qualities with and without added far-red or UV-A light, and then exposing them to many different light spectra revealed that spectral acclimation influenced photosynthetic rates in lettuce. Adding far-red or UV-A to PAR based spectra after acclimation did not directly modify photosynthetic rates compared to those achieved with just the base spectra. However, plants that were acclimated to UV-A light achieved significantly higher photosynthetic rates in blue or red-based spectra, compared to the PAR or PAR+FR acclimated plants. Findings in strawberry acclimation were similar but found RB+RGB acclimated plants to achieve significantly higher photosynthetic rates than RGB acclimated plants in blue light, though this enhancement was less profound than the effect of UV acclimation observed in lettuce.

The observed increase in photosynthetic rates in lettuce after acclimating plants to UV-A light is a novel finding with academic and applied implications. This response defies any obvious evolutionary, molecular, or biochemical explanation, and certainly warrants more investigation. Applying this concept to the design of lighting systems in controlled environment production could potentially significantly increase yields and ultimately profits.
The findings in cannabis regarding bud yield and modifications to the secondary metabolite profile are of obvious value. Unfortunately, given that the control treatment in that study differed in both light quality and intensity, it is difficult to make strong conclusions about underlying biological mechanisms that lead to these results. This is certainly only the beginning of research into optimizing light spectra for cannabis production; hopefully future studies on this topic will have greater flexibility in experimental design to obtain clearer conclusions than were found here.

The overarching objective of achieving a “perfect” light spectrum for a given production task has not yet been achieved, but the findings presented in this thesis bring our community of plant physiologists and environmental scientists a little bit closer to this goal.


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APPENDIX I

Controlled Environment Systems Research Facility “Bluebox 2” technical summary.

(http://www.ces.uoguelph.ca/TechNotes/GuelphBlueBox_TechnicalSpecifications_SALSA_rev00.pdf)

Controlled Environment Systems Research Facility
Guelph BlueBox SALSA System

These plant growth chambers were originally designed as two totally sealed and environmentally controlled spaces in which some of the contributions of plants to the function of life support are studied. More recently, the SALSA BlueBoxes have been retrofitted with multispectral, double layer LED systems in a variety of configurations, however their first lighting systems utilized plasma (microwave) lamps to study the effect of inner canopy irradiation on crop production. The walk-in plant growth chambers are capable of a high degree of closure and are dedicated specifically to plant canopy lighting studies and nutrient recycling analysis and control in planned environment agriculture.

Technical Specifications

- Volume = 29 m$^3$ (430 ft$^3$) (4.5 m x 2.8 m x 2.3 m) per chamber.
- Plant Growing Area = 5 m$^2$ (54 ft$^2$) (2 m x 2.5 m) per chamber.
- Argus Titan control system - full data graphing and recording of all sensors and actuators.
- BB1 holds twelve Intravision 1600 Watt water-cooled multispectral and programmable LED lighting systems on two levels and with individually addressable UV (308 & 380nm), blue (448nm), white (5650k), green (568nm), red (660nm) and far red (735nm) irradiation sources.
- BB2 is equipped with twelve Intravision fixed wavelength Blade LED (three growing levels) and four spectrum adjustable Aurora LEDs, both on custom racking systems with integrated NFT hydroponics.
- Carbon dioxide enrichment and continuous recording from 0 to 20,000 ppm using LI-COR NDIR gas analyzers.
- Temperature control range from 15°C to 35°C +/- 0.5°C.
- VPD control from 0.2 to 1.5 kPa.
- Constructed of primarily non-off gassing inert materials to reduce plant effects from VOCs common to many building materials.

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APPENDIX II

Controlled Environment Systems Research Facility “Small Chamber” technical summary.

(http://www.ces.uoguelph.ca/TechNotes/GuelphBlueBox_TechnicalSpecifications_SAL-SA_rev00.pdf)

Controlled Environment Systems Research Facility
Guelph BlueBox System: SEC9

Initially developed for low pressure studies of plant growth for space applications, the SEC9 growing system consists of 9 individually controlled circular growth chambers capable of operating from ambient pressure to a full vacuum. Their primary function is the short-term (days) quantification of plant photosynthetic response to a variety of environment variables (side bar). These growth chambers are an integral part of the CESRF hardware collection used to study of plant growth and development, photosynthetic gas exchange, air quality, and hydroponic solution remediation technologies under atmospheric conditions common to both Earth-based studies and extraterrestrial exploration and habitation.

Technical Specifications

- 1600 Watt water-cooled multispectral and programmable LED lighting system with available UV (368nm), blue (440 and 460nm), cyan (490nm), green (568nm), red (630 and 660nm) and far red (735nm) irradiation
- Carbon dioxide enrichment from 0 – 10,000 ppm
- Continuous CO₂ (0-20,000ppm) data recording
- Temperature control range from 15°C - 35°C +/- 0.5°C
- VPD control from 0.2 - 1.5 kPa
- Variable speed air flow with bottom up distribution
- Integrated Argus Control System - full data graphing and recording of all sensors and actuators
- Made of primarily non-off-gassing inert materials
- 1900 x 450 mm (HxD) growing volume can accommodate a wide variety of crops
- Ability to custom blend the amount of CO₂, nitrogen and oxygen
- Vacuum ports available for custom system modifications depending on the experimental protocols required
- Off-line LED cooling system that runs independent of building chilled water supply
APPENDIX III

Controlled Environment Systems Research Facility “Phridge” technical summary.

(http://www.ces.uoguelph.ca/TechNotes/GuelphBlueBox_TechnicalSpecifications_PS1000_rev01.pdf)

Controlled Environment Systems Research Facility
Guelph BlueBox model PS1000

The PS1000 plant growth chamber is the sixth generation of whole plant photosynthesis systems to be designed at the University of Guelph’s Controlled Environment Systems Research Facility (CESRF). These growth chambers are capable of high-resolution measurement of whole plant photosynthesis and evapotranspiration. They are used as a precision tool to better study plant physiological responses in response to manipulation of multiple variables including:

Temperature
Humidity
Carbon dioxide
Oxygen
Light (quantity, quality)

Nutrients
Plant water status
Insect predation
Pathogen application/response
Chemical application (pesticide, bio control, fertilizer)

Technical Specifications

- 1600 Watt water-cooled multispectral and programmable LED lighting system with available UV (368 & 380nm), blue (448nm), white (5650K), green (568nm), red (655nm) and far red (735nm) irradiation
- Passive pressure compensation to improve system closure
- Carbon dioxide enrichment from 0 – 10,000 ppm
- Continuous CO₂ (0-20,000ppm) data recording
- Temperature control range from 15°C - 35°C +/- 0.5°C
- VPO control from 0.2 - 1.5 kPa
- Variable speed air flow with bottom up distribution
- Available active venting system to reduce O₂ and ethylene buildup during long term studies
- Integrated Argus Control System - full data graphing and recording of all sensors and actuators
- Made of primarily non-off gassing inert materials
- 1200 x 600 x 800 mm (HxDxW) growing volume can accommodate a wide variety of crops

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APPENDIX IV

Light intensity mapping within the strawberry and basil production racks for A) RB; B) RGB; C) RB+RGB; and D) FR Blade spectra. Light maps for the RB, RGB, and RB+RGB spectra were produced by measuring PPFD at many points on the rack at soil level before the plants were placed in the rack. This mapping was not completed for the FR Blade spectra, but has been estimated by averaging the light distribution pattern of the measured RB, RGB, and RB+RGB spectra in their racks. Each measured value has been expressed as a percentage of the point with the greatest PPFD in the rack, which is expressed as 100%. Bands of intensity are indicated in different colours in steps of 10%, as indicated on each graph. Light intensities for each spectral light treatment were based on measurements taken in the brightest region in each rack. The x- and y-axes of the following surface graphs are position coordinates for each measured position, and carry no units.
Light intensity mapping within the Small Chambers for each individual LED colour in the Snowflake 2.0 arrays. Light maps for each colour were produced by measuring PPFD at many points on in the chamber 100 cm away from the light source. Each measured value has been expressed as a percentage of the point with the greatest PPFD in the rack, which is expressed as 100%. Bands of intensity are indicated in different colours in steps of 10%, as indicated on each graph. Light intensities for each spectral light treatment were based on the average PPFD for each colour at 100 cm from the source. The x- and y-axes of the following surface graphs are position coordinates for each measured position, and carry no units.
APPENDIX VI

Light quality schedule for response photosynthetic measurements in lettuce, post-acclimation (described in section 5.2.2.1).

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