THE EFFECTS OF LIGHT SPECTRA ON THE GROWTH AND DEVELOPMENT OF GREENHOUSE CBD HEMP

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by
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ABSTRACT

The legalization of *Cannabis sativa* L. in some forms at both the state and federal level has caused a demand in cannabis-derived products, including grain, fiber, and bioactive compounds such as cannabidiol (CBD) and tetrahydrocannabinol (THC). Little scientific information is available to support best practices for cultivation. For greenhouse growers, the relatively high prices of CBD and THC make growing for cannabinoids economically appealing. This study aims to look at how supplemental light quality can improve cannabinoid yield and physiological traits desirable for greenhouse CBD hemp production. Cannabis (*C. sativa L.* var. 'TJ's CBD') was grown in a greenhouse environment under supplemental lights with a background of sunlight. Eight supplemental lighting treatments (60:40 red:blue LED, 70:30 red:blue LED, 80:20 red:blue LED, 90:10 red:blue LED, metal halide (MH), high pressure sodium (HPS), White LED, and Shift) were used in a randomized block design. Plants were established under HPS and then moved to a short-day photoperiod to induce flowering under the light quality treatments for the last nine weeks before harvest. Plant heights were taken throughout the experimental treatments; fresh and dry weight data were collected after nine weeks of short days. HPS, White LED, and Shift treatments resulted in taller plants than the MH, 70:30, and 80:20 treatments. Fresh weights and dry weights were also higher in the Shift treatment when compared with 70:30, 80:20, and MH treatments. Dry weight partitioning remained approximately the same across treatments. The results of this study could guide further research into the optimal supplemental light for greenhouse CBD hemp production.

BIOGRAPHICAL SKETCH

John (Jack) Wellhofer, who grew up just outside of Philadelphia, Pennsylvania, has always had a passion for math and science. This passion led him to pursue a degree in chemical engineering at Rensselaer Polytechnic Institute in Troy, New York. In the summer before his senior year, he worked in the plant science laboratory of Dr. Tessa Pocock, where he gained an appreciation for the chemical engineering abilities of plants. Upon graduation, he joined the laboratory as the full-time technician before coming to Cornell University to pursue further education in controlled environment agriculture.

Jack's first experience in hydroponics was a science fair experiment in eighth grade. A simple deep water culture system was compared with a soil-based one using mustard greens and fluorescent lights in the basement of his parents' house. He continued to pursue this interest in high school, where he set up an aquaponics system in the greenhouse of the biology room along with his friend. They grew tilapia and a variety of leafy greens.

His work with Dr. Pocock at the Lighting Enabled Systems and Application plant science laboratory connected him with Dr. Neil Mattson at Cornell University through the Greenhouse Lighting and Systems Engineering consortium. Jack worked on projects at RPI that were collaborations with researchers at Cornell. This overlap ultimately led to his decision to pursue a Master of Professional Studies degree program in the field of Horticulture at Cornell.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Neil S. Mattson for his guidance and mentorship in this project and in my professional career. He committed time and effort to getting my project underway, making the connections and offering advice that proved invaluable. Neil has always been approachable and made me feel welcome in the world of plant science. I am so thankful that he was my mentor throughout my MPS experience.

Second, I would like to thank Nickolas Kaczmar, to whom I truly owe the success of this project. Without his assistance, knowledge, and dedication to my success, this project would not have reached the scale and success it did. New to the Mattson Lab but not Cornell research, Nick provided the clairvoyance needed to establish a greenhouse full of hemp. When pandemic struck, Nick remained committed to the experiment and completed data collection when I needed to leave. I also want to thank him for some shared runs on the slopes of Greek Peak. I hope we can meet on the snow again sometime soon.

Third, I would like to thank my parents for supporting my path. I love engineering and it was a difficult decision to move out of that world and into the new. Throughout the process, my parents supported the decisions I made, knowing that I had thought them through and that those decisions were the best for me. I am thankful for their trust, faith, and support in me. I love you mom and dad.

Fourth, I would like to thank Dr. Tessa Pocock for inspiring me to take the leap into plant science. Tessa was welcoming and inspiring from the start, encouraging a passion for plant science she knew I had. She dedicated time to mentoring me and ensuring that my path to Cornell and beyond was clear. I am ever grateful for her guidance and faith in me.

Fifth, I want to thank the hemp team and greenhouse staff at Cornell. When we needed plants, advice, or a quick diagnosis on some leaf spot, the hemp team was there for us. Though we are greenhouse folk and not field folk, they welcomed us with open arms. I must also thank the greenhouse staff at Kenneth Post Laboratory and the Guterman Bioclimatic Laboratories for caring for my plants every single day. Without their care, dedication, and extensive growing experience, my plants would not have been as successful as they were.

Last, I want to thank LumiGrow and Hawthorne Gardening Co. for providing the LEDs used in this experiment. This experiment required an extensive amount of lighting capacity. When I started, I thought I would only have access to a few spare lights unused in other experiments. Through their generosity and dedication to research at Cornell, they allowed this experiment to take on a scale I had never imagined.

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60:40	Red to blue ratio of 60 percent red, 40 percent blue
70:30	Red to blue ratio of 70 percent red, 30 percent blue
80:20	Red to blue ratio of 80 percent red, 20 percent blue
90:10	Red to blue ratio of 90 percent red, 10 percent blue
CBD	Cannabidiol
HID	High Intensity Discharge
HPLC	High Performance Liquid Chromatography
HPS	High Pressure Sodium
LED	Light Emitting Diode
MH	Metal Halide
PFD	Photon Flux Density
PPFD	Photosynthetic Photon Flux Density
THC	Tetrahydrocannabinol

INTRODUCTION

With the legalization of cannabis in many forms across the United States, there is now a demand for cannabis products including grain, fiber, and bioactive compounds derived from cannabis, such as cannabidiol (CBD) and tetrahydrocannabinol (THC). While grain and fiber cannabis varieties are grown in the field, the relatively higher prices of CBD and THC make growing those cannabis varieties in greenhouses more economically appealing. Growing in a greenhouse allows control over air temperature, humidity, nutrients, carbon dioxide, and light, all of which can be modified and adjusted to improve crop yields. For this study, the effects of light quality on the physiology of hemp and the production of cannabinoids are studied in order to make recommendations on supplemental lighting for greenhouse CBD hemp production.

The Cannabis Plant, Its Nomenclature, and Its Uses

Cannabis sativa L. is a flowering, annual, herbaceous plant native to eastern Asia that has been cultivated for thousands of years (Edwards, 1990). *C. sativa* is a short-day, dioecious plant, producing flowers when the day length is below a critical photoperiod. Colloquially, *C. sativa* can be referred to as cannabis, marijuana, or hemp, depending on the cultivar (strain) and sex of the plant (Small, 2015). The term cannabis is used when referring to any *C. sativa* plant, including the subspecies, and will be used interchangeably with hemp throughout this paper when referencing the crop grown.

Marijuana refers specifically to female plants containing high levels of the psychoactive compound tetrahydrocannabinol (THC), sometimes also referred to as drug-type marijuana or cannabis or medical marijuana or cannabis. Hemp, sometimes industrial hemp, refers to cannabis

grown specifically for industrial use and with low levels of THC, legally defined as having less than 0.3 percent THC (Legal Information Institute).

Hemp can be grown for fiber, grain, and, more recently, cannabidiol (CBD). In fiber and grain production, male and female plants are allowed to populate the same field, as pollination needs to occur for grain production. The harvested fiber is used in a variety of products, ranging from textiles, to fiber reinforced concrete (Jarabo et al., 2012), to composite plastics (Panthapulakkal and Sain, 2006). Grain is harvested from pollinated females and is used to produce hemp seed products high in oils and protein (Wang and Xiong, 2019). In CBD production, only the unpollinated female flowers are useful, producing the highest concentration of all the cannabinoids, including CBD, when compared to the leaves and other parts of the plant, including grain (Turner et al., 1980). The use of feminized seeds or cuttings propagated from female plants is preferred, as it eliminates labor and wasted resources of growing and scouting for male plants to be removed (Toth, 2020).

Cannabis Legalization

Although a number of federal and state laws regulated the production and use of marijuana, the Controlled Substances Act of 1970 prohibited even the medical use of it by classifying it as a Schedule 1 drug, one with a high potential for abuse and no accepted medical use. Notwithstanding this classification, just three years later, Oregon decriminalized the possession of marijuana, followed shortly thereafter by Alaska, Maine, Colorado, California, and Ohio. In the past two decades, the medical marijuana movement has taken hold, starting with California in 1996. At the time of writing, 33 states, four US territories, and the District of Columbia have legalized medical marijuana (Hanson and Garcia, 2020). In 2012, Colorado and

Washington became the first states to legalize recreational marijuana through ballot measures. Since then, another nine states, as well as Canada, have legalized recreational marijuana (Berke and Gould, 2020).

Another major legal development occurred in 2018, when Congress passed the Agriculture Improvement Act of 2018, also referred to as the 2018 Farm Bill, which descheduled some cannabis products (including CBD and other non-THC cannabinoids). The 2018 Farm Bill established a new hemp regulatory system under the United States Department of Agriculture, allowing the cultivation, processing, and marketing of hemp products (Legal Information Institute). As commercial interest in hemp has grown since the enactment of the bill, so has the demand for scientific research on hemp production which was previously unavailable (Industrial Hemp Market).

Until recently, studies related to cannabis were limited in number and scope as research was not allowed at research universities in the United States. The research that has been conducted (by private companies or in other countries such as Canada) was also weighted toward marijuana, rather than hemp (Bidodeau et al., 2019, Caplan et al., 2019). Much of the information growers are using to grow cannabis comes from the illicit market, as noted by Potter in his 2009 dissertation on the production of cannabis as a phytopharmaceutical (Potter, 2009). Forums, hobbyist websites, and word-of-mouth have been the main source of information regarding cultivars, environmental conditions, and other growing parameters, lacking basis in scientific publications.

Market for CBD

CBD and the other cannabinoids interact with the human body through the activation of the cannabinoid receptors, CB1 and CB2 (Pertwee, 1997). There is evidence to suggest that CBD has medical benefits, including the prevention of vomiting, reduction of inflammation, and the mitigation of symptoms of some neurological diseases such as epileptic seizures (Burstein, 2015; Grotenhermen and Müller-Vahl, 2016). CBD taken orally has been proven to reduce vomiting in other mammals (Parker et al., 2006) and is currently undergoing trials to reduce chemotherapy-induced nausea and vomiting (Mersaides et al., 2018). CBD applied as a topical gel was demonstrated to reduce inflammation due to arthritis (Hammell et al., 2015). The Food and Drug Administration has approved the drug Epidiolex with CBD as the active ingredient as treatment for Lennox-Gastaut syndrome and Dravet syndrome, two rare types of epilepsy. Case studies have also suggested that CBD can be used to reduce anxiety and insomnia, although clinical studies are still needed to confirm the case studies (Shannon 2016, 2019). CBD appears to have a wide range of medical uses in humans, although further, more extensive studies are still needed to confirm results, determine effective dosage, and measure effect size.

In 2018, the CBD market was estimated at $4.6 billion and is expected to grow to $20 billion by 2024 (Grand View Research, 2019; Dorbian, 2019). CBD products have appeared in many forms, ranging from the smokable whole flower to CBD extracts in the form of tinctures, edibles, and oils. It is possible to purchase these products online, in corner stores, and at dispensaries (CBDoil.org). At this time, Epidiolex remains the only FDA approved drug with CBD as the active ingredient.

Even though the FDA warns consumers of unfounded medical claims and reminds consumers that CBD cannot be a food additive or be labeled as a dietary supplement (FDA,

2020), there is a substantial, expanding market for CBD products. Given this potential, growers will want to produce the highest quality crop with the highest yield. One way greenhouse growers may remain competitive versus the field-grown market is through the modification of environmental parameters, allowing for improved yield when compared to the same crop grown in the field.

Horticultural Lighting

The quantity, quality, and duration of light regulate plant growth and development (Pocock, 2015). In general, if a plant does not receive enough light it will become stunted, have reduced pigmentation, or begin a shade-avoidance response (Zelenskii, 1987). Besides using light as an energy source for photosynthesis, plants can use the quality of the light to sense information about the environment around them. Light quality refers to the spectral distribution of light given to a plant. Light quality is grouped into colors based on wavelength; 320–400 nanometers (nm) is UVA, 400–500 nm is blue, 500–600 nm is green, 600–700 nm is red, and 700–750 nm is far red, sometimes referred to as near-infrared. These wavelengths are sensed by photoreceptors within the plant leaf and are involved in a wide variety of physiological responses (Pocock, 2015). A plant that does not receive the right quality of light may exhibit physiological differences when compared to the same plants grown under optimal lighting conditions (Aphalo, 1999).

Electrical lighting is common in controlled environment agriculture, where lighting may provide part (supplemental lighting) or all (sole-source lighting) of the radiation necessary for plant development. Historically, the sole purpose of supplemental lighting was to reach the necessary light quantity required for optimum plant growth (Both, 2000). In some cases, such as

floriculture, supplemental lighting is used to maintain or inhibit flowering through photoperiodic lighting (Blanchard and Runkle, 2009). High pressure sodium (HPS) lamps, a type of high intensity discharge (HID) lamp, are the most common supplemental lights used in greenhouses (van Iersel and Gianino, 2017). More recently, technological improvements in light emitting diodes (LEDs) have made them a commercially viable option for growers, offering more biomass (yield) per unit energy input than HPS (Hernandez et al., 2020). LEDs often have a lower power requirement than comparable HID lamps (Gomez et al., 2013). They can provide a tunable light spectrum, useful in horticulture research and some commercial operations. Furthermore, they produce much less heat than HID lamps, which is particularly desirable in vertical farming operations where cooling is a problem (Singh et al., 2015).

For this study, the effects of blue light (400–500 nm) are of particular interest. Blue light is sensed by cryptochrome and regulates plant height and secondary metabolite production (Pocock, 2015). Previous studies on supplemental blue light in food crops found that blue light exposure can influence the production of anthocyanins (Jones, 2018) and reduce the height of flowering and vegetable crops (Mortensen and Strømme, 1987).

The studies are more limited in cannabis, but some recent studies found high blue light LED treatments cause higher CBD and THC bioaccumulation when compared to HPS, a low blue light source (Magagnini et al., 2018). An older study, using colored light filters to apply treatments, found no differences in the accumulation of THC when comparing blue light, red light, and sunlight, but did note an increase in the production of cannabichromene (CBC) under filtered red, blue, and green light, as well as darkness. (Mahlberg and Hemphill, 1983). Hawley noted an increase in cannabinoid concentration when red, blue, and green LED subcanopy lighting is used compared to a control without subcanopy lighting (Hawley, 2018). However, a

meta-analysis by Backer et al. found a positive correlation between THC and CBD yield per square meter with increasing light intensity (Backer, 2019), suggesting intensity might account for the differences in Hawley's study. Lastly, a study comparing blue-enriched LEDs to HPS found differences in internode length and the cannabinoid and terpene profiles (Namdar et al., 2019). Using supplemental lighting, particularly lighting with a high blue photon flux density (PFD) may be a suitable way to increase cannabinoid yield in greenhouse CBD hemp.

Greenhouse Production

Greenhouse production poses a number of benefits for growing hemp over field production. First is the ability to control the growing environment. Hemp is photoperiod and temperature sensitive, and in the field must be planted in the late spring to ensure the survival of seedlings in northern climates (van der Werf et al., 1995). With limited growing days and unavoidable weather events, planting or harvesting can be delayed, resulting in reduced or lost yield. In a greenhouse, cold temperatures, excessive rain, and other environmental factors are better regulated or even eliminated (Resh, 2016). Depending on the greenhouse setup (such as the use of blackout curtains to impose short day flower-inductive photoperiods between the spring and fall equinox), or with the use of autoflowering hemp cultivars, it may be possible to produce multiple crops per year, increasing the yield per acre of land. Greenhouses also allow control over lighting conditions, which may allow for improved yield over field-grown crops.

Although there are benefits to greenhouse production, greenhouses may not be economically viable for hemp production in the long term. As regulation restrictions ease and more field growers turn to hemp, the price of hemp products will naturally decline. The cost of producing in a greenhouse is significantly higher than in a field due to capital and energy costs,

making only high-margin crops economically viable. Currently, field hemp is labor intensive, often needing to be harvested by hand, driving prices up. But companies producing agricultural combines are able to automate the harvest process (CBD Hemp Harvester), which would substantially lower the labor costs of field production. If greenhouse growers want to remain in the CBD market, they will need to find a way to become more competitive with field growers. This may be in the form of improved efficiency, through automation, increased plant productivity, and reduced energy and capital expenses. It may also be through the selection of niche cultivars that could be sold at high prices.

The information on the effects of supplemental light quality on CBD hemp production is still limited and in need of further investigation. Modern LEDs allow the precise application of light spectra, allowing better comparisons of narrow wave band lighting to HID lamps, rather than relying on filtered light or broad spectrum lamps to make spectrum comparisons. The objective of this experiment was to study the effect of supplemental light source, including quality (such as R:B ratio and narrow waveband lighting vs. broadband light) and type (LED vs. HPS vs. MH) on the yield, morphology, and cannabinoid content of greenhouse CBD hemp.

MATERIALS AND METHODS

In this experiment, the response of *Cannabis sativa* to supplemental lighting treatments was examined. Due to restrictions on the purchasing of seeds and propagation of cultivars, only one cultivar, 'TJ's CBD,' was used in this experiment. This cultivar was selected due its high cannabidiol (CBD) and low tetrahydrocannabinol (THC) content, as well as due to a Material Transfer Agreement with the supplier, TJ's Gardens, allowing Cornell University to propagate the cultivar for experimental purposes.

Propagation, Establishment, and Growth

Cuttings from 'TJ's CBD' were taken from mother plants maintained by the Larry Smart Laboratory at Cornell AgriTech in Geneva, New York on November 15, 2019. Stem cuttings were rooted in the propagation house at the Kenneth Post Laboratories at Cornell University in Ithaca, New York. Shoots of 10–16 cm length were taken from the mother plant, dipped in Clonex, and inserted into 3.8 x 3.8 cm Grodan rockwool plugs. The leaves were slightly trimmed to reduce water loss due to transpiration. The new cuttings were placed in 1020 trays under a mist system that supplied clear water every 20 minutes. The cuttings received bottom heat at 26.5 °C and natural daylight supplemented by incandescent bulbs to maintain a 15 h photoperiod, to prevent premature flowering. Plants were maintained in the propagation house for three weeks. During the second and third weeks, a weak nutrient solution, 50 mg/L N from 21 N - 2.2 P - 16.6 K Jack's All-Purpose Liquid feed (JR Peter's Inc., Allentown, PA), was applied to the cuttings to prevent chlorosis.

After rooting, plants were transported to the Guterman Bioclimatic Laboratories at Cornell University, Ithaca, New York. Rooted cuttings were transplanted into 10 cm pots with a

commercial peat:perlite potting mix (LM-111, Lambert Peat Moss, Rivière-Ouelle, Canada) and put on grow benches under high pressure sodium (HPS) lights at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on December 6, 2019. A photoperiod of 16 h was maintained, along with 25.5/16.5 C day/night temperatures. Plants were watered daily on an as-needed basis with a 200 $mg \cdot L^{-1}$ nitrogen fertilizer made from 10 N- 13.2 P - 16.6 K Jack's Blossom Booster (JR Peter's Inc., Allentown, PA). After two weeks of establishment, plants were transplanted into eleven-liter pots in LM-111. Plants remained under the same environmental conditions and fertilizer for four weeks to allow vegetative growth to occur. On January 13, plants were moved into lighting treatments (described below) and a change in photoperiod to 12 h (with a background of ambient short days) to induce flowering. Other environmental conditions remained the same. A top dressing of calcium sulfate was applied on January 31 to address a possible calcium deficiency (marginal necrosis noticed in leaves). Fertilizer was stopped on March 2 to begin a flushing cycle prior to harvest in line with commercial practice. The plants were watered with clear water for the remainder of the experiment until harvest on March 16.

Lighting Treatments

Eight lighting treatments, including the control, were applied for 63 days (January 13 to March 16) in this experiment. The treatments were HPS (control, HS2000 600W, U.S. Global Resources, Florida, Texas), LED red to blue ratios of 90:10, 80:20, 70:30, and 60:40 (LumiGrow Pro 650e 585W, LumiGrow, Emeryville, California), a phosphor converted white LED (Gavita Pro 1700e 645W, Gavita, Vancouver, Washington), a metal halide (PARsource 315W, Petaluma, California), and a shift treatment. The shift treatment received seven weeks of HPS and two weeks of the highest blue light treatment, a red to blue ratio of 60:40. Each light treatment had

two fixtures, except for the metal halide which required three, and the white LED which required one to meet DLI targets.

Each light fixture was adjusted to produce 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) in the center of the treatment area at one meter above the bench height. An Apogee PS-300 spectroradiometer (Apogee Instruments, Inc., Logan, Utah) recorded the spectrum and light intensity of each plant location in each treatment. Measurements were taken at least 30 minutes after sunset to ensure the only light source was from the light treatment fixture. One at a time, each light treatment was turned on and measurements for each plant location were taken. Each plant location was positioned to give the maximum amount of distance from its neighbors, including those of neighboring treatments. A layout of the experiment is in Figure 1. The average spectrum for each light treatment taken from the eight plant locations is available in Appendix 1.

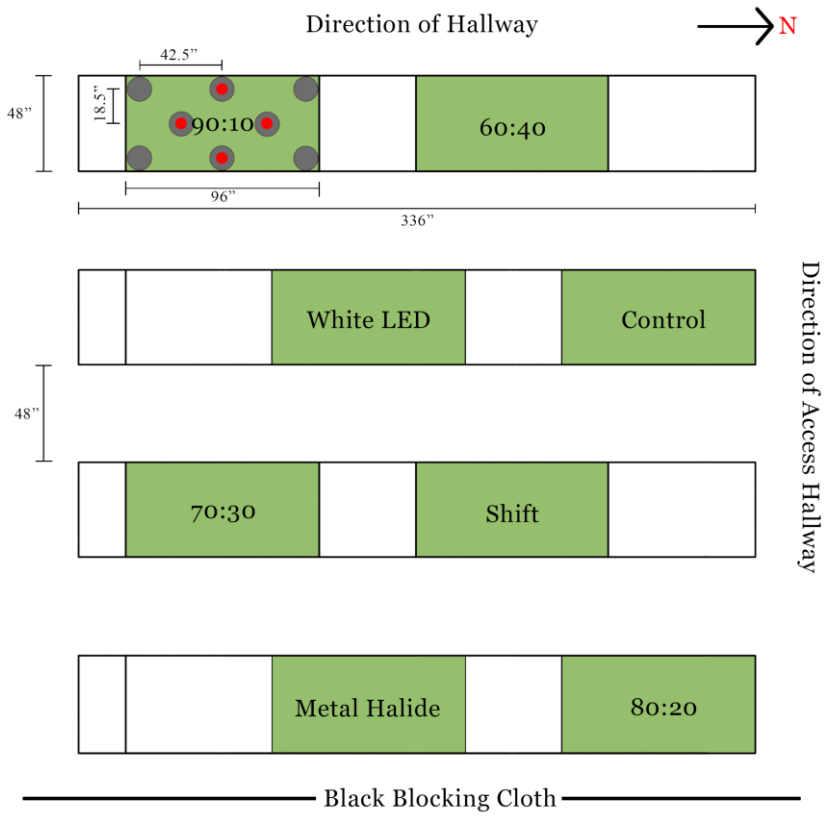

Figure 1: The layout of the lighting treatments. Treatments and plant placement were spaced to allow maximum plant growth and minimal interference from neighboring treatments. Placement of the eight plants per treatment was as shown in the 90:10 treatment. Plant numbers for each treatment were assigned as if reading from left to right, top to bottom. The four red dots indicate the four plants chosen for HPLC analysis for each treatment. The fifth plant was chosen based on distance from neighboring light treatments. A two foot buffer was included on the left side in the diagram to allow for additional spacing away from the greenhouse exhaust fans.

Prior to beginning the 12 h photoperiod for flowering, measures were taken to block light from adjacent greenhouses. The greenhouse to the east was on a 16 h photoperiod with HPS lamps hung at approximately three meters above the floor. Black plastic was taped along the glass wall between the experiment location and the adjacent greenhouse, up to one meter above the neighboring HPS lamps. Light was blocked such that walking with a light sensor around the greenhouse after sunset and with the treatment lights off showed a light level below 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, below the critical threshold for flowering (Borthwick and Scully, 1954).

While this experiment was ongoing, another hemp experiment was running in the same greenhouse. Due to limited space, there was not enough room to space these experiments far enough apart so that they did not interact with one another. The other experiment was growing under HPS lamps and would have greatly affected the spectra for the 80:20 and MH treatments in particular due to proximity. To remedy this, a large black tarp was placed between the two experiment sections to mitigate the light bleed. The black tarp is noted above in Figure 1. While this was effective at stopping the light bleed, it also blocked most of the morning sun that the 80:20 and MH treatments received.

Harvest Procedure

All plants were harvested on March 16, nine weeks after the start of short day flower inductive photoperiods, in accordance with prior studies (Potter, 2009). Plant heights were measured from the soil line to the tip of the apical meristem. Certain plants were chosen for high performance liquid chromatography (HPLC) analysis of their apical flowers. The chosen plants were those receiving the highest portion (light quantity) of their designated light treatment and the least light from a neighboring treatment. For all treatments, plants 2, 4, 5, and 7 were chosen (noted in Figure 1), with the fifth plant chosen based on the treatment location. The top ten centimeters of the apical flower was removed, placed in a previously weighed and labeled brown paper bag, and weighed. The bags were then placed in a drying oven at 30 ℃ until the weights remained steady between measurements. The samples were then stored in an airtight container at 16 ℃ until HPLC analysis. Cold storage at 4 ℃ was not used due to limited space availability.

Whole plant fresh weight was measured at the time of harvest, including the apical flower. Plants were cut at the base of the stem just above the soil line. All plant material (fresh

weight) was weighed on a scale to the nearest tenth of a gram and then hung upside down to air dry on metal wire stretched across the length of the greenhouse, in accordance with commercial practices, to obtain dry weight measurements. Due to space limitations, a dark space to prevent UV degradation of the cannabinoids was not used, however, the HPLC samples were already collected and the cannabinoid profiles of the remaining plant would not be tested. Plants were dried in the greenhouse at 10 ℃ and less than 40 percent relative humidity was maintained. Box fans and installed greenhouse fans were used to keep air circulation high to prevent mold. Plants were considered dry when the stems would snap, not bend, upon applying pressure, in accordance with commercial cannabis drying practices. Once dry, plants were individually stripped and the dry plant material was partitioned into stems, leaves, and flowers. The stem, leaves, and flowers were each weighed on a scale to the nearest tenth of a gram. Whole plant dry weights were calculated by summing the plant parts.

Due to the COVID-19 pandemic, HPLC data collection was not completed in time to be included in this report. It is the intention to complete HPLC at a later date and include the results, and thus the methods that will be used are included in this report. HPLC extractions will be performed in the laboratory of Dr. Jocelyn Rose according to the procedure outlined in Toth et al., 2020 to determine concentrations of cannabinoids and terpenes.

Experimental Design and Statistical Analysis

The experiment was designed in a randomized block design. Due to the limits of available greenhouse space and lighting fixtures there was only one block per lighting treatment. The eight lighting treatments were randomized across the greenhouse space. Within each lighting treatment there were eight replicate plants that were randomly selected and placed. All statistical

analyses were performed in RStudio version 3.6.1 (R Core Team, 2019, Vienna, Austria). An analysis of variance (ANOVA) was performed on heights, fresh weights, and dry weights for each treatment. A post-hoc analysis to separate means was conducted using Tukey's honestly significant difference test (alpha = 0.05). Results were plotted using RStudio.

RESULTS

At the end of the growing period, there was a statistically significant difference between the Shift treatment and the 60:40 R:B, 70:30 R:B, 90:10 R:B, and White LED treatments. The average Shift treatment height was 1065 mm, and the average 60:40, 70:30, 90:10, and White LED treatment heights were 979, 978, 981, and 979 mm, respectively. No other significance in treatment heights was observed at the end of flowering. Figure 2 shows the average plant heights at the end of the experiment. To determine whether the Shift treatment affected the final height of the plants, average plant heights from February 26, the seventh week of short days (which was the last week of HPS light for the Shift treatment before moving to a 60:40 R:B treatment for the remaining two weeks) were also tested for significance. The Shift treatment was significantly different from the 60:40 R:B, 70:30 R:B, and 90:10 R:B, but not the White LED treatment or the HPS treatment on Feb 26. Thus, it appears much of the high differences in the Shift treatment were due to the first seven weeks of HPS lighting—as the Shift treatment was not significantly different from HPS at either week seven or at the final harvest.

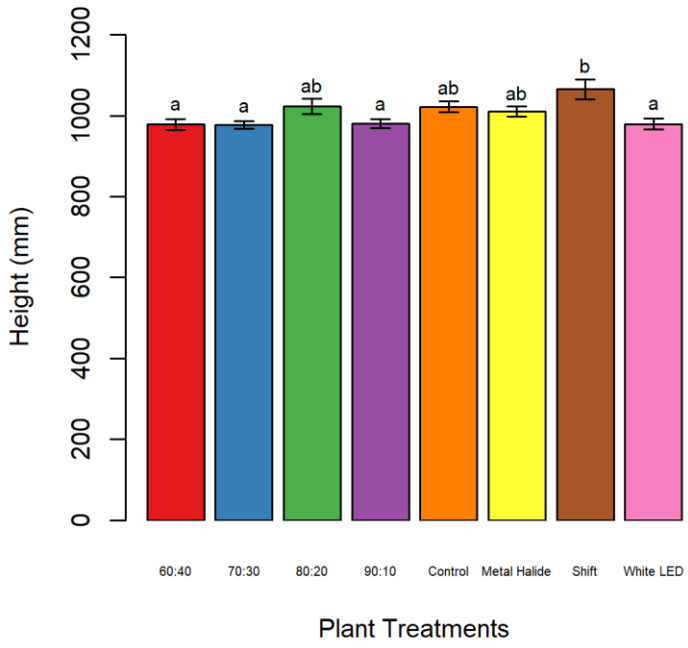

Figure 2: Mean plant heights of 'TJ's CBD' hemp grown in a greenhouse under eight lighting treatments for 63 days of short-day photoperiods prior to harvesting. Data represent the means (± std. err.) of eight plants per treatment. Letters represent mean separation comparison using Tukey's HSD (alpha = 0.05). The lighting treatments included four ratios of R:B LEDs, control (high pressure sodium, HPS), metal halide, shift (7 wks HPS followed by two weeks 60:40 R:B), and white LED.

The greatest plant fresh weights were in the 60:40, Control (HPS), Shift, and White LED treatments (Figure 3). The Shift treatment had significantly greater fresh weight than the 70:30, 80:20, and MH treatments. The Control, HPS, and White LED treatments had significantly greater fresh weight than the MH treatment.

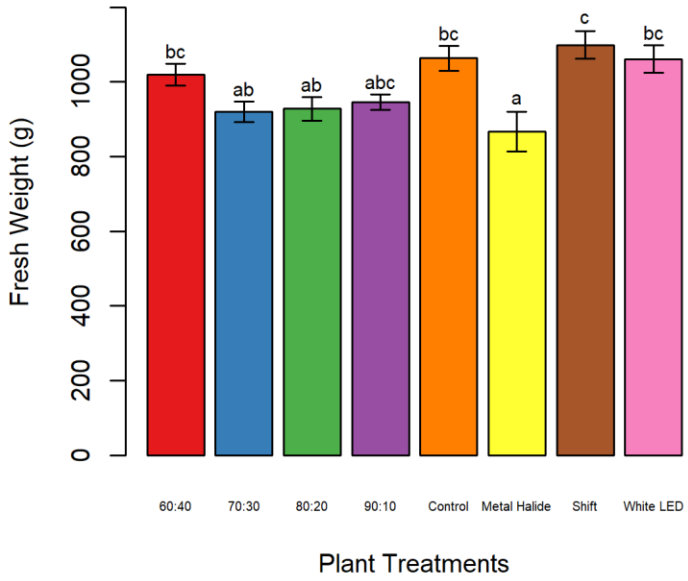

Figure 3: Mean fresh weights of 'TJ's CBD' hemp grown in a greenhouse under eight lighting treatments for 63 days of short-day photoperiods prior to harvesting. Data represent the means (± std. err.) of eight plants per treatment. Letters represent mean separation comparison using Tukey's HSD (alpha = 0.05). The lighting treatments included four ratios of R:B LEDs, control (high pressure sodium, HPS), metal halide, shift (7 wks HPS followed by two weeks 60:40 R:B), and white LED.

The dry weights of the stems, leaves, and flowers followed similar trends as the fresh weights. The dry stems of the Control, Shift, and White LED treatments were greater than the Metal Halide treatment. The Shift and White LED treatments also had higher dry stem weights than the 70:30 treatment. The trend was similar in the leaf dry weight, with more dry biomass in the Control, Shift, and White LED when compared to the 70:30 and 80:20 treatments. For the dry flower weights, the White LED was weighed more than the 70:30, 80:20, and the Metal Halide. The Shift treatment had more dry flower weight than the Metal Halide, but not any other treatment. Graphs of the dry weights of the plant parts are presented in Figures 4, 5, and 6.

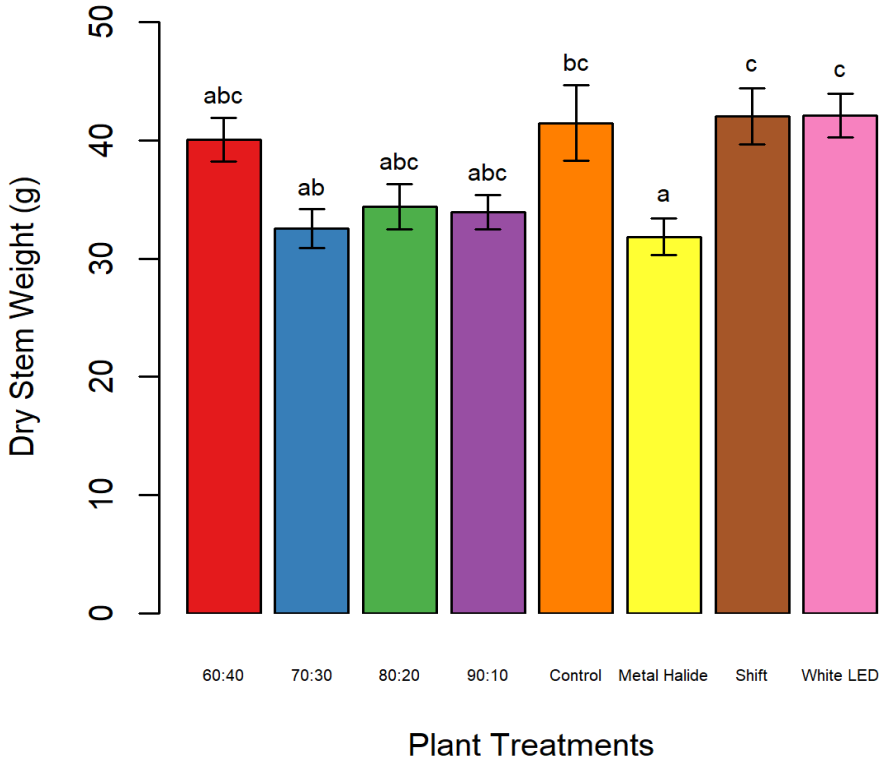

Figure 4: Mean dry stem weights of 'TJ's CBD' hemp grown in a greenhouse under eight lighting treatments for 63 days of short-day photoperiods prior to harvesting. Data represent the means (± std. err.) of eight plants per treatment. Letters represent mean separation comparison using Tukey's HSD (alpha = 0.05). The lighting treatments included four ratios of R:B LEDs, control (high pressure sodium, HPS), metal halide, shift (7 wks HPS followed by two weeks 60:40 R:B), and white LED.

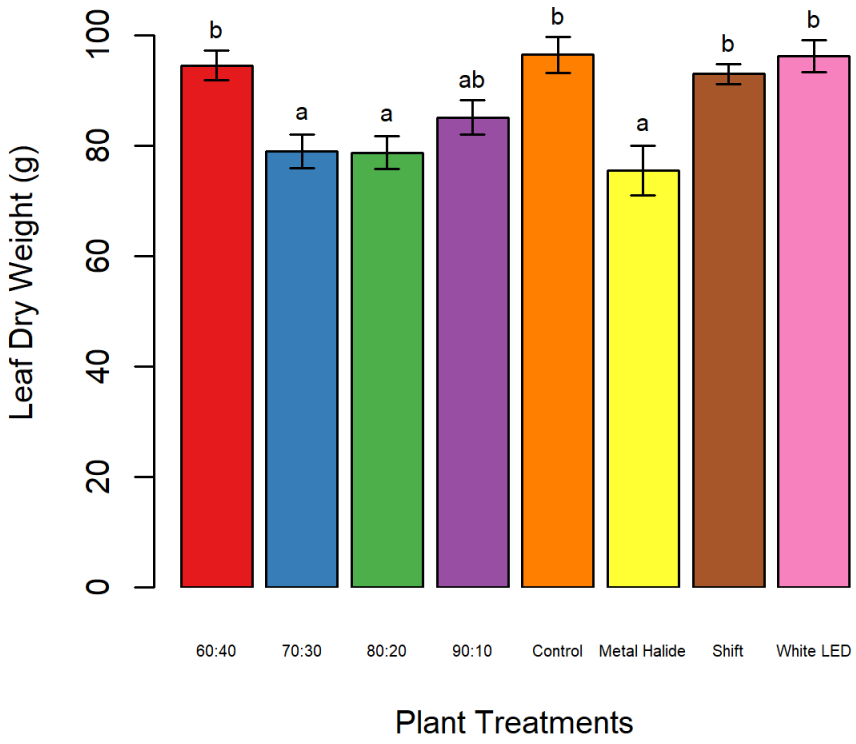

Figure 5: Mean dry leaf weights of 'TJ's CBD' hemp grown in a greenhouse under eight lighting treatments for 63 days of short-day photoperiods prior to harvesting. Data represent the means (± std. err.) of eight plants per treatment. Letters represent mean separation comparison using Tukey's HSD (alpha = 0.05). The lighting treatments included four ratios of R:B LEDs, control (high pressure sodium, HPS), metal halide, shift (7 wks HPS followed by two weeks 60:40 R:B), and white LED.

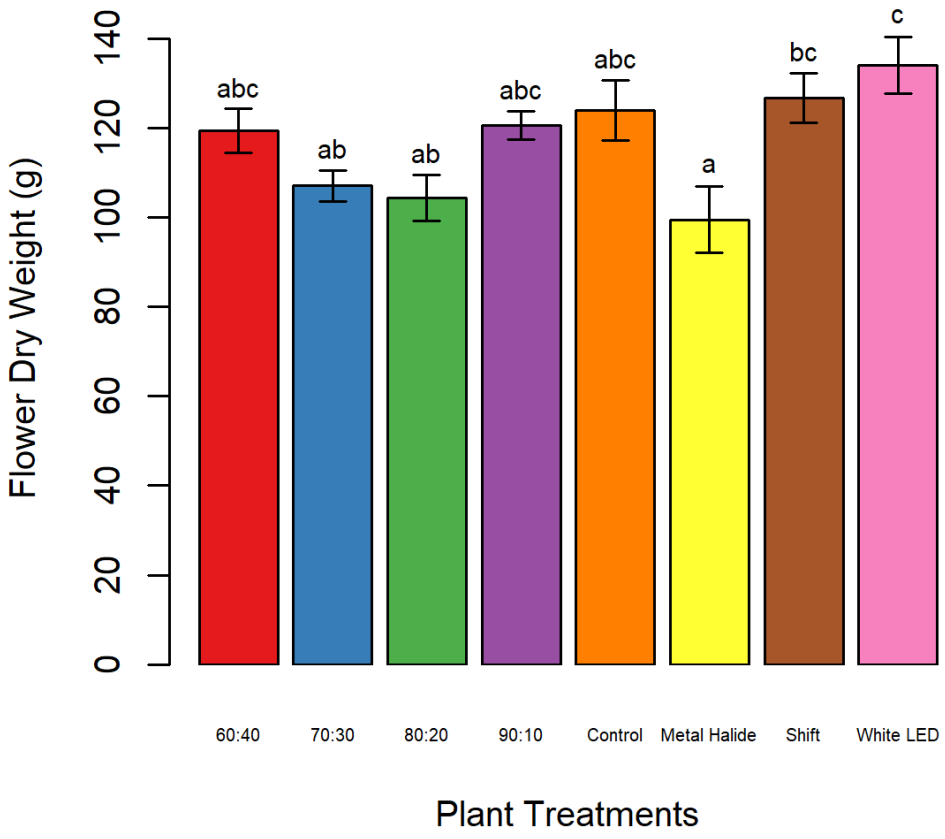

Figure 6: Mean dry flower weights of 'TJ's CBD' hemp grown in a greenhouse under eight lighting treatments for 63 days of short-day photoperiods prior to harvesting. Data represent the means (± std. err.) of eight plants per treatment. Letters represent mean separation comparison using Tukey's HSD (alpha = 0.05). The lighting treatments included four ratios of R:B LEDs, control (high pressure sodium, HPS), metal halide, shift (7 wks HPS followed by two weeks 60:40 R:B), and white LED.

Total mean dry weights are presented in Figure 7. These means follow similar trends as the fresh weights and dry weights of the plant parts. There was significantly more dry weight than the Control, Shift, and White LED when compared to the 70:30, 80:20, and Metal Halide treatments. Figure 8 presents the mean dry weights of the plant parts as a percent of the mean total dry weight of the plants.

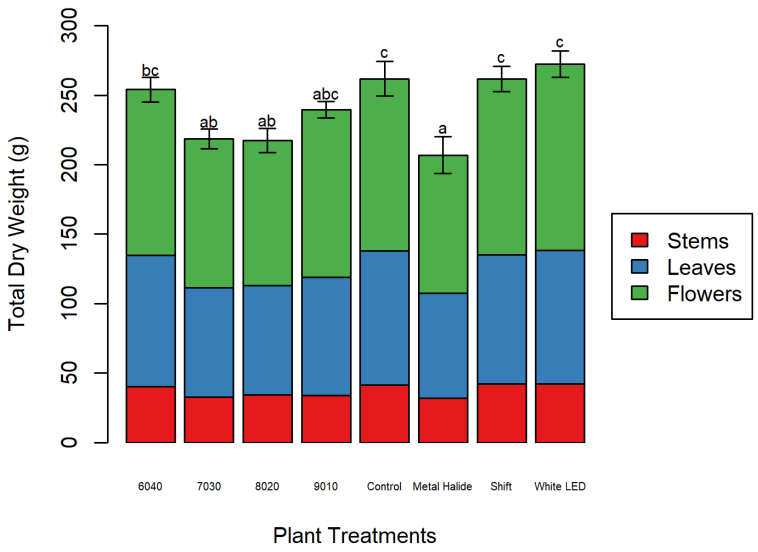

Figure 7: Mean total dry weights with plant part partitioning of 'TJ's CBD' hemp grown in a greenhouse under eight lighting treatments for 63 days of short-day photoperiods prior to harvesting. Data represent the means (± std. err.) of eight plants per treatment. Letters represent mean separation comparison using Tukey's HSD (alpha = 0.05). The lighting treatments included four ratios of R:B LEDs, control (high pressure sodium, HPS), metal halide, shift (7 wks HPS followed by two weeks 60:40 R:B), and white LED.

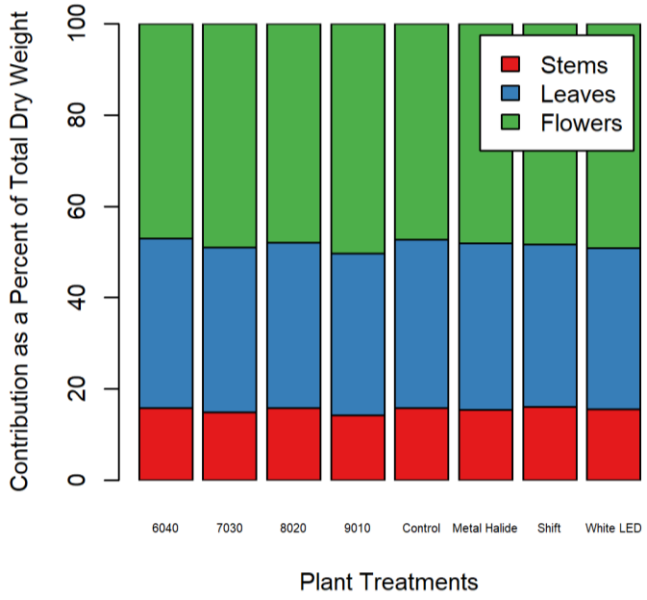

Figure 8: Biomass allocation, i.e. Mean dry weight of plant parts as a percent of total dry weight of 'TJ's CBD' hemp grown in a greenhouse under eight lighting treatments for 63 days of short-day photoperiods prior to harvesting. The lighting treatments included four ratios of R:B LEDs, control (high pressure sodium, HPS), metal halide, shift (7 wks HPS followed by two weeks 60:40 R:B), and white LED.

Throughout the experiment, Apogee light meters (SQ-520, Apogee Instruments Inc., Logan, Utah) recorded instantaneous light data in five minute intervals. These data were recorded to determine if each treatment was receiving approximately the same DLI in the greenhouse (note that supplemental lighting treatments provided a very similar DLI as measured in the center under each fixture, however, patterns related to shading of ambient light and position of light treatments in the greenhouse may have affected overall DLI). A representative sunny day is presented in Figure 9. For six of the eight treatments, the DLI was quite similar and varied from 18.1 to 21.0 $mol \cdot m^{-2} \cdot d^{-1}$. For the 80:20 treatment DLI was 13.8 $mol \cdot m^{-2} \cdot d^{-1}$, and for the White LED the DLI was 23.4 $mol \cdot m^{-2} \cdot d^{-1}$.

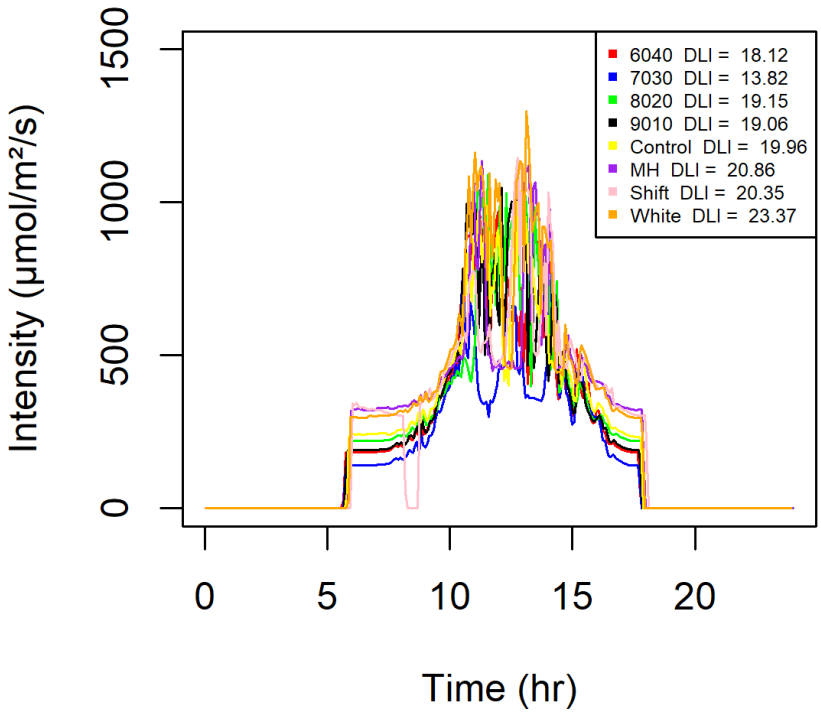

Figure 9: A representative sunny day of light intensities in the greenhouse. Data were taken from light readings on February 3, 2020. The figure legend includes the DLI each treatment received. The zero readings at approximately 7 hours on the Shift treatment were likely caused by a disconnected spectroradiometer battery.

Treatments were spaced as far from one another as possible given the space available, however a bit of light spillover between treatments occurred. Appendix 1 contains graphs of the average spectrum of each treatment taken individually at night. It was the goal of this experiment to include treatments consisting of 90 percent red, 10 percent blue, and so on for the 80:20, 70:30, and 60:40 treatments. With all of the treatment lights on, some bleed over occurred. Table X shows the absolute UV, blue, green, red, and far red $\mu mol \cdot m^{-2} \cdot s^{-1}$ quantities each treatment was receiving, on average.

Table 1. The average $\mu mol \cdot m^{-2} \cdot s^{-1}$ each treatment was receiving, divided into UV (320–400 nm), blue (400–500 nm), green (500–600 nm), red (600–700 nm), and far red (700–750 nm).

	UV	**Blue**	**Green**	**Red**	**Far Red**
60:40	0.29 ± 0.05	57.83 ± 7.22	7.41 ± 2.30	79.15 ± 9.47	1.55 ± 0.44
70:30	0.32 ± 0.03	40.75 ± 5.12	3.93 ± 0.90	79.14 ± 11.32	0.99 ± 0.13
80:20	0.28 ± 0.02	26.54 ± 3.72	6.77 ± 2.91	85.93 ± 13.93	1.90 ± 0.53
90:10	0.12 ± 0.02	16.58 ± 2.29	3.51 ± 1.10	106.04 ± 16.35	0.96 ± 13
HPS	0.53 ± 0.04	8.72 ± 0.71	95.96 ± 7.57	82.48 ± 7.07	16.64 ± 1.46
MH	8.03 ± 0.57	45.32 ± 2.97	74.04 ± 5.08	35.92 ± 3.00	15.12 ± 1.19
Shift	0.73 ± 0.04	9.85 ± 0.54	95.68 ± 11.34	96.53 ± 9.46	21.12 ± 2.17
White LED	0.33 ± 0.04	24.28 ± 3.41	52.45 ± 7.44	59.07 ± 7.34	3.94 ± 0.53

Due to the coronavirus pandemic, the Rose laboratory was unable to process high performance liquid chromatography samples from the apical flowers. Data on the cannabinoid profiles of the treatments will be included in a later revision.

DISCUSSION

Recommendations for optimal supplemental lighting for greenhouse cannabis/hemp production has not previously been reported in the scientific literature. This study is only the beginning of extensive research that would need to occur in order for lighting recommendations to be made. From the data, early indications would suggest that White LEDs or HPS lamps are both suitable as supplemental lighting options for greenhouse CBD hemp. Both lighting treatments produced the highest fresh and dry weights, resulting in the highest yield among treatments.

For the plant heights, while statistically significant differences were observed, the height difference was small enough as to be insignificant in practice. The treatment with the highest average plants was the Shift, with an average plant height of 104.9 cm. The shortest treatment mean was the 90:10 treatment at 96.8 cm, making the gap between the two only 8.1 cm. These results are similar (in result and effective size) to those observed by Magagnini et al. (2018), who found HPS to be taller than blue-rich LED treatments under sole-source lighting. The difference of a few centimeters in plants that reach one meter tall is likely not relevant for commercial greenhouse growers. Importantly, there does not appear to be a trend in decreasing height as blue light increases, suggesting that blue light may not impact plant elongation as much as in other species such as chrysanthemum and tomato (Mortensen and Strømme, 1987). However, it can be difficult to determine a trend under the sunlight background. In addition, there was no significant change in height between the Shift and Control treatments after the blue light treatment was applied to the Shift, although plants were likely finished with internode elongation growth by the time the blue light treatment was applied. While most of the impact of the blue light was expected to influence the cannabinoid profile of the flowers, as previous studies have suggested

(Magaginini et al, 2018; Namdar et al., 2019), it is important to note that height was relatively unaffected.

The White LED and HPS treatments remained the best performers in fresh weight. This effect size was larger than the height differences. The Control, Shift, and White LED had fresh weights of 1063 g, 1099 g, and 1060 g, respectively, compared to the two lowest treatments, 70:30 and MH, which had 920 g and 866 g fresh weight, respectively. This is a yield difference of more than 100 g (or >10% fresh weight), which, when scaled to commercial production levels, would provide a significant increase in profit. It should be noted that these differences may be due to differences in DLI. From Figure 3, the highest fresh weight treatments correspond with higher DLI, and vice versa for the lower fresh weights. The positive correlation on DLI and yield is known in cannabis and in other greenhouse crops (Backer, 2019; Hawley, 2018). Better DLI control in future work would help determine if portions of the White LED and HPS spectra cause an increase in biomass when compared with other lighting treatments.

The dry weights followed similar trends as the fresh weights for each treatment, indicating similar water content in each plant part across treatments. The increase in dry weight (>10%) under HPS and White LED is of particular interest to commercial growers, as dry weight increases correlate directly to increased profit. The dry weight partitioning as a percentage of total dry weight was also approximately equal in all treatments, suggesting that the light treatments did not induce a response that created more flowers than stems or leaves. It has been reported by Magagnini et al. that HPS lighting increases stem and leaf elongation, and therefore dry weight. This effect is also observed in other food crops by Tibbitts et al. and Wheeler et al, and is likely due to a low R:FR ratio, which can induce shade avoidance responses such as leaf expansion and stem elongation. Because this experiment was performed under a background of

sunlight, that ratio was less affected than in the cited literature and an effect on the plants was not observed.

It should be noted that many of the plants suffered from leaf necrosis late in the growing cycle (Appendix 2). Some of this may have been due to the switch to clear water at the end of the growing cycle, however many plants were already showing signs of necrosis prior to the switch. The MH treatment was particularly affected, even before the other treatments were showing damage. The MH lights, which were initially closer to the plants than the other treatments to achieve the same PPFD, were moved further from the leaves as the plant grew when damage was observed. As most of the upper leaves were necrotic at the end of the experiment, this may have significantly influenced the yield data from the MH treatments.

The main intention of this study was to look at how cannabinoid profiles could be manipulated using supplemental lighting. Unfortunately, at the time of writing, the HPLC data was not available to include. Results and discussion for HPLC will be included at a later date.

Follow up studies to our experiment with greenhouse light quality for CBD hemp should be conducted Due to the time and photoperiod requirements of this experiment, it was not feasible to perform two additional blocks (or replicates over time) that would increase the statistical significance of the results. Especially accounting for the effects of patterns of shading of ambient lighting in the greenhouse, it would be important to randomize the location of the lighting treatments in further replication. As it currently stands, the layout of the greenhouse made it such that some treatments received more or less sun depending on the time of day. Figure 9 shows data taken from February 3, a representative sunny day, showing that all but the 80:20 lighting treatment was receiving approximately the same DLI. However, better control of DLI would eliminate any effect light quantity had on the parameters of interest. It is also possible

that some of the treatment's results were skewed due to the spectral influence from their neighbors, particularly from the spectra of the HPS and the MH. Figure 10 shows two plant locations which received a noticeable amount of light from a neighboring treatment. Appendix 1 lists the average spectrum of each treatment was receiving.

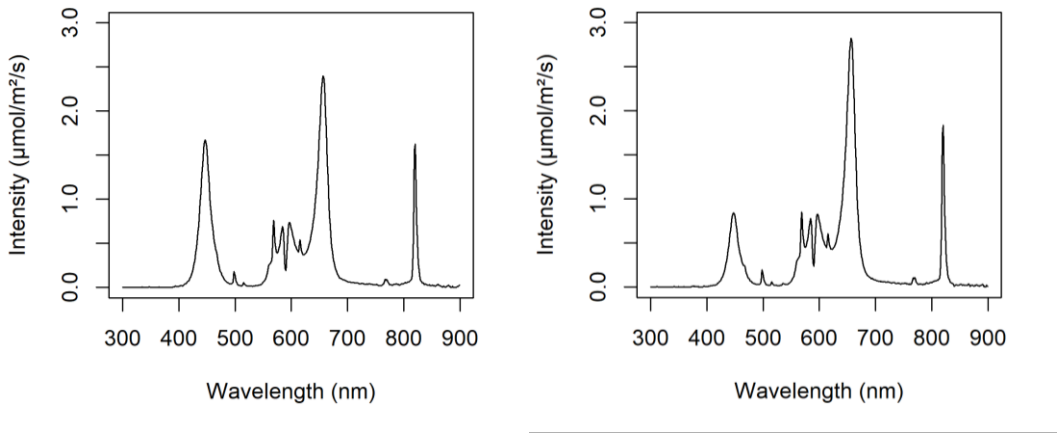

Figure 10: Spectra from the 60:40 treatment plant location 8 (left) and 80:20 treatment plant location 1. Both of these plant locations are close to the HPS treatments, Control and Shift. From the graph, the 850 nm line, a tell-tale of HID lamps, is clearly visible and is influencing the spectrum of the plant location.

Greenhouse experiments with light quality are difficult to execute due to patterns of shading and changing ambient light conditions. However, our objectives are to study light quality with a background of sunlight (representative of greenhouse hemp production). If the objective was to determine the impact of light quality under sole-source lighting (representative of indoor/warehouse production) then the experiment should be conducted in controlled environment chambers where sole-source lighting could be implemented. Only 8–9 $mol \cdot m^{-2} \cdot d^{-1}$ out of the 18–20 $mol \cdot m^{-2} \cdot d^{-1}$ were provided by the supplemental lighting. Under full sunlight, the effect of the supplemental light on the physiology of the crop is muted. This is similar to findings of greenhouse researchers working with other crops such as lettuce (Hernandez et al., 2020). Effects of the spectra on the plants would be best observed under sole source eliminating background effects of sunlight, as well as eliminating light bleeding from other treatments. It

was, however, our objective to observe effects in greenhouse production setting, and to determine if any effects were noticeable and significant such that a particular style of lighting should be used. Initial results suggest there may be differences based on between supplemental light quality treatments for greenhouse CBD hemp.

CONCLUSION

At this time, lighting studies on cannabis, and CBD hemp cultivars in particular, are limited. In the current experiment, it was shown that supplemental light quality can have an effect on the height, fresh weight, and dry weight of CBD hemp. The 70:30 and 80:20 red to blue ratio LEDs, as well as the MH lamps, kept the studied plants shorter than the White LED, HPS, and 90:10 and 60:40 lighting treatments. Fresh weight was improved under both White LED and HPS when compared to the other treatments. Dry weights were improved following a similar trend as fresh weights, indicating no change in relative water content based on lighting treatments. Biomass partitioning did not appear to be affected by lighting treatments. In future work to validate these results, effort should be concentrated on isolating treatments from one another and controlling for DLI differences due to greenhouse architecture. Doing so would better ensure that observed differences are due to light quality. Understanding the effects that supplemental lighting has on greenhouse CBD production will allow greenhouse CBD producers to remain competitive against field grown CBD.

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APPENDIX

Appendix 1: Average Spectrum of Light Treatments

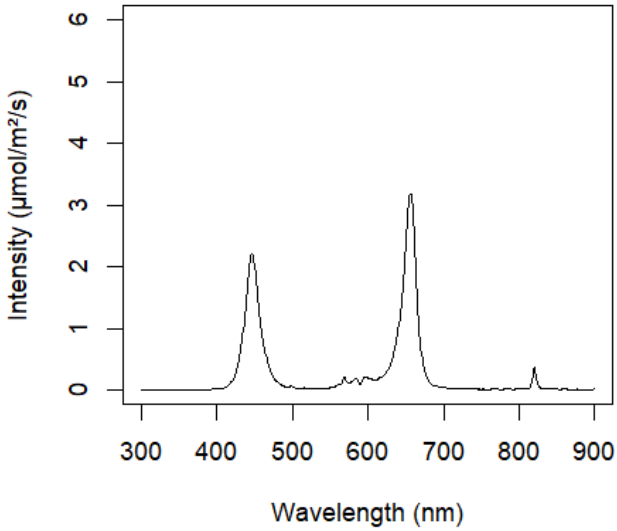

Figure 1a: Average spectrum of eight plant locations for the 60:40 R:B lighting treatment.

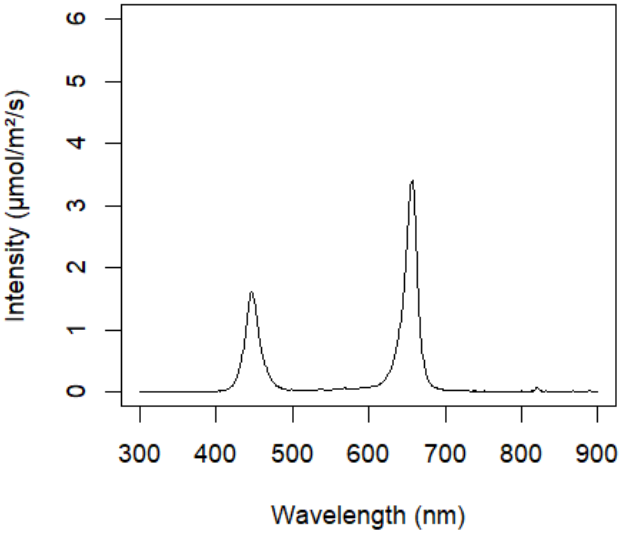

Figure 1b: Average spectrum of eight plant locations for the 70:30 R:B lighting treatment.

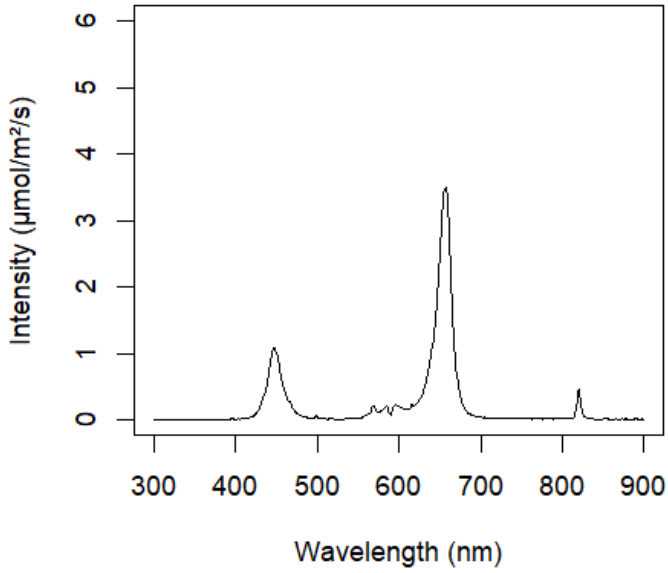

Figure 1c: Average spectrum of eight plant locations for the 80:20 R:B lighting treatment.

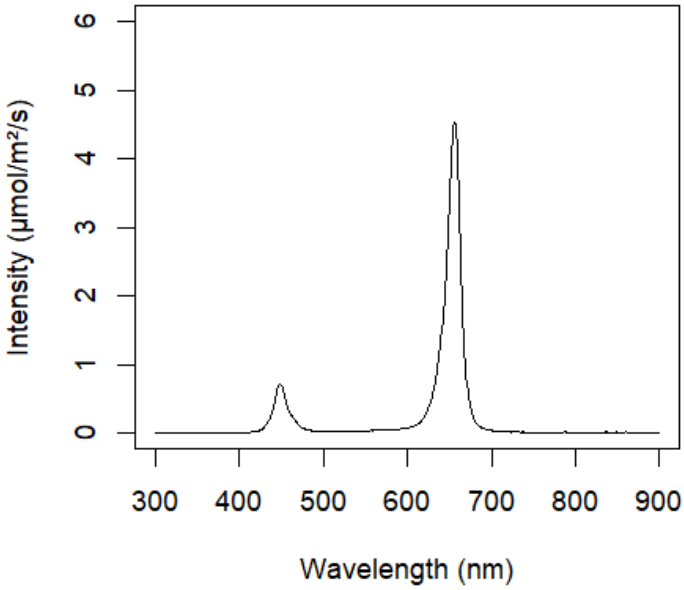

Figure 1d: Average spectrum of eight plant locations for the 90:10 R:B lighting treatment.

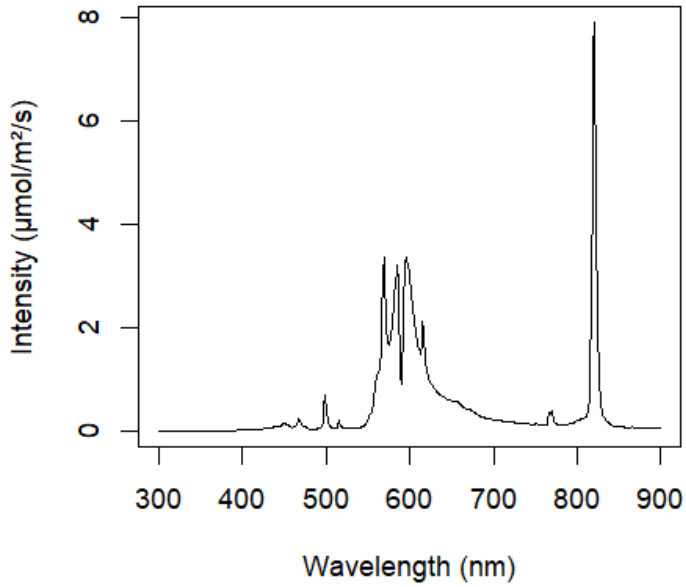

Figure 1d: Average spectrum of eight plant locations for the Control (HPS) lighting treatment.

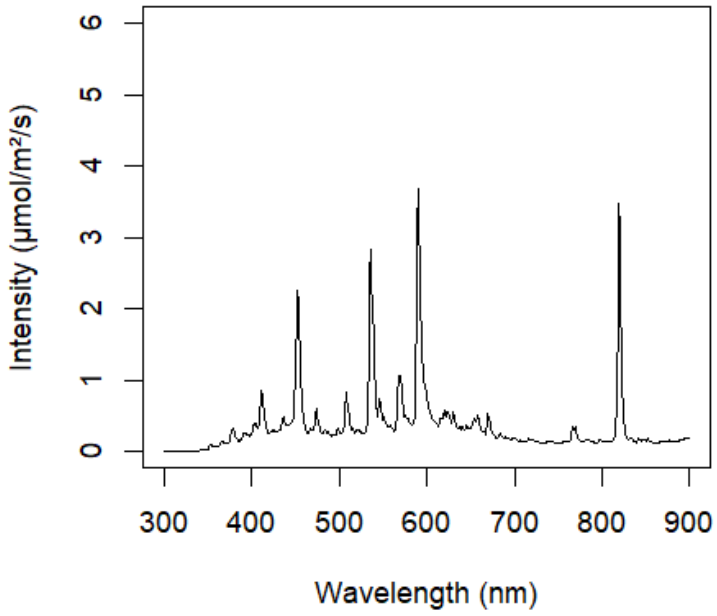

Figure 1e: Average spectrum of eight plant locations for the Metal Halide lighting treatment.

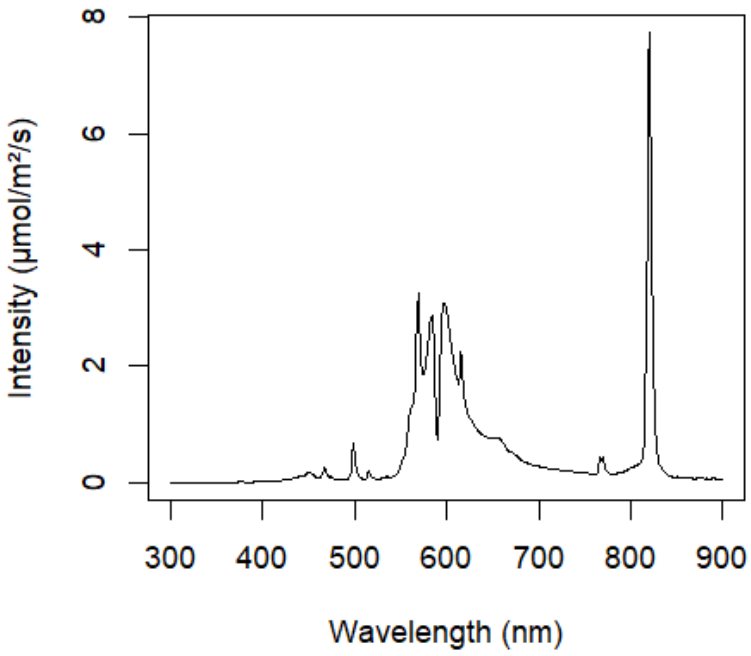

Figure 1f: Average spectrum of eight plant locations for the Shift lighting treatment. For the last two weeks of the short day light treatments, a 60:40 R:B spectrum was applied.

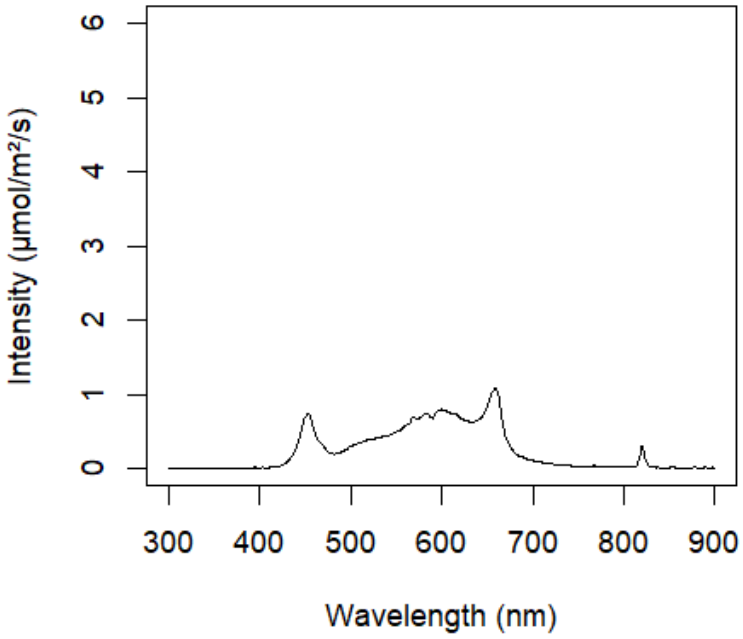

Figure 1g: Average spectrum of eight plant locations for the White LED lighting treatment.

Appendix 2: Select Project Images

Figure 2a. ‘TJ’s CBD’ placed in plant locations under supplemental lighting treatments on January 13, 2020.

Figure 2b. ‘TJ’s CBD’ fully grown on the day of the harvest, March 16, 2020.

Figure 2c. Plants hung upside down in the greenhouse to air dry after harvest on March 16, 2020.


Figure 2d. Severe nutrient deficiency, possibly a potasium deficiency, observed on the larger fan leaves.

Figure 2e. Major chlorosis after fertilizer treatments were stopped for the flushing cycle prior to harvest. Taken March 13, 2020.