Optimized lighting strategies to increase yield and chemical uniformity in *Cannabis sativa*

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List of Abbreviations

CBD	cannabidiol
CBDA	cannabidiolic acid
CBG	cannabigerol
CBGA	cannabigerolic acid
CEA	controlled environment agriculture
DM	dry mass
EC	electrical conductivity
FM	fresh mass
HI	harvest index
HPS	high pressure sodium
LC	lower canopy
LED	light-emitting diodes
LUE	light use efficiency
MC	middle canopy
pH	potential of hydrogen
PPFD	photosynthetic photon flux density
PUE	power use efficiency
RCBD	randomized complete block design
SD	standard deviation
THC	tetrahydrocannabinol
THCA	tetrahydrocannabinolic acid
UC	upper canopy

Abstract

Cannabinoid uniformity plays an important role on the quality and marketability of commercial cannabis. Energy consumption is a primary concern as lighting is one of the main input costs for controlled environment cannabis production systems. The objective of this study was to evaluate lighting strategies to maximize yield and uniformity of cannabinoids in cannabis plants. Four lighting treatments included high pressure sodium (HPS) toplight, light-emitting diodes (LED) toplight, HPS toplight + interlight and LED toplight + interlight. Two cycles were completed with a treatment randomization. No differences were observed in canopy temperatures between treatments. Plants grown under HPS lighting had an increase in plant height. LED treatments significantly increased dry inflorescence production compared to HPS treatments. The addition of interlighting increased biomass produced in the lower canopy resulting in a significant increase in biomass produced per plant. Significantly higher photosynthetic photon flux density (PPFD) values were achieved under LEDs which explains the increase in inflorescence production. When comparing light use efficiency (grams per mol), HPS lighting significantly increased the biomass produced per mol of light received. Further analysis of power use efficiency expressed in grams per kilowatt-hour (kWh) revealed that LEDs led to higher profitability. LED toplighting led to a 7% increase in secondary metabolite production for delta-9-tetrahydrocannabinol (THC). Lower canopy THC values were slightly increased by 7% with the use of interlighting although no significant differences were observed. LED treatments were more energy efficient at producing grams of THC when analyzing THC power use efficiency. Cannabigerol (CBG) concentrations were significantly increased in LED treatments. Major terpenes had no significant differences between treatments. This study investigated the added benefit from implementing interlighting and LED toplighting in indoor controlled environment cannabis production systems. LED toplight and interlight strategies show potential to compete with conventional HPS lighting strategies.

Résumé

L'uniformité des cannabinoïdes joue un rôle important sur la qualité et la valeur marchande du cannabis récréatif. La consommation d'énergie devient une préoccupation majeure car l'éclairage est l'un des principaux coûts des systèmes de production de cannabis en environnement contrôlé. L'objectif de cette étude est d'évaluer les stratégies d'éclairage pour maximiser le rendement et l'uniformité des cannabinoïdes dans les plantes de cannabis. Quatre traitements d'éclairage inclus une lampe horticole classique de sodium à haute pression (HPS) éclairage suspendue dont la lumière vient du haut, la lumière de diode électroluminescente (DEL) éclairage suspendue, HPS éclairage suspendue + intercalaire et DEL éclairage suspendue + intercalaire. Deux cycles ont été complétés avec une randomisation des traitements. Aucune différence n'a été observée dans les températures des feuilles supérieures entre les traitements. Les plantes cultivées sous éclairage HPS ont eu une augmentation de la hauteur des plantes. Les traitements DEL ont considérablement augmenté la production d'inflorescences sèches par rapport aux traitements HPS. L'ajout d'un éclairage intercalaire dans le bas des plantes a augmenté la biomasse produite dans l'intérieur du feuillage, entraînant une augmentation significative de la biomasse produite par plante. Des valeurs de densité de flux de photons photosynthétiques (DFPP) significativement plus élevées ont été obtenues sous les lumières DEL, ce qui explique l'augmentation de la production d'inflorescences. En comparant l'efficacité d'utilisation de la lumière exprimée en grammes par mole, l'éclairage HPS ont significativement augmenter la production de biomasse par mole de lumière. Une analyse plus approfondie de l'efficacité de la consommation d'énergie exprimée en grammes par kilowattheure (kWh) a révélé que les DEL était plus rentable. L'éclairage DEL a entraîné une augmentation de la production de métabolites secondaires du delta-9-tétrahydrocannabinol (THC). Les valeurs de THC obtenu du bas des plantes ont légèrement augmenté avec l'utilisation de l'inter-lumière, mais aucune différence significative n'a été observée. Les traitements DEL étaient plus efficients en énergie pour la production totale de THC. Les concentrations de cannabigérol (CBG) ont significativement augmentées dans les traitements DEL. Aucune différence significative n'a été observé entre les traitements pour les principaux terpènes. Cette étude a examiné les avantages de la mise en œuvre d'éclairage combinant l'éclairage intercalaire et l'éclairage suspendue DEL dans les systèmes de production de cannabis en environnement contrôlé. Les stratégies d'éclairage DEL suspendue et en intercalaire ont un potentiel de concurrence avec les stratégies d'éclairage HPS conventionnelles.

Contribution of Authors

Justin Bohemen carried out the room preparation according to the design. Dr. Mark Lefsrud contributed to the design and conception of the experiment in addition to the editing of the document. Justin Bohemen performed the experiment, analysis and writing of the paper. Justin Bohemen managed the plants daily. Dr. Philip Wiredu contributed to the editing of the document. Vincent Desaulniers-Brousseau contributed to the editing of the document. Dr. Sabrina Carvalho contributed to the editing of the document.

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Conflict of Interest

The authors have no conflict of interest to declare.

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Chapter 1. Introduction

Cannabis sativa can be traced back to Central Asia nearly 12,000 years ago where it was cultivated for its fibers and psychoactive properties (Crocq, 2020). The term cannabis is used to describe the female cannabis plants which produce the psychoactive and therapeutic compounds commonly used for consumption. *C. sativa* is divided into three subspecies which include *C. sativa* spp., *C. indica* spp. and *C. ruderalis* spp. (McPartland, 2018). The most known subspecies are sativa and indica which are differentiated with distinct physiological traits and varying secondary metabolite profiles. Selective crossbreeding between subspecies has made their distinctions nearly meaningless (McPartland, 2017). C. *ruderalis* spp. has the unique ability to initiate flowering without reference to photoperiod (McPartland, 2018).

Canada legalized cannabis for medical use in 1999 (Hawley et al., 2020). Recreational cannabis was legalized in 2018 making Canada the second country in the world to achieve this status. The production and sale of cannabis were controlled by implementing a strict legal framework (Hawley et al., 2020). Controlled environment agriculture (CEA) has been widely adopted to increase efficiency and output through year-round production. CEA is considered one of the key factors that will influence the transition to a greener and more sustainable future (Cohen et al., 2022). There is an urge to increase energy efficiency regarding electrical light provided for photosynthesis (Vatistas et al., 2022).

Cannabis inflorescences contain several specialized secondary metabolites produced in glandular trichomes such as cannabinoids and terpenes (Livingston et al., 2020). Cannabinoid precursor molecules, olivetolic acid and geranyl-pyrophosphate (GPP), are synthesized from the polyketide pathway and the deoxyxulose phosphate/methyl-erythritol phosphate (DOXP/MEP) pathway respetively (Flores-Sanchez & Verpoorte, 2008). Cannabigerolic acid (CBGA) is formed by the geranylation of olivetolic acid by GPP. CBGA is converted to either cannabidiolic acid (CBDA), Δ9-tetrahydrocannabinolic acid (Δ9-THCA), or cannabichromenic acid (CBCA) by oxidocyclase enzymes. Acidic cannabinoids undergo decarboxylation to neutral cannabinoids (Jin et al., 2020).

In 1964, Δ^9-tetrahydrocannabinol (THC) was discovered by Mechoulam and Gaoni in Israel (Gaoni & Mechoulam, 1964). This cannabinoid is known for its psychoactive effects which

are produced by interacting with endocannabinoid receptors (Livingston et al., 2020). Cannabidiol (CBD) is the therapeutic cannabinoid that has been gaining popularity recently due to its medicinal properties (Vuckovic et al., 2018). CBD has little binding affinity for cannabinoid receptors (CB_1 and CB_2 receptors) limiting the undesirable psychotropic effects such as euphoria, anxiety and paranoia (Volkow et al., 2014). CBD acts as an anti-inflammatory, antiepileptic, and anxiolytic molecule (Bridgeman & Abazia, 2017). Cannabigerol (CBG), an important cannabinoid, has been studied for its potential antimicrobial activity and use for treating neurological disorders and inflammatory bowel disease (Nachnani et al., 2021).

Approximately 140 terpenes have been identified, which contribute to the enhanced aromas that are specific to certain cannabis accessions (Goncalves et al., 2019). Although several terpenes have been quantified in cannabis, there is still the need to further characterize these secondary metabolites (Desaulniers Brousseau et al., 2021). Sequence analysis of 5 accessions identified 33 cannabis terpene synthase (CsTPS) genes that produce mono and sesquiterpenes which have an important effect on resin glands which alter the quality of cannabis inflorescence (Booth et al., 2020). Important monoterpenes include α-pinene, β-myrcene, limonene and linalool. Major sesquiterpenes include α-humulene and β-caryophyllene (Sommano et al., 2020). β-Caryophyllene has potential impacts on several chronic diseases such as mental health disorders, type 2 diabetes, chronic pain, and neurodegenerative disorders, however more human trials are required to validate effectiveness (Blake, 2021). Several studies have reported that α-Humulene has anti-tumor activity as well as anti-inflammatory and antimicrobial activities (Mendes de Lacerda Leite et al., 2021). Limonene is a monoterpene that has pharmacological activities involved in the therapeutic and protective ability of neurodegenerative disorders (Eddin et al., 2021). Linalool and pinene have the potential to treat psychiatric and neurological illnesses although evidence is limited to preclinical trials; well designed clinical trials are needed to assess effectiveness (Weston-Green et al., 2021).

Accumulation of secondary metabolites in glandular trichomes is affected by genetic and environmental factors (Bernstein et al., 2019a). Plant genetics determine the production potential while environmental conditions govern the quantity, quality, and distribution of secondary metabolites within the plant (Bernstein et al., 2019a). The main environmental factors including light type and intensity, plant density, fertilization, pot size and length of flowering period will

have an impact on the yield, THC content and distribution of cannabinoids for cannabis plants (Backer et al., 2019).

The number of licensed producers nationwide has steadily increased since legalization, now counting over 800 (Government of Canada, 2022). Energy consumption has become a primary concern as lighting is one of the major input costs for indoor cannabis production (Rodriguez-Morrison et al., 2021a). LEDs have been identified as a way to increase energy efficiency in cannabinoid production but needs additional tuning to successfully implement this energy efficient lighting system to alleviate the increasing energy demand (Morello et al, 2022). Lighting plays a major role on biomass production and potency in cannabis emphasizing the importance of selecting the appropriate lights for an indoor facility (Rodriguez-Morisson, 2021). Inflorescence and phytocannabinoid yield appear to have an inverse relationship where cannabinoid concentrations are reduced with an increase in yield caused by the dilution effect (Trancoso et al., 2022).

Cannabinoid uniformity is an important factor that impacts the marketability of the final product (Danzinger & Bernstein, 2021b). The levels of secondary metabolites produced by plants are not governed solely by its genetic background (Backer et al., 2019). Several other environmental conditions such as light intensity, light spectrum, mineral nutrition, and elicitation of stress impact the production of secondary metabolites (Backer et al., 2019). Stress-related examples include drought stress, insect herbivory and extreme heat which were shown to have differing outcomes on secondary metabolite production (Park et al., 2022). Chemical profile variability has been observed at differing heights in cannabis plants (Bernstein et al., 2019b). Local environments sensed by plant organs will impact their physiology and production of secondary metabolites. Inter-shoot shading is one of the main causes of micro-climate variability within the plant canopy which in turn decreases cannabinoid uniformity (Danzinger & Bernstein, 2021b). Danzinger and Bernstein in 2021 showed that low cannabinoid concentrations in the lower inflorescence is correlated to low light penetration. Methods used to increase cannabinoid concentrations in the lower canopy include defoliation and the removal of bottom branches (Daninger & Bernstein, 2021b). These strategies can be used with large cannabis plants to increase cannabinoid standardization (Danzinger & Bernstein, 2021b).

With overhead lighting strategies, the quantity of light that penetrates the lower canopy is limited resulting in lower yields and THC content. A combination of lighting strategies that favor higher light intensities in the lower canopy of cannabis plants would increase cannabinoid uniformity (Nicole-de Groot, 2023). Biomass production is expected to increase due to higher light intensities. Increasing light intensities results in a linear increase in inflorescence density and a higher proportion of marketable aboveground biomass which in return lowers the need to remove unmarketable product in the lower canopy facilitating the cultivation process (Rodriguez-Morrisson, 2021a).

The objective of this study was to evaluate different lighting systems on cannabis and the cannabinoid profile. Specifically, to determine the added value of implementing LED lighting for a controlled environment cannabis production system. The study evaluated the effects of toplight LEDs and interlight LEDs on biomass yield and cannabinoid content of inflorescence and explored different lighting strategies to increase uniformity of cannabinoids spatially in cannabis plants.

Hypothesis

1) LEDs will increase THC concentrations with similar marketable dry inflorescence mass compared to HPS lighting systems.
2) Interlighting strategies will increase inflorescence production as well as cannabinoid uniformity in cannabis plants.

Chapter 2. Literature review

Photosynthesis is a plant process that involves numerous steps to absorb, transfer and store solar energy to be used by plants as carbohydrates (Tanaka & Makino, 2009). This complex reaction takes place within the chloroplasts (Tanaka & Makino, 2009). Plant pigments are categorized into two groups known as vital pigments (Chlorophyll a) and accessory pigments (Chlorophyll b, carotenoids and phycobilin) (Kochhar & Gujral, 2020). Different wavelengths of light are absorbed by pigments according to their specific absorption spectrum (Tanaka & Makino, 2009). For example, the peak absorption for chlorophyll a is 449 nm and 660 nm while chlorophyll b is at 453 nm and 642 nm. Other accessory pigments such as carotene and xanthophyll have their peaks at 440 nm and 470 nm (Kochhar & Gujral, 2020). Photosystems I and II are responsible for harvesting photons and using light energy to generate high energy compounds such as NADPH and ATP by extracting electrons from water (Caffari et al., 2014). The preliminary reaction takes place in PSII where in the presence of light energy, 2 H_2O + CO_2 is converted to O_2 and energy in the form of an electron (Caffari et al., 2014). The electron is then transported to PSI through the electron transport chain to form ATP and NADPH (Caffari et al., 2014). These high energy compounds are used to fix carbon dioxide by the chloroplast's metabolism (Caffarri et al., 2014). Carbon assimilation by fixation of CO_2 is catalyzed by the enzyme Rubisco which plays an important role in plant photosynthesis (Tanaka & Makino, 2009).

Light intensity is measured as photosynthetic photon flux density (PPFD) which includes the quantum units of moles of photons in the wavelength range of 400-700nm (photosynthetically active radiation; PAR) per surface area per time (Wu et al., 2019). Cannabis (*Cannabis sativa*) responds well to increasing light intensity values although there is a trade-off point between crop productivity and input costs which make it critical to find the optimal light intensity for a specific growing environment (Rodriguez-Morrison, 2021a). Typically, inflorescence production increases with increasing light intensity for cannabis and far exceeded the added cost of electricity (Eaves et al., 2019). Inflorescence mass increases in a linear trend up to light intensities of 1800 µmol m^{-2} s^{-1} although leaf level photosynthesis saturated well below this level (Rodriguez-Morrison, 2021a). Chandra et al. (2008) reported that temperatures ranging between 25 to 30 ℃ and a light intensity of ~1500 µmol m^{-2} s^{-1} are the optimal environmental conditions for *C. sativa*.

Plants absorb light which drive the photosynthetic process with wavelengths from 400 to 700 nm (PAR) (McCree, 1972). This range includes violet (~400-450 nm), blue (~450-520 nm), green (~520-560 nm), yellow (~560-600 nm), orange (~600-625 nm), and red (~625-700 nm) (McCree, 1972; Eichorn Bilodeau et al., 2019). McCree established the basis of understanding PAR with his findings in the 1970s (McCree, 1972). Several factors such as the action spectrum, absorptance and spectral quantum yield of CO_2 were measured for 22 plants species which found 2 peak absorbance areas at 440 and 620 nm (McCree, 1972). The photosystems involved in photosynthesis contain chlorophyll and carotenoid antenna pigments responsible for capturing and redirecting light optimally (Caffari et al., 2014). Chlorophyll a and b have a higher absorbance in the red and blue wavelengths than in the green (Eichorn Bilodeau et al., 2019). The optimal spectrum required for all stages of cannabis growth to achieve the optimal yield is not fully understood (Backer et al., 2019). Red and amber seem to be optimal in driving vertical growth in vegetative stage, whereas blue wavelength drives phytochemical accumulation (Morello et al, 2022).

Plants can activate a network of photosensory pathways based on light quality and quantity (Folta & Carvalho, 2015). Photomorphogenesis is an important plant process that causes differences in plant development because of light (Eichorn Bilodeau et al., 2019). It is regulated by five plant photoreceptors that will impact hormone levels and in return mediate alterations in metabolism, plant morphology, physiology, and development (Folta & Carvalho, 2015). Photoreceptor pigments have specific wavelength absorbance patterns which impacts their ability to absorb different light spectrums (Kang et al., 2020). Phytochromes (PHYs) are responsible for absorbing red (R) and far red (FR) wavelengths (Li et al., 2011). FR photons have been well studied for their effect on stem elongation due to increased auxin production (Zhen et al., 2021). Low red:far-red ratios are important in altering photomorphogenesis due to shade type morphology which induces etiolation for plants to extend past neighboring plants (Franklin and Whitelam, 2005). Cryptochromes (CRYs), phototropins (PHOTs) and the Zeitlupe Family Proteins (ZTL/FKF1/LKP2) absorb light in the blue and ultraviolet-A (UV-A) wavelengths. UV resistance Locus 8 (UVR8) absorbs ultraviolet B (UV-B) wavelength (Paradiso & Proietti, 2022).

Several electrical lighting systems can be used for indoor cannabis production, such as incandescent bulbs, fluorescent bulbs, high pressure sodium (HPS) lamps, and LEDs (Eichorn

Bilodeau et al., 2019). Traditionally, high pressure sodium (HPS) lighting has been adopted by the cannabis industry due to its high light intensity and uniform light distribution over large areas (Nelson & Bugbee, 2014). HPS lights emit radiant heat which can help reduce heating costs for facilities and greenhouses (Katzin et al., 2021). There have been improvements over time with HPS lighting, where double-ended HPS fixtures with electronic ballasts are 1.7 times more efficient than earlier based HPS fixtures (Nelson and Bugbee, 2014). However, there still are several disadvantages associated with HPS lighting including high energy consumption, frequent bulb changes, and reduced useful life with excessive voltage (power surges) and frequent starts (Eichorn Bilodeau, 2019). HPS lights span the entire spectrum of sunlight although they have higher light output in specific regions (Amrein et al., 2020). They are unable to modulate their spectrum (Dutta Gupta & Agarwal, 2017). Light in the yellow (560-600 nm), red (600-700 nm) and infrared spectrum (700-830 nm) are the most prevalent (Amrein et al., 2020). Blue spectrum (400-500 nm) is contained in smaller quantities (Amrein et al., 2020). HPS fixtures are not placed near the canopy due to their high operating temperature and are a fire hazard due to the presence of pressurized gas in a glass bulb (Paradiso & Proietti, 2022).

The use of high-powered LED lighting has been expanding in most indoor growing environments due to its advantages over HPS lighting such as longer lifespan, improved carbon footprint, efficient energy utilization, increased safety due to solid state, lower heat emission and narrow spectral emission (Magagnini et al., 2018; Paradiso & Proietti, 2022; Eichorn Bilodeau et al., 2019). Many studies have been conducted on the response of several plant species under LED lighting (Lazzarin et al., 2021; Massa et al., 2008; Rahman et al., 2021;). This lighting technology allows producers to control light intensity and spectrum with dimmable features to match the desired spectral quality needed at various stages of the plant's growth to modify the production of inflorescence mass and secondary metabolites (Darko et al., 2014). Due to reduced heat loads, LEDs can easily be placed close to or within the plant canopies (Paradiso and Proietti, 2022). Historically, LEDs have been estimated to cost approximately 5 to 10 times more as an initial investment, based on the capital cost per deliverable photon, compared to HPS lighting (Nelson & Bugbee, 2014). The trade off for this initial investment is the reduction of long-term costs associated with maintenance and replacement of bulbs due to their longer lifespan (Gomez & Izzo, 2018). On average, LEDs can last for approximately 50,000 to 100,000 hours while HPS can last up to 24,000 hours (Danila & Lucache, 2013). LED fixtures reduce energy consumption with

increased efficiencies of 3.4 µmol J^{-1} for white and red fixtures and 4.1 µmol J^{-1} for blue and red fixtures (Kusuma et al., 2020). This new technology outperforms 1000W double ended HPS fixtures which have efficiencies of 1.7 µmol J^{-1} (Kusuma et al., 2020). LED lights emit a limited amount of radiant heat which creates a larger dependence on heating sources which could lead to an increase in cost for controlled environment agriculture (CEA) (Katzin, 2021). Under LEDs, leaf temperature is reduced by 1.3℃ on average when compared to HPS lighting causing a possible undesired reduction in transpiration (Nelson & Bugbee, 2015).

A study by Magagnini et al. (2018) compared HPS lighting to AP673L and NS1 LED fixtures made by Valoya (Helsinki, Finland). The plants under HPS had an increase in yield and height. The plants grown under LED's had higher CBD and THC concentrations (Magagnini et al., 2018). Namdar et al. (2019) showed that, cannabis plants grown under blue-enriched LED fixtures (Jugano Ltd, Tel Aviv, Israel) with a blue-to-red ratio of 4:1 had increased CBGA accumulation and derived products increased in cytotoxic activity (Namdar et al., 2019). These studies demonstrated that current LED technologies had significant differences in chemical profiles and morphological growth compared to HPS lighting (Magagnini et al., 2018, Namdar et al., 2019). In a study by Wetsmoreland et al. (2021), yield differences were correlated to the percentage of blue photons in the light source used therefore several plant light interactions still need to be understood.

Plant canopies receive light radiation as direct or diffuse light (Li & Yang, 2015). Direct light emits photons which are not scattered by the environment (Li & Yang, 2015). Whereas diffuse light is scattered by the specific light source before it reaches the canopy (Iqbal, 1983). Diffuse light has been reported to improve photosynthetic rates and productivity since it penetrates deeper and more uniformly into the canopy (Li & Yang, 2015). Leaves in the lower canopy receive higher light intensities with diffuse light (Li & Yang, 2015). Certain LEDs use diffusers to produce diffuse light. HPS fixtures use a reflector to redirect the emitted light towards the plants creating a diffuse light. Direct light creates shading for the lower canopy which in turn reduces light intensity in the lower canopy (Li et al., 2014).

Diffuse and direct light penetrate leaf tissues at different depths (Li & Yang. 2015). Leaf tissue absorption is strongly influenced by wavelength, leaf anatomy and direction of light (Brodersen & Vogelmann, 2010). Incident light rays perpendicular to the leaf penetrate deeper

than light rays at lower angles (<60°) (Brodersen & Vogelmann, 2010). An increase in light penetration depth facilitates the channeling of light into the mesophyll thus increasing photosynthetic capacity (Brodersen & Vogelmann, 2010).

Cannabis has been traditionally grown using overhead lighting strategies (Nelson & Bugbee, 2014). Inefficiencies occur in the vegetative stage as light can decrease within the plants and is not used for photosynthesis (Massa et al., 2005). As the plants grow, shading occurs creating a gradient in light intensity between the upper and lower canopies (Massa et al., 2005). Adding additional overhead lighting might cause light saturation in the upper canopy where absorption of the emitted rays happen before reaching the lower canopy (Massa et al., 2005). Danzinger and Bernstein (2021b), showed that the upper inflorescence produce higher quantities of cannabinoids compared to inflorescence located lower along the stem. This has a very important impact on the standardization of cannabis products for medical patients (Namdar et al., 2018). New technologies such as interlighting LEDs can easily be positioned within the canopy to increase light intensity in the lower canopy (Nicole-de Groot, 2023).

Biomass production increased by 33% with the use of interlights in a cowpea crop (Massa et al., 2005). In soybeans, crop productivity was increased by 23-87% by emitting light to the inner canopy (Stasiak et al., 1998). The use of LED interlighting has been studied on fruit and vegetable crops showing quality and biomass optimization in *Solanum lycopersicum* (tomato) (Paponov et al., 2020, Deram et al., 2014), *Capsicum annuum* (sweet pepper) (Guo et al., 2016; Ratner et al., 2020) and *Cucumis sativus* (mini cucumber) (Hao et al., 2015). Interlighting has yet to be studied extensively for its potential in controlled environment cannabis systems. A fraction of the light emitted by interlights is absorbed by the abaxial side of leaves (Paradiso & Marcellis, 2012). Research studies on rose plants demonstrated that higher net photosynthetic rates were achieved on the adaxial side of leaves rather than the abaxial side (Paradiso & Marcellis, 2012). Nonetheless, supplementing light to the lower canopy helps to homogenize the light intensity spatially optimizing photosynthesis in local environments to increase secondary metabolite standardization (Terashima & Hikosaka, 1995).

In a study conducted by Hawley et al. (2018), the use of LED lighting as a subcanopy lighting strategy was tested. The results seemed promising with an increase in inflorescence mass and accumulation of specific cannabinoids of interest to the market (THC) although further

research needs to be carried out to determine the benefits that new lighting strategies may bring to the industry (Hawley et al., 2018). The authors list future research opportunities which include supplemental lighting used within the crop (interlighting) and modified overhead light sources (Hawley et al., 2018).

Chapter 3. Materials and methods

3.1 Plant materials

Two 3-month growing cycles were performed with identical procedures and a randomization of treatments. Two hundred and eighty-eight clones of *Cannabis sativa* 'Kush Mint F2' (Chemotype 1 THC dominant) were propagated by cuttings from mother plants. Cuttings were dipped in Technaflora Rootech cloning gel with 0.55% Indole-3 Butyric Acid (IBA) (Technaflora, Mission, B.C., Canada). Cuttings were rooted in trays of 72 Jiffy Preforma plugs (Winnipeg, MB, Canada). Cuttings were placed under domes with a 24 h d^{-1} photoperiod supplied by a Lightstick LED grow light EDK 48W at an intensity of 94 ± 10 µmol m^{-2} s^{-1} for 15 days. PPFD was measured using a LI-COR LI 250A light meter (Lincoln, Nebraska, USA). Rooted cuttings were transplanted to a starter phase in 1 L pots with PRO-MIX HP mycorrhizae peat based growing medium (Premier Tech, Rivière-du-Loup, QC, Canada) with the addition of 5 ml of Dynomyco mycorrhiza inoculant (Mazor, Israel). Plant population was reduced to 192 plants based on uniformity. Plants were grown in pre-vegetative stage for 23 days under a mixture of Lightstick LED grow light EDK 48 W and Sunblaster EDJT5 96W grow lights with nanotech T5 reflectors (Langley, BC, Canada). The plants were exposed to 18 hr d^{-1} photoperiod with a light intensity of 273 ± 26 µmol m^{-2} s^{-1}. The pre-vegetative plants were transplanted in 8.5 L pots in their final production room. Each treatment contained plants that were obtained from identical mothers.

3.2 Cultivation conditions

Figure 1 demonstrates the cultivation parameters and plant maintenance interventions at the various stages of the experiment. Table 1 presents the environmental parameters used during different stages of plant growth.

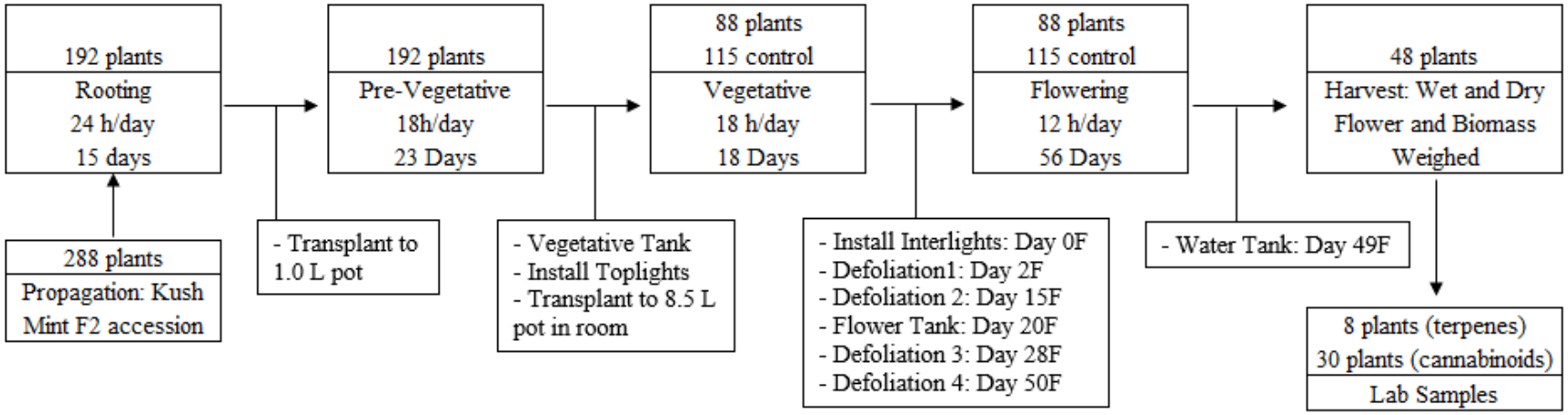

Figure 1. Cultivation parameters and plant maintenance interventions in each stage.

Stage	Temperature (°C)	Relative humidity (%)	Light intensity (µmol)	Carbon dioxide (ppm)	Photoperiod (h 24^{-1})
Mothers	23	55	344 ± 22	400	18
Rooting	23	55	94 ± 10	400	24
Pre-vegetative	23	55	273 ± 26	400	18
Vegetative	24	50	Variable	750	18
Flowering	24	50	Variable	1000	12

Table 1. Environmental conditions during every stage of growth

Every treatment was separated by 2 pieces of polyethylene film along the ends (1.2 m x 1.8 m) and along the inside edge (0.8 m x 3.0 m). A picture of the polyethylene film layout is included in Figure B1 (Appendix B). Stray light was measured with all the lights on except for the specific zone being tested. Light interference accounted for an average of 15%. A slight gap of 32 cm was left for ventilation purposes under the polyethylene film at the ends of each block. Nets were installed as a support structure to avoid broken stems due to the mass of the inflorescence. Once the nets were installed, the polyethylene films were raised above the net at a height of 39 cm above the base of the plant.

The plants remained in the vegetative stage with a photoperiod of 18 h d^{-1} for 18 days. The flowering stage was initiated through photoperiod modifications. The flowering plants received 12 h d^{-1} photoperiod. The growing area for each treatment was 3.0 m x 1.2 m. Each block had 11 plants resulting in a final plant density of 3.1 plants per m^2. Images of the plant life cycle taken at 2 week-intervals are presented in Figure B2 (Appendix B). Nets were installed on the first day of the flowering stage at a height of 50.8 cm from the table. During the net installation, final plant height was unknown which led to the assumption that 2 nets would be required to support the plants.

The interlights were installed 5.1 cm above the first net to facilitate logistics. The plants grew above the interlights requiring a guidance intervention to strategically position branches around the interlights 14 days into the flowering stage. Only one layer of netting was required to support the inflorescence. The interlight setup is presented in Figure B3 of the Appendix. The environmental parameters in the room (temperature and relative humidity) were measured with data loggers approximately 6 cm above the canopy level for each plot (Table 2). Environmental parameters in the intercanopy were not measured due to data logger availability.

The first experiment took place from May to July 2022 and the second experiment was conducted between August and October 2022. Most environmental observations were similar between both cycles. There was one exception where reduced height and cooler canopy temperatures were observed during the data collection at day 14 of the flowering stage. Temperature setpoints were increased by 1°C for 14 days in the second growing cycle to account for the shorter growth rate. The setpoint was returned to its original setting after the plants measured a comparable height to the first growing cycle.

	Day		Night	
	Temp. (℃)	R.H. (%)	Temp. (℃)	R.H. (%)
Cycle 1	25.8 ± 0.5	48.6 ± 4.4	22.6 ± 1.6	46.3 ± 3.3
Cycle 2	26.0 ± 0.2	48.6 ± 2.9	21.6 ± 0.2	46.5 ± 1.2

Table 2. Average day and night temperature values for both growing cycles. Data is presented as mean ± standard deviation (SD) (n=5).

All treatments received four defoliation events. With reference to a study by Danzinger and Bernstein (2021b), this experiment used the bottom branches and leaves removal (BBLR) plus defoliation. The first defoliation process was done two days into the flowering stage. It involved biomass removal from the bottom (13 cm above the base) of the plant and three fan leaves at the top of the plants to increase light penetration. A second defoliation intervention was performed 15 days into the flowering phase. It consisted of a final lower biomass removal. Quantities removed varied based on treatments. Treatments without interlight were defoliated up to 37 cm from the base of the plant to replicate a conventional production where light is only provided above the plants. Treatments with interlight were minimally defoliated up to 15 cm below the interlight to allow lower canopy inflorescence to develop. The third defoliation event occurred 28 days into the flowering stage. A small portion of large fan leaves were removed evenly for all treatments. The defoliation procedure followed industry standards to increase light levels and air movement (Danzinger and Bernstein, 2021b). Treatments with interlight had larger quantities removed due to the additional biomass left at the previous intervention 15 cm below the interlight. The fourth defoliation event removed all fan leaves in preparation for harvest.

3.3 Fertigation

A fertigation system was used for all stages of growth. Fertilizers were applied through the irrigation network. During the cloning phase, Cyco nutrient B1 boost (Vancouver, WA, USA) and Canna Start (Oosterhout, NB, NL) were used at recommended rates of 1 mL L^{-1} and 4 mL L^{-1} respectively. Cyco nutrients A (1.5 mL L^{-1}), B (1.5 mL L^{-1}) and B1 Boost (1 mL L^{-1}) (Vancouver,

WA, USA) were used during the pre-vegetative phase. The plants followed a personalized fertilizer recipe formulated by Plant Products (Leamington, Ontario, Canada) using a dripper irrigation system at a set time through several pulses daily once transplanted into the flowering room. At every stage of growth, irrigation amounts were determined based on a 20 % runoff target. Irrigation ranged between 1.0 - 2.5 L day^{-1} during both cycles. The plants received a vegetative formulation containing potassium nitrate, calcium nitrate, magnesium sulphate, mono potassium phosphate, iron chelate, manganese sulphate, sodium tetraborate, zinc sulphate, sodium molybdate and copper sulphate for the vegetative phase and the first 3 weeks of flowering. The vegetative tank was set to an EC of 1.8 and a pH of 5.9. Macronutrient concentrations in the vegetative formulation after laboratory analysis were 142 ppm of nitrogen, 49 ppm of phosphorus and 233 ppm of potassium. Micronutrient concentrations consisted of 167 ppm of calcium, 77 ppm of magnesium, 14 ppm of sodium, 0.6 ppm boron, 0.2 ppm copper, 2.5 ppm iron, 1.1 ppm manganese, <0.1 ppm molybdenum (0.04), 0.7 ppm zinc, 318 ppm sulphate, 14 ppm sodium and 3 ppm of silicon. After the third week of flowering, plants received a flowering formulation until the last week before harvest. The flowering formulation contained calcium nitrate, epsom salts (magnesium sulphate), potassium sulphate, mono potassium phosphate, iron chelate, manganese sulphate, sodium tetraborate, zinc sulphate, sodium molybdate and copper sulphate. The flowering tank was set at an EC of 1.8 mS cm^{-1} and a pH of 5.9. Macronutrient concentrations in the flowering formulation were 82 ppm of nitrogen, 83 ppm of phosphorus and 322 ppm of potassium. Micronutrient concentrations consisted of 175 ppm of calcium, 76 ppm of magnesium, 16 ppm of sodium, 0.7 ppm boron, 0.3 ppm copper, 2.7 ppm iron, 1.2 ppm manganese, 0.1 ppm molybdenum, 0.8 ppm zinc, 564 ppm sulphate, 16 ppm sodium and 3 ppm of silicon. The plants were watered using fresh water with no fertilizer for the last week prior to harvest. Identical fertilizer regimes were maintained during the entire growth cycle for all treatments to reduce the number of variables in the study. The treatments with higher light intensities may have benefited from increased fertilizer rates, but were not performed in this study. Based on a study by Bevan et al. (2021), the optimal nitrogen and phosphorus rates for soilless cannabis production is 194 and 59 ppm respectively. Several studies have observed a yield increase with high nitrogen rates which comes at the expense of lower cannabinoid production (Dilena et al., 2023; Caplan et al., 2017; Saloner & Bernstein, 2021).

3.4 Light treatments

Plants were grown under four different lighting treatments: HPS toplight, LED toplight, HPS toplight with LED interlight and LED toplight with LED interlight treatments. The control in the experiment was overhead HPS treatment which is the current lighting strategy employed at ROSE Lifescience Inc (Huntingdon, Quebec). Figure 2 depicts the treatment layout that was used for both cycles of the experiment. A row of HPS lights was used as control on the middle table of the experiment during both growing cycles where plants grown on this area were excluded from the analysis. A randomized complete block design (RCBD) with 2 treatment factors (toplight type and the use of interlights) was used for the study. Block effect was determined. The treatments were randomly assigned to their respective locations.

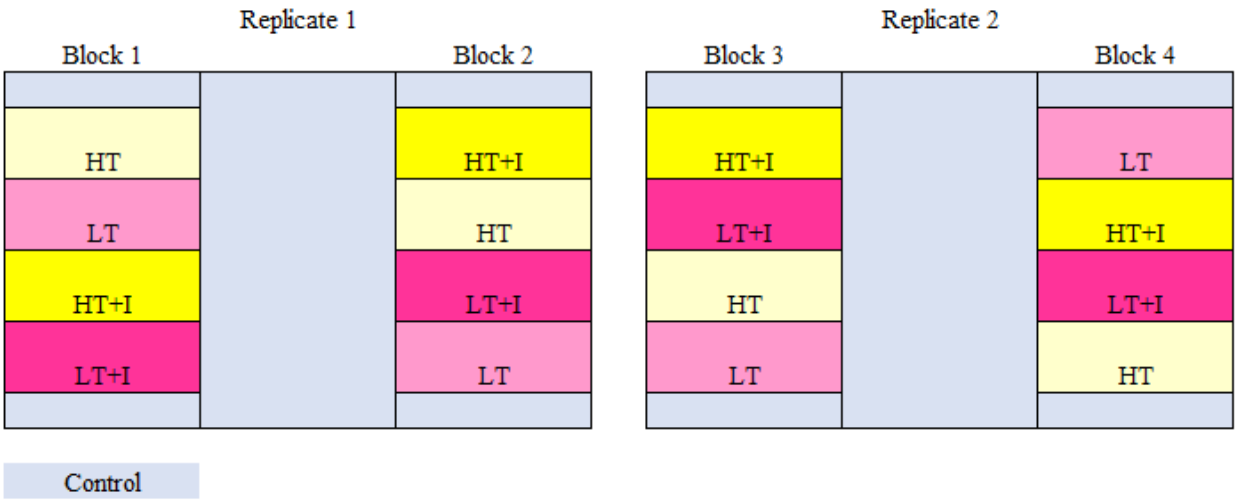

Figure 2. Treatment definitions and locations for both replicates. HT: HPS toplight (control), LT: LED toplight, HT+I: HPS toplight + interlight and LT+I: LED toplight + interlight.

Toplight and interlight LEDs were provided by Signify, Philips Horticulture LED Solutions (Eindhoven, Netherlands). The LED toplights were the Top Light Compact type. HPS luminaires used were 1000W NXT2 (P.L. Lights, Beamsville, ON, Canada). The light spectrum emitted from the LEDs can be broken down in percentages. The LEDs used were 13% blue, 5% green, 82% red and 1% far-red. The HPS lights consist of a double-ended Lightspeed 1000W HPS bulb and an aluminium reflector (Mirabel, Qc, Canada). The lights spectrum for the HPS lights were 4% blue,

45% green, 51% red and 7% far-red. GreenPower LED interlighting module 2.5-meter model were used as supplemental lighting within the canopy (Mississauga, Ontario, Canada). The interlights were specifically formulated to emit light horizontally at an angle of 180°. Treatments without interlights had a reduction in total photosynthetic photon flux density (PPFD) which is a known limitation. This study was designed to evaluate the performance of supplemental interlighting in addition to toplighting.

The plants were exposed to the toplight treatments on the day of transplant to the vegetative stage according to the layout of the experiment. Due to a limitation in the design, light output was greater for the HPS lights when comparing LED and HPS fixtures one-to-one therefore an additional LED toplight fixture was required to have comparable PPFD between both toplight types. Interlights were installed and activated on the first day of flowering. Two fixtures were installed between the three rows of plants resulting in a higher light intensity for the middle row of plants. Figure 3 below depicts the toplight and interlight orientation and plant positioning in the experimental treatments. To ensure homogeneity of treatments, harvested plants were selected based on their positioning to reduce light interference. One plant was harvested from the inside row of plants. Two plants were harvested on the row next to the wall and three plants were harvested from the middle row. Corner plants were used as a buffer for the measured plants. Plant selection varied based on block position. Harvested plants correspond to B, C, E, F, G and J positions on Figure 4.

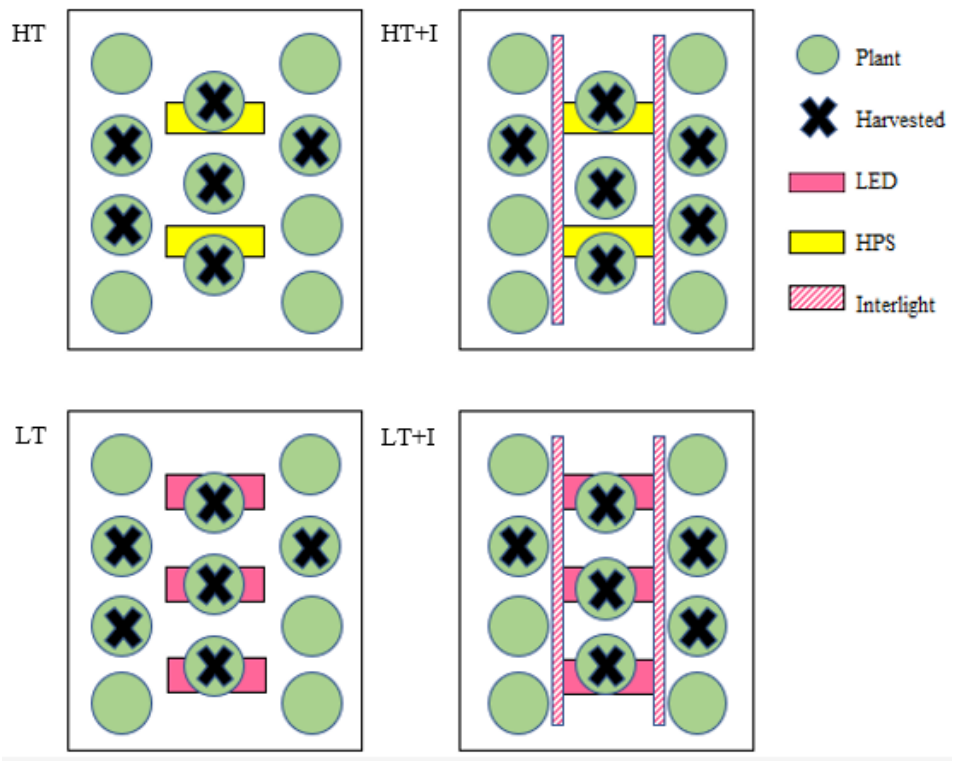

Figure 3. Light orientation and plant positioning within experimental growing area

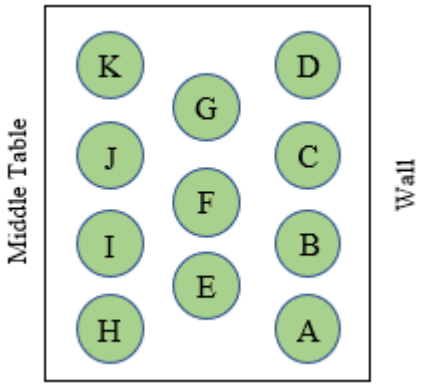

Figure 4. Plant identification within each treatment. Identical mothers were used to propagate the replicates of same letters in every treatment.

3.5 Plant measurements

Several plant measurements were collected throughout the production phase. Plant heights were measured from the base of the plant to the apical meristem using a Mastercraft measuring tape (Toronto, ON, Canada) with an accuracy of 0.1 cm. Plant height was measured on four different occasions during both growth cycles to evaluate the effect of light on plant growth rate. Plant

height measurements were taken from 6 plants per treatment, a total of 96 measurements per treatment. Measurements were taken on the day of transplant to the vegetative phase to ensure uniform plants were used, the day before flowering, and 14 and 27 days into the flowering stage. The plants had reached their final height before the final measurements.

Canopy temperatures were measured at 6 occasions per cycle using a laser infrared temperature device (2278-20, Milwaukee, Brookfield, WI, USA) for temperature variability between treatments due to light sources. Canopy temperatures were measured on the top full fan leaf on 4 plants per treatment (48 total observations per treatment). Canopy temperatures were taken on a weekly basis for the first three collection events and at 2-week intervals for another three collection events during both replicates. Room temperature and relative humidity were monitored using Omega Nomad OM-73 data loggers (Omega, Norwalk, CT, USA) placed above the apical meristem of a plant in the center of every block. Data points were recorded every 45 minutes and collected every other week for a total of 6 collections for each replicate.

Photosynthetic photon flux density (PPFD) was measured using a LI-COR LI 250A light meter (Lincoln, Nebraska, USA). Light intensity measurements were collected for every individual plant above the apical meristem. Light intensities were measured every other week for a total of 5 collections in each replicate. Eleven plants were measured per treatment for a total of 88 observations per collection. Leaf chlorophyll content was measured at the bottom, middle and top of 3 plants per treatment for a total of 72 observations per collection. This was performed on day 9 and 35 of flowering for both cycles using a SPAD 502Plus device (Chiyoda City, Tokyo, Japan). At the end of every defoliation event, fresh biomass removed was calculated for each treatment using an A&D EK-15KL scale (Ann Arbour, MI, USA) with an accuracy of 0.1 grams. At harvest, wet biomass was measured using the scale. Six plants were harvested per treatment.

Plants with interlights were partitioned into 2 segments (lower and upper canopy). The segregation was done at the level of the net. Biomass below the net was removed on treatments without interlights therefore harvested inflorescence correspond only to the upper canopy portion (Figure 5). The lower canopy segment of treatments with toplights only corresponds to the middle canopy positioning for treatments with interlights. The net was positioned 37 cm above the plants and an extra 15 cm was left below the net for LC inflorescence on the treatments with interlights. Stems were removed using a commercial cannabis bucking machine presented in Figure B4 of the Appendix (Mobius, Surrey, BC, Canada). Flowers underwent a 5-day drying process in a regulated

environment at 18 ℃ and 55 % R.H.. Flowers were dried untrimmed on baker's racks. Samples were isolated before trimming following an identical randomized sampling pattern for every treatment. Plant partitions were trimmed in individual batches using a M108S trimmer presented in Figure B4 of the Appendix (Mobius, Surrey, BC, Canada). Samples were hand-trimmed. Dry flowers were manually separated by size with a cut-off length of 2.2 cm to differentiate large and small flowers (Figure B5 in the Appendix). Dry inflorescence mass was weighed using an A&D EJ-200 precision balance (Ann Arbour, MI, USA) with an accuracy of 0.01 grams. Dried cannabis samples from 3 plants in every treatment were sent to Phytochemia Laboratories for cannabinoid and terpene analysis (Saguenay, QC, Canada). Random selection was used to create a representative sample for every treatment. Cannabinoid samples for THC, CBD and CBG comprised of eight UC samples and six LC/MC samples per treatment. For terpenes, 4 samples per treatment were quantified for a total of 88 terpenes.

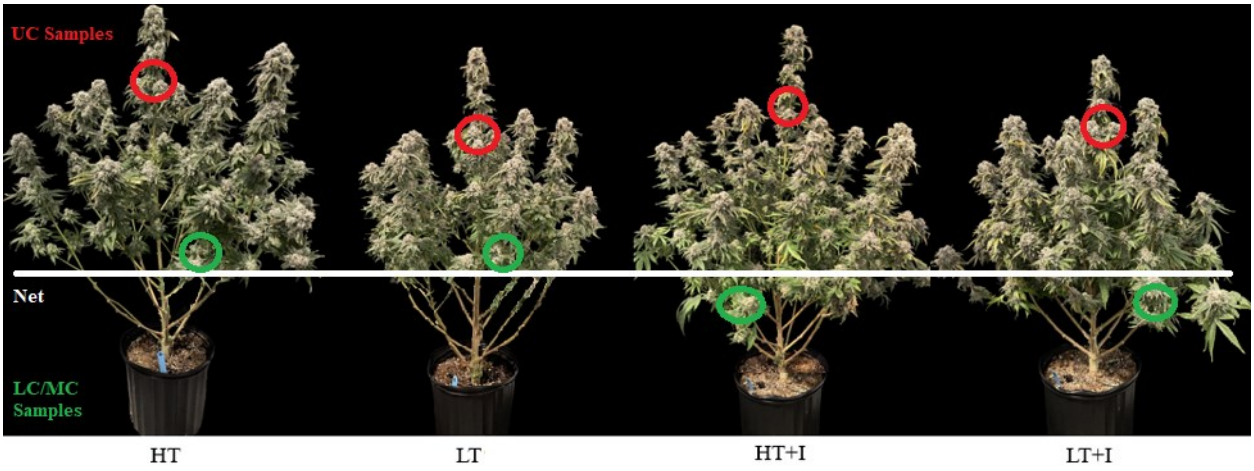

Figure 5. Sampling locations and net height for different lighting treatments. Due to increased removal of biomass on treatments without interlights during defoliation 2, the samples obtained from these plants were more elevated (in the middle canopy) than HT+I and LT+I samples.

Cannabinoid and terpene concentrations were analyzed for both lower and upper portion of the plant. Seven grams of dry inflorescence was sent for both cannabinoid and terpene analysis. Cannabinoids that were measured include tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabigerol (CBG) and cannabigerolic acid (CBGA). Total THC is defined as THCa x 0.877 + THC. Total CBD and CBG values were obtained using the same equation. A total of 16 samples per treatment were analyzed for cannabinoid content. Eight samples were obtained from both the upper canopy as well as the

lower canopy. Cannabinoid samples were obtained from plants in the E and G positions according to Figure 4. Terpene analysis included the quantification of 88 terpenes including β-caryophyllene, α-humulene, limonene, linalool and several other important terpenes. Four samples per treatment were analyzed for terpene content. Terpene samples were obtained from plants in the F position.

3.6 Statistical analysis

The statistical analysis was performed using SAS 9.4 (Cary, NC, USA) to identify any significant difference between treatments. One-way and two-way analysis of variance (ANOVA) test using proc GLM at a significance level of $p = 0.05$ was used for statistical analysis. For statistical analysis, mean values were used for each treatment from a large sample of observations depending on the variable being measured. Main effects and interaction effects were tested for the two independent factors: toplight type and the use of interlight. Every treatment was replicated four times (twice in space, twice in time) where every replicate acted as a block. Results for two-way ANOVAs are presented in Table A1 in the Appendix. Tukey's HSD post-hoc tests was used for pairwise comparison.

Chapter 4. Results

Cannabis sativa "Kush Mint F2" plants were cultivated under four different lighting treatments for over 10.5 weeks (18 vegetative days and 55 flowering days) to evaluate the effects of lighting and interlighting on plant height, inflorescence mass and secondary metabolites (cannabinoids and terpenes). Light treatments included HPS toplight (HT), LED toplight (LT), HPS toplight and LED interlight (HT+I) and LED toplight and LED interlight (LT+I). A repeated cycle with randomized treatment locations was performed to evaluate differences between treatments.

4.1 Environmental conditions

One-way ANOVA ($p=0.075$) showed that room temperature at canopy level was not significantly affected by the different light treatments. Mean temperature values recorded by the data loggers for both growing cycles are presented in the Appendix Figure A1. The mean temperature was highest in HT+I (26.6 ℃ ± 0.6), followed by HT (25.8 ℃ ± 0.1), LT (25.6 ℃ ± 0.5) and LT+I (25.5 ℃ ± 0.7). Two-way ANOVA analysis demonstrates that HPS lighting significantly increased temperature compared to LED's ($p=0.041$). Interlighting had no impact on temperature recorded with the data loggers ($p=0.274$).

Figure 6 summarizes mean relative humidity collected from data loggers for both replicates. Significant differences were observed between treatments ($p=0.031$). The highest relative humidity was achieved in treatment LT+I (50.6% ± 1.6), followed by LT (48.7% ± 1.8), HT (48.5% ± 1.5) and HT+I (46.6% ± 0.3). Mean relative humidity had significant differences between LT+I and HT+I ($p=0.02$). LED toplights led to a significant increase in relative humidity compared to HPS ($p=0.02$). The interaction effect between toplight type and the use of interlight was significant ($p=0.035$)

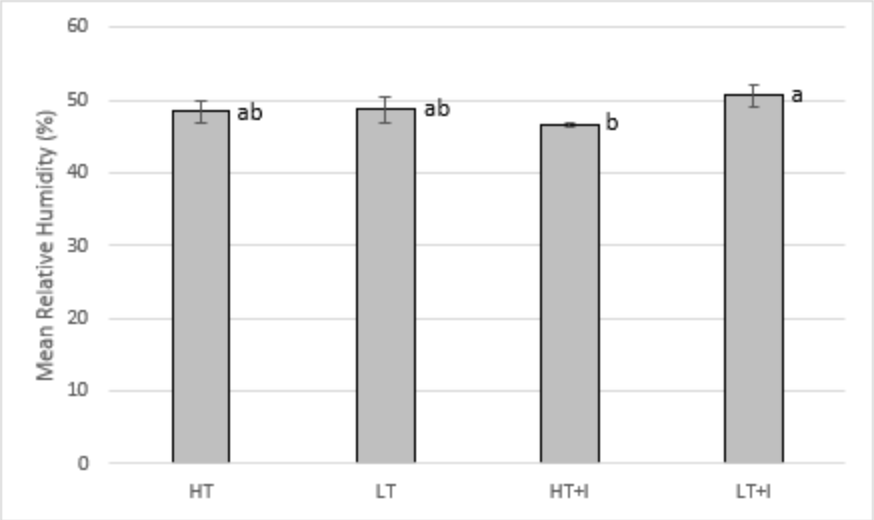

Figure 6. Effect of lighting type on relative humidity. Data is presented as mean ± SD in percentage (n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

No significant differences were observed in mean canopy temperatures between treatments which are presented in Figure A2 in the Appendix ($p=0.263$). The highest canopy temperature was for HT+I (25.8 ℃ ± 0.4) and HT (25.8 ℃ ± 0.2), followed by LT+I (25.4 ℃ ± 0.7) and LT (25.2 ℃ ± 0.4). No significant differences were obtained for two-way ANOVA's comparing toplight type ($p=0.063$) and the use of interlight ($p=0.682$) for canopy temperatures.

4.2 Light intensity

Mean light intensity is presented in Figure 7. The highest light intensity was achieved for treatment LT+I (972 µmol m^{-2} s^{-1} ± 26), followed by HT+I (881 µmol m^{-2} s^{-1} ± 18), LT (746 µmol m^{-2} s^{-1} ± 26) and HT (664 µmol m^{-2} s^{-1} ± 24). All treatments were significantly different from each other ($p \leq 0.001$). Two-way ANOVAs showed that the use of LED's resulted in an increase in light intensity ($p \leq 0.001$). The addition of interlighting significantly increased light intensity ($p \leq 0.001$)

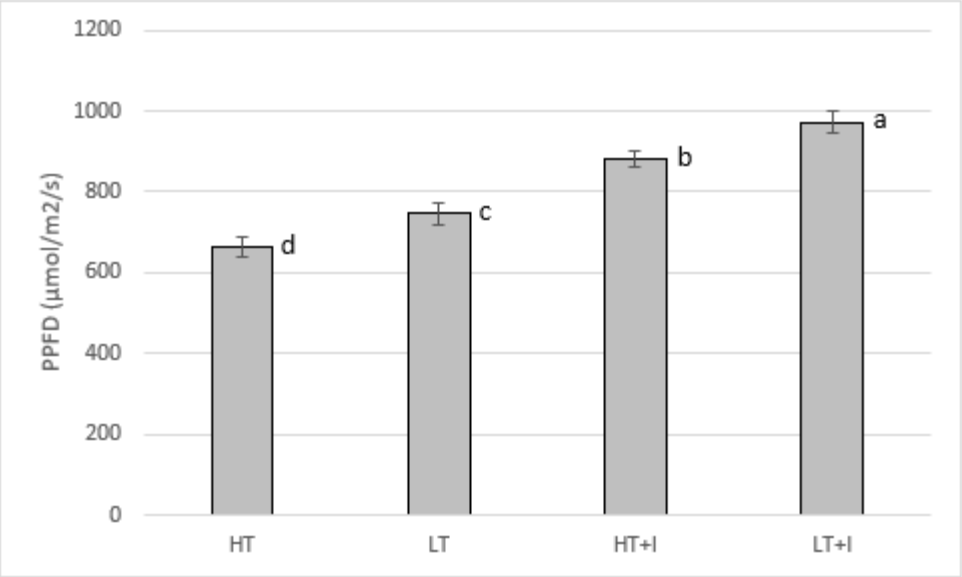

Figure 7. Effect of lighting type on light intensity. Data is presented as mean ± SD in PPFD (n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

4.3 Plant height

Significant differences between treatments in plant height are presented in Figure 8 and 9 ($p \leq 0.001$). The treatment with the tallest plants was HT (91.85 cm ± 0.99), followed by HT+I (87.30 cm ± 0.34), LT (82.20 cm ± 0.34) and LT+I (79.15 cm ± 2.79). Plants grown under HPS toplighting were significantly taller than under LED's ($p \leq 0.001$). The use of interlights had a significant decrease on plant height ($p=0.005$). HT treatment was significantly taller than HT+I ($p=0.048$), LT ($p \leq 0.001$) and LT+I ($p \leq 0.001$). HT+I was taller than LI ($p=0.027$) and LT+I ($p=0.002$). No differences were observed between both treatments using LED toplight ($p=0.22$).

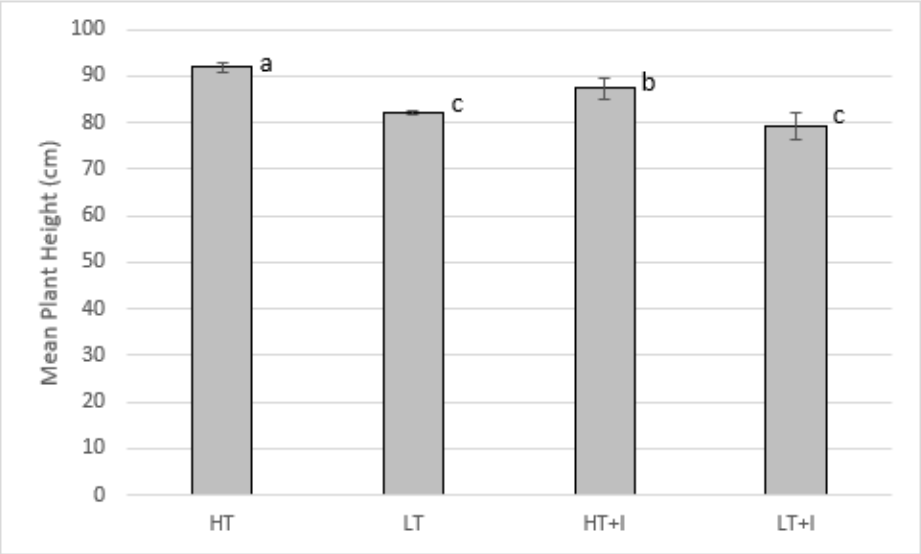

Figure 8. Effect of lighting type on plant height. Data is presented as mean ± SD in centimeters (n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at α = 0.05.

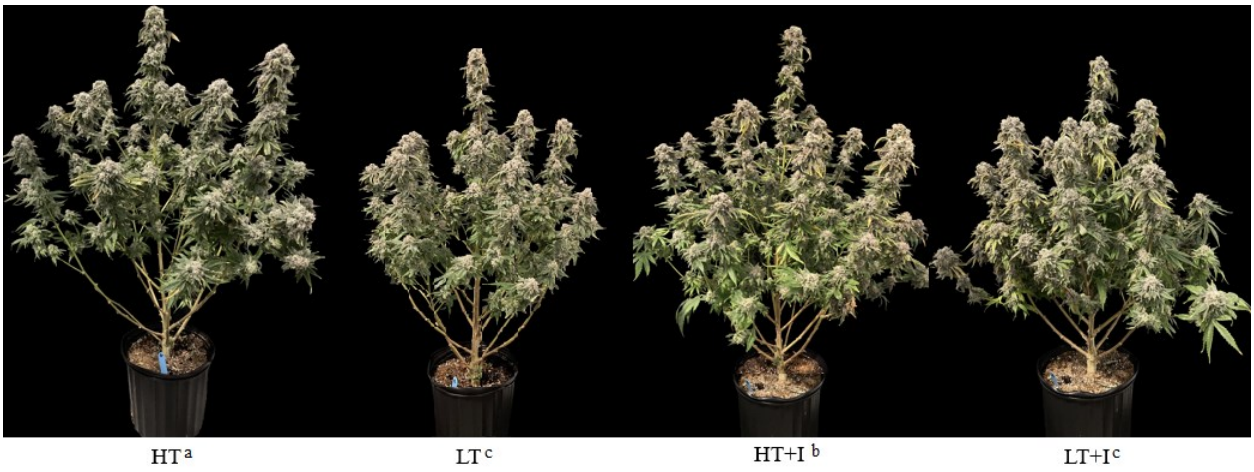

Figure 9. Plant height comparison at harvest. Different small letters represent significant differences between treatments determined by Tukey HSD test at α = 0.05.

4.4 Chlorophyll content

SPAD meter measurements were collected during the growing phase to identify differences in chlorophyll production at different heights in the plant canopy (bottom, middle and top). There were no significant ($p > 0.05$) differences in chlorophyll content between the lighting treatments. For these reasons, the results have been omitted from this report.

4.5 Plant biomass

4.5.1. Biomass and inflorescence mass

Total fresh biomass (inflorescence, trim and stem), fresh inflorescence (inflorescence and trim) and dry inflorescence mass per plant at harvest are reported in Figure 10. For total fresh biomass, significant differences were observed between treatments ($p \leq 0.001$). The highest total biomass produced was LT+I (1279.0 g ± 21.3), followed by HT+I (1255.3 g ± 54.8), HT (1084.5 g ± 35.8) and LT (1039.9 g ± 53.9). Significant differences were observed for both fresh and dry inflorescence mass ($p \leq 0.001$). For fresh inflorescence mass, the treatment with highest production was LT+I (1021.0 g ± 35.1), followed by HT+I (992.2 g ± 46.9), HT (838.0 g ± 42) and LT (820.3 g ± 49.8). Similar trends were observed with dry inflorescence mass although treatment HT had the lowest quantity produced. The highest dry inflorescence mass produced was LT+I (148.9 g ± 22.7), followed by HT+I (137.5 g ± 14.9), LT (120.2 g ± 13.5) and HT (117.3 g ± 14.8). P values for dry inflorescence mass one-way ANOVA's can be found in Table A2. Interlights significantly increased total fresh biomass ($p \leq 0.001$) as well as fresh and dry inflorescence production ($p \leq 0.001$). No significant differences were observed between type of toplights used for total fresh biomass ($p=0.432$) and fresh inflorescence mass ($p=0.502$). However, LED lighting led to a significant increase in dry inflorescence mass compared to HPS ($p=0.032$). An interaction effect was observed between toplight type and the use of interlights for total fresh biomass ($p=0.025$) and fresh inflorescence mass ($p=0.016$). The total fresh biomass can be separated into three segments known as the inflorescence, stem and trim mass. Due to limitations with the harvest schedule and procedure, only fresh values were obtained for stem mass. For fresh stem mass, the treatment with highest production was HT+I (263.1 ± 8.3 g), followed by LT+I (258.0 ± 17.1 g), HT (246.5 ± 19.7 g) and LT (219.7 ± 9.2 g). Conversely, only dry values were obtained for trim since the process to separate the trim from the flower was only completed once the product was dry. For dry trim mass, the treatment with highest production was HT+I (83.0 ± 8.1 g), followed by LT+I (81.4 ± 14.0 g), HT (61.2 ± 10.5 g) and LT (57.5 ± 7.5 g). The biomass removed during defoliation events is reported in Figure A4 of the Appendix, which is discussed in Section 4.5.4.

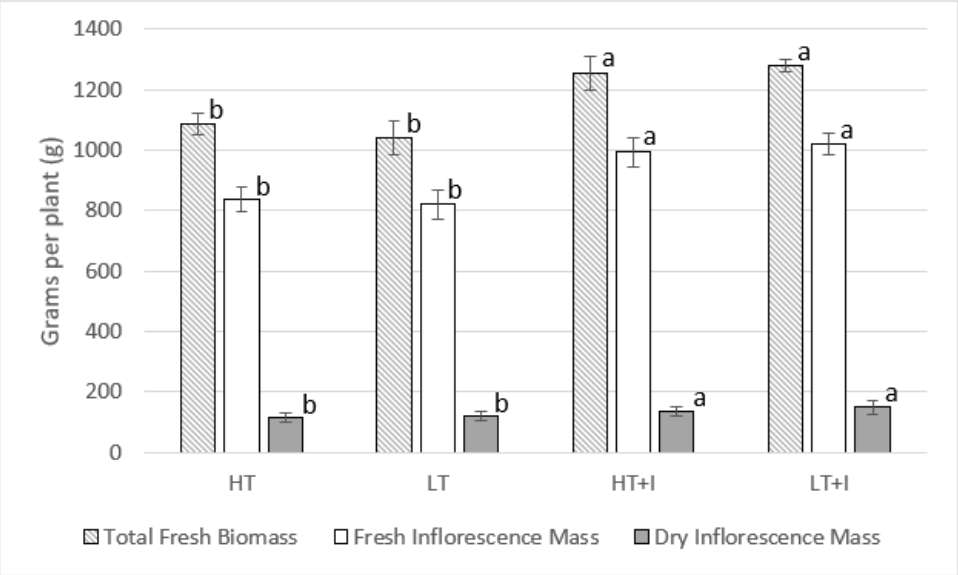

Figure 10. Effect of lighting type on mass for total fresh biomass, fresh inflorescence and dry inflorescence. Data is presented as mean ± SD in grams (n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

Comparing treatments for upper canopy dry inflorescence mass values with one-way ANOVA, no differences were observed ($p=0.059$). However, two-way ANOVAs demonstrated a significant increase in production for LED compared to HPS toplights ($p=0.029$). Nevertheless, no differences were observed with the use of interlights ($p=0.131$) (Figure A3).

At harvest, plants were partitioned at the level of the net resulting in lower and upper canopy segments (Figure 11). No significant differences were observed for upper canopy partitions. Treatments without interlights (HT and LT) had their lower canopy segment removed during defoliation 2. Treatments with interlights including HT+I and LT+I, had an extra 25.9 g and 29.7 g respectively in lower canopy dry inflorescence.

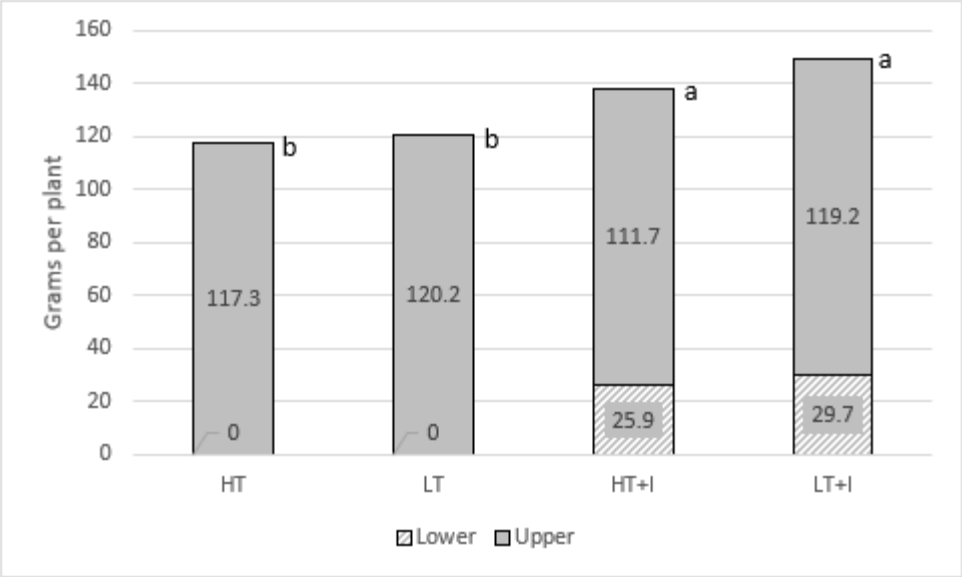

Figure 11. Effect of lighting type on dry inflorescence mass separated in lower and upper canopy partitions. Data is presented as mean ± SD in grams (n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

Inflorescence was separated into small and large flowers with a cut-off size of 2.2 cm (Figure 12). Large inflorescence is destined for the premium market where they are sold as whole flower. Whereas small inflorescence would be marketed for pre-roll production resulting in a less profitable outcome. LED lighting increased the large flower proportion significantly by an average of 3% compared to HPS treatments ($p=0.009$). Interlights resulted in a slightly larger proportion (1%) of small flowers although no significant differences were observed ($p=0.094$). Statistics were performed on the percentage of large and small flowers.

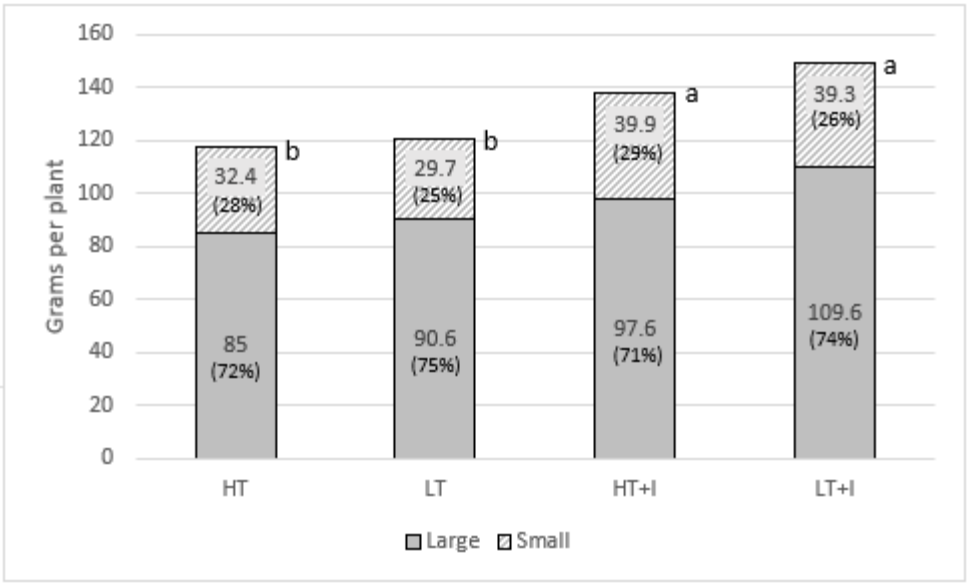

Figure 12. Effect of lighting type on dry inflorescence mass in grams separated by size into small and large inflorescence. Data is presented as mean in percentage (n=4). Different small letters represent significant differences for the sum of small and large inflorescences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

4.5.2 Harvest index

Ratios between dry inflorescence and total plant mass known as harvest index (HI) were calculated (Figure 13). An estimation of dry plant biomass was measured using the difference between fresh and dry stems. Dry stems had a mass loss of 74%. Fresh stems were disposed using a bucking machine to facilitate the harvesting process. Fresh stem mass was converted to dry mass to estimate HI. The treatment with the highest HI was LT (0.51 ± 0.04), followed by LT+I (0.50 ± 0.07), HT (0.48 ± 0.06) and HT+I (0.47 ± 0.04). No significant differences were observed with one-way ANOVAs (p=0.098). LED toplights significantly increased HI with two-way analysis of variance (p=0.023). The use of interlights had no significant impact on HI (p=0.331).

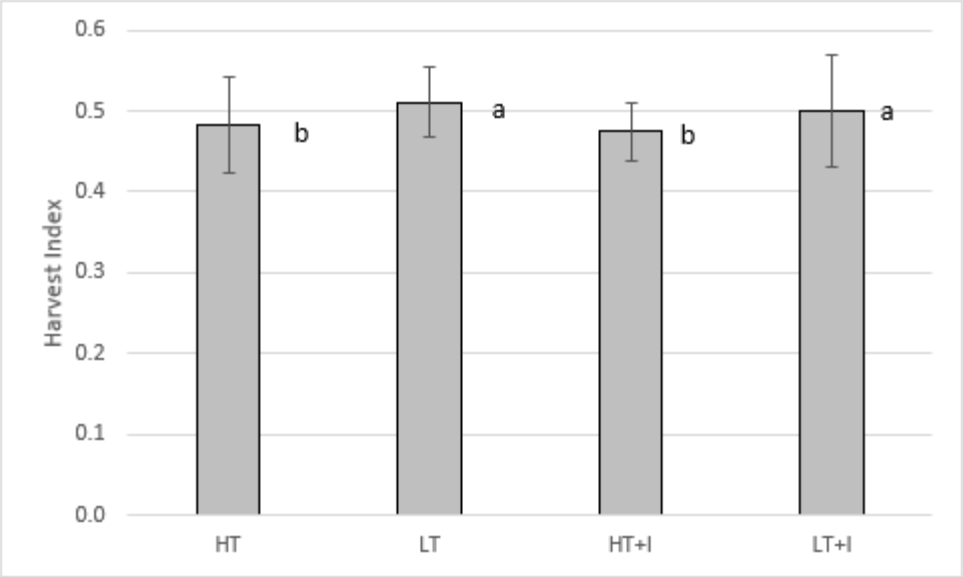

Figure 13. Effect of lighting type on harvest index (HI). Data is presented as mean ± SD in grams per mol (n=4). Different small letters represent significant differences from two-way ANOVAs between treatments determined by Tukey HSD test at $\alpha = 0.05$.

4.5.3 Light use efficiency (LUE) and power use efficiency (PUE)

Analysis of light use efficiency (LUE) revealed some differences between treatments ($p \leq 0.001$) which are presented in Figure 14. This calculation considers the quantity of light used for inflorescence production in each treatment in grams per mol. Thus, the treatment with the highest LUE was HT+I (0.168 g mol^{-1} ± 0.019), followed by HT (0.156 g mol^{-1} ± 0.019), LT (0.145 g mol^{-1} ± 0.011) and LT+I (0.142 g mol^{-1} ± 0.014). Treatment HT+I is significantly different than HT ($p=0.028$), LT ($p \leq 0.001$) and LT+I ($p \leq 0.001$). Treatment HT was significantly different than LT ($p=0.048$) and LT+I ($p=0.016$). Both LED toplight treatments (LT and LT+I) were not different from each other ($p=0.883$). Toplight type had a significant difference on LUE ($p \leq 0.001$) where HPS led to increased values. By contrast, the use of interlights did not have an impact on LUE ($p=0.081$). Additionally, an interaction effect between toplight type and the use of interlight was observed ($p=0.015$).

The effects of lighting type on power use efficiency (PUE) led to highly significant differences in one-way ANOVAs (Figure 14). This calculation considers the efficiency of the

lighting fixtures as it includes the quantity of kilowatt hours consumed for inflorescence production in grams. Lighting system designs were limited based on available space for each treatment (discussed in Section 5.7.) which may have influenced the results regarding power consumption. Highest PUE value was achieved by treatment LT+I (0.828 g kWh^{-1} ± 0.13), followed by HT+I (0.725 g kWh^{-1} ± 0.08), LT (0.713 g kWh^{-1} ± 0.08) and HT (0.657 g kWh^{-1} ± 0.08). Treatment LT+I significantly increased g kWh^{-1} production compared to HT ($p \leq 0.001$), LT ($p=0.002$) and HT+I ($p=0.005$). LED toplight type had a significant increase on PUE ($p \leq 0.001$). Similarly, the use of interlight significantly increased PUE ($p \leq 0.001$).

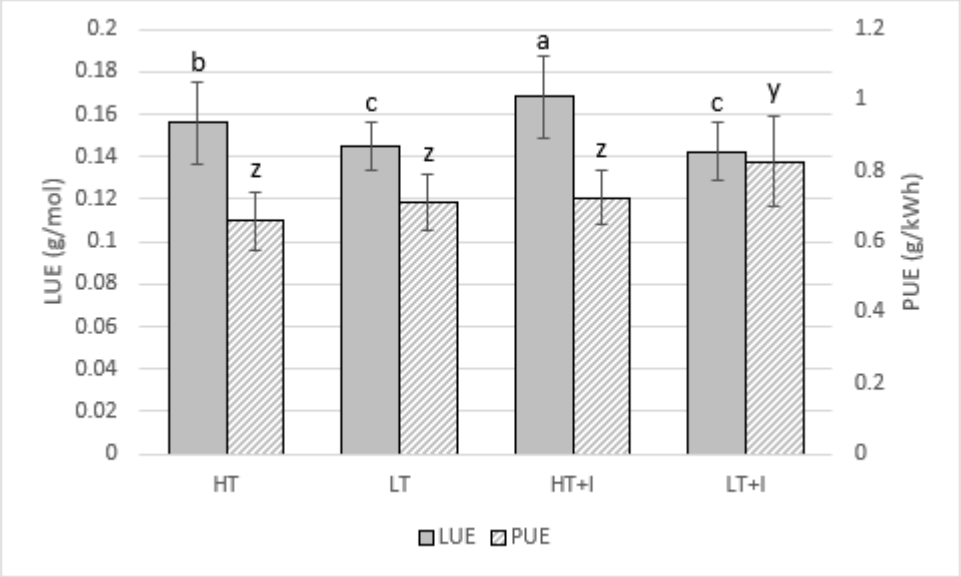

Figure 14. Effect of lighting type on light use efficiency (LUE) and power use efficiency (PUE). Data is presented as mean ± SD in grams mol^{-1} and grams kWh^{-1} (n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

4.5.4 Additional information

Biomass removed at every defoliation event was measured to ensure consistency between treatments and replicates. The mean quantities of defoliated biomass per plant are presented in Figure A4 in the Appendix. Significant differences between treatments were present ($p=0.004$). Most abundant quantities were removed on HT (481 g ± 23), followed by LT (472 g ± 15), LT+I (426 g ± 25) and HT+I (417 g ± 12). More biomass was removed on treatments without interlights to mimic a realistic approach to cultivating with overhead lighting solely. The interlighting

treatments had significantly less biomass removed ($p \leq 0.001$). Quantities removed varied based on treatments and defoliation time. The third defoliation event had a large impact on the differences observed. On average, 988 g of large fan leaves were removed for treatments with no interlight while 1440 g was removed for treatments with interlights.

Additionally, labour requirements were monitored for defoliation events. The mean time per plant required for defoliation is summarized in Figure A5 in the Appendix. Significant differences were observed between treatments ($p \leq 0.001$). The longest time required to defoliate was achieved by LT+I (18.9 min ± 1.3), followed by HT+I (18.4 min ± 1.5), HT (14.3 min ± 1.6) and LT (14.0 min ± 2.9). The use of interlights significantly increased labour requirements for this trial ($p \leq 0.001$).

4.6 Phytochemicals

4.6.1 THC cannabinoid

Cannabinoid analysis was partitioned into upper and lower canopy sections. Mean upper canopy THC concentrations are presented in Figure 15. No significant differences were observed in upper canopy THC content with one-way ANOVA's ($p=0.188$). The highest THC content was achieved by treatment LT (28.2 % ± 2.2), followed by LT+I (28.1 % ± 2.3), HT+I (26.5 % ± 1.5) and HT (26.1% ± 2.7). LED toplighting produced significantly higher upper canopy THC concentrations than HPS from two-way ANOVA's ($p=0.039$). However, the addition of interlight had no impact on upper canopy THC content ($p=0.829$).

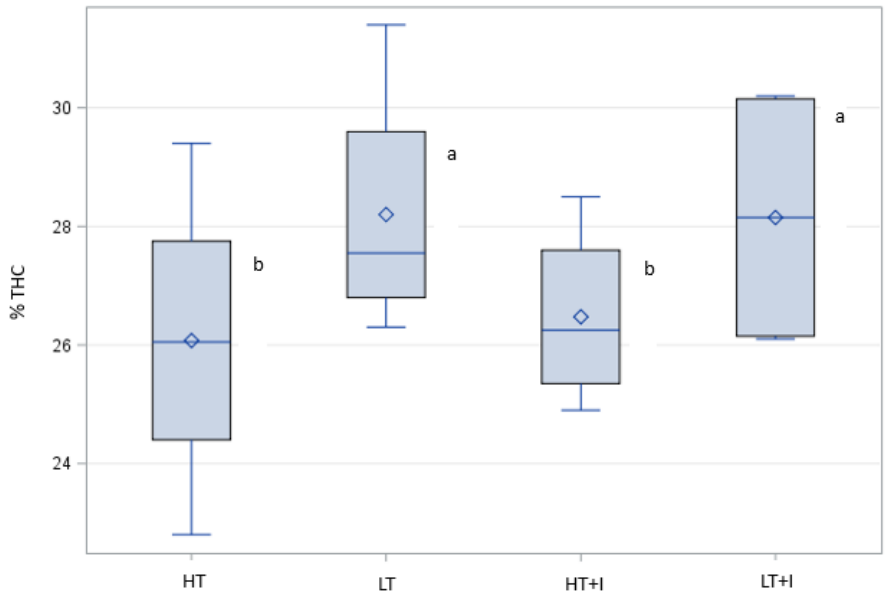

Figure 15. Effect of lighting type on upper canopy THC content. Anhydrous values are presented. Data is presented as mean ± SD in percentage (n=4). Different small letters represent significant differences from two-way ANOVAs between treatments determined by Tukey HSD test at $\alpha = 0.05$.

It is important to note that lower canopy samples were collected from different heights within the plant canopy located along the plants in treatments HI and LI (middle canopy) compared to treatments HI+I and LI+I (lower canopy) which is presented in Figure 5. Lower canopy THC content is summarized in Figure 16. No differences were observed between treatments ($p=0.619$). Likewise, Two-way ANOVAs showed no differences between toplight type ($p=0.891$) and the use of interlights ($p=0.242$). The highest lower canopy THC content was achieved by HT+I (24.0 % ± 2.7), followed by LT+I (23.5 % ± 2.0), LT (22.5 % ± 2.7) and HT (21.7 % ± 2.1). Chemical uniformity can be determined by analyzing the percent difference between upper and lower canopy THC content. The most uniform cannabinoid production was from treatment HT+I (2.5% difference), followed by HT (4.3% difference), LT+I (4.7% difference) and LT (5.7% difference).

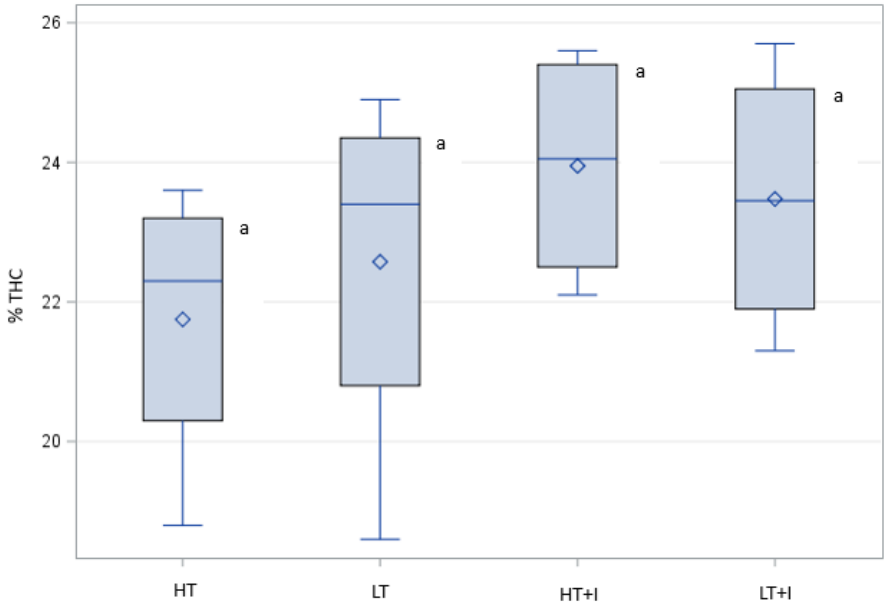

Figure 16. Effect of lighting type on middle and lower canopies THC content. The lower canopy segment of treatments with toplights only corresponds to the middle canopy positioning for treatments with interlights. Anhydrous values are presented. Data is presented as mean ± SD in percentage (n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

In terms of chemical uniformity, treatment HT+I was the most uniform with only a 2.5% difference between lower and upper canopies (Figure 17). This treatment was followed by HT (4.3% difference), LT+I (4.7% difference) and LT (5.7% difference). All lower canopy concentrations were similar while the upper canopies had a 2% difference between LED and HPS. Treatment LT+I had an increase in lower canopy THC although this effect didn't impact chemical uniformity to the same extent since the upper canopy values were also increased. It is important to emphasize the difference in sampling locations (Figure 5) which may have affected chemical uniformities for this study. Lower canopy samples obtained from the treatments with interlights had similar THC profiles as the middle canopy samples from treatments without interlights. In terms of this trial, interlights increased chemical uniformity and produced more inflorescence mass with similar secondary metabolites concentrations as treatments without interlights.

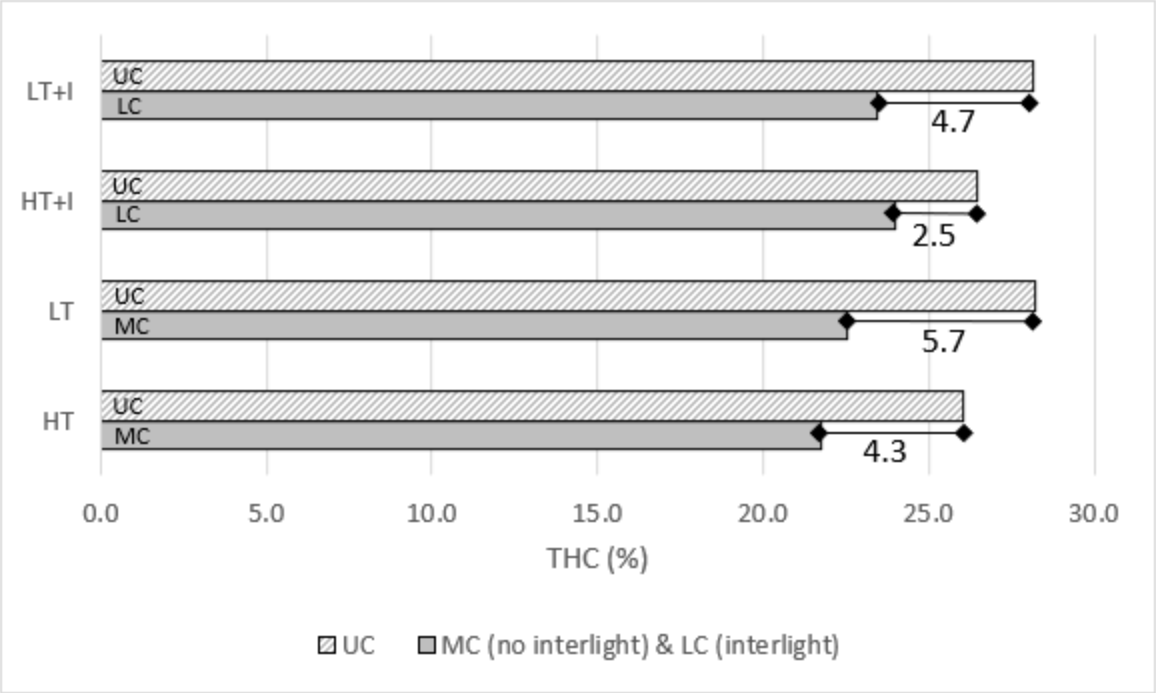

Figure 17. Chemical uniformity between different plant canopies. Treatments with interlights compare the upper canopy THC values to the lower canopy while treatments without interlights compare the upper canopy (UC) to the middle canopy (MC). Diamond bars represent the difference in THC percentages between both canopy locations respective of their treatment. Data is presented as mean ± SD in percentage (n=4).

4.6.2 Total THC and THC PUE

Total THC per plant was calculated by accounting for the mean inflorescence mass multiplied by specific THC content for every treatment. The results are displayed in Figure 18. Highest total THC per plant was achieved by LT+I (3.4 g THC plant^{-1} ± 0.3), followed by HT+I (3.1 g THC plant^{-1} ± 0.3), LT (2.9 g THC plant^{-1} ± 0.3) and HT (2.6 g THC plant^{-1} ± 0.4). Significant differences in one-way ANOVA's were observed between treatments ($p \leq 0.001$). LT+I significantly increased total THC compared to HT+I ($p=0.006$), HT ($p \leq 0.001$) and LT ($p=0.005$). HT treatment was significantly lower than LT ($p=0.038$) and HT+I ($p=0.002$). No significant differences were observed between treatments LT and HT+I ($p=0.233$). Additionally, LED toplights significantly increased total THC per plant compared to HPS toplights ($p \leq 0.001$). Moreover, the use of interlighting significantly increased total THC values ($p \leq 0.001$).

Highly significant differences ($p \leq 0.001$) were observed with the analysis of PUE in grams of THC per kWh. This calculation considers the efficiency of the lighting fixtures as it includes the quantity of kilowatt hours consumed for inflorescence production in grams of THC per kWh. The best treatment was LT+I (0.019 g THC $kWh^{-1} \pm 0.001$), followed by LT (0.017 g THC $kWh^{-1} \pm 0.002$), HT+I (0.016 g THC $kWh^{-1} \pm 0.001$) and HT (0.015 g THC $kWh^{-1} \pm 0.002$). One-way analysis of variance revealed that Treatment LT+I significantly increased THC production per kWh compared to treatments HT ($p \leq 0.001$), LT ($p=0.012$) and HT+I ($p \leq 0.001$). Treatment LT was significantly superior to control treatment HT ($p=0.003$). For two-way ANOVAs, LED toplights increased THC PUE compared to HPS ($p \leq 0.001$). Similarly, the use of interlights significantly increased THC production efficiencies ($p \leq 0.001$).

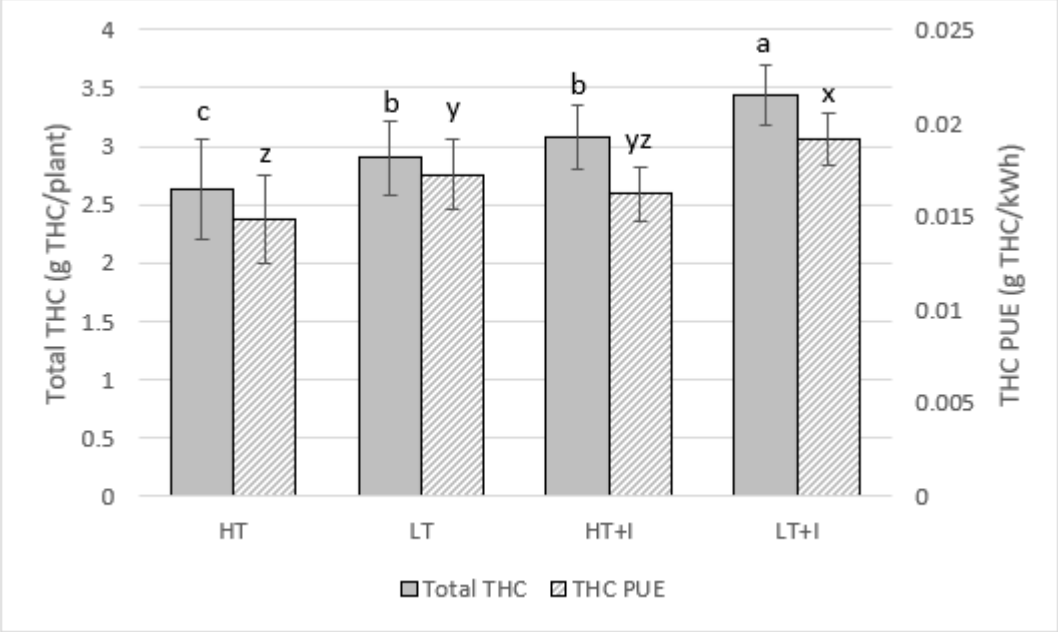

Figure 18. Effect of lighting type on total THC and THC power use efficiency (PUE). As is cannabinoid values were used to calculate results. Data is presented as mean (g THC $plant^{-1}$ and g THC kWh^{-1}). Different small letters represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

4.6.3 CBG and CBD

Other quantified cannabinoids include CBD and CBG which were analyzed in two partitions (upper and lower canopy). CBD concentrations were either undetected or detected in

very low quantities for the majority of samples sent for analysis therefore they have been omitted from these results. CBG was detected in lower concentrations than THC values. For the upper canopy, differences were observed between treatments for mean CBG content ($p \leq 0.001$). Upper canopy CBG values are presented in Figure 19. Treatments with the highest CBG content included LT (0.38% ± 0.03) and LT+I (0.38% ± 0.02), followed by HT (0.30% ± 0.03) and HT+I (0.29% ± 0.03). LT and LT+I produced significantly higher CBG concentrations than HT and HT+I respectively ($p=0.002$, $p \leq 0.001$, $p=0.002$, $p \leq 0.001$). The use of interlighting did not impact CBG production ($p=0.691$). In contrast, the use of LED toplights significantly increased CBG production compared to HPS lighting ($p \leq 0.001$).

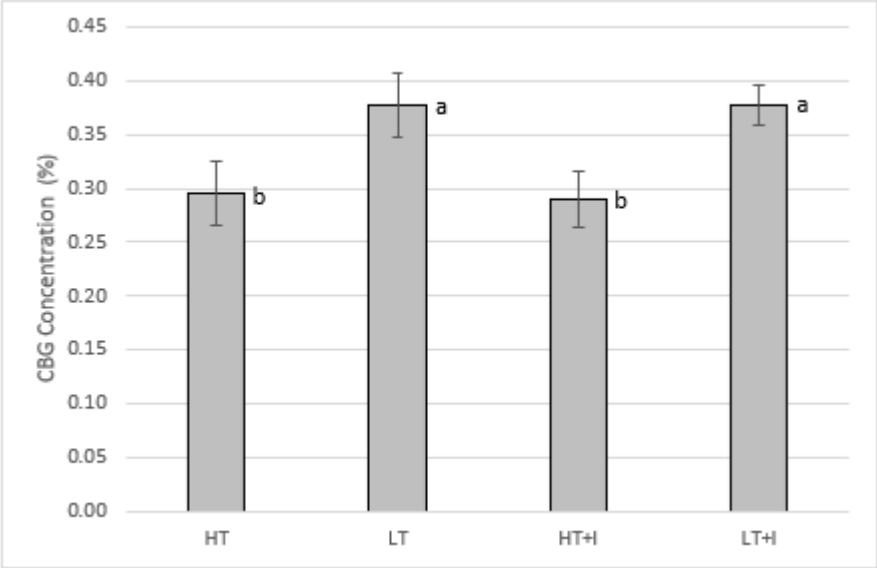

Figure 19. Effect of lighting type on upper canopy CBG content. Anhydrous values are presented. Data is presented as mean ± SD in percentage (n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

No differences were observed for lower canopy CBG values ($p=0.876$). Similarly, two-way ANOVAs had no differences between toplight type ($p=0.615$) and the use of interlights ($p=0.779$). Refer to Figure A6 in the Appendix for a graph on lower canopy CBG content.

4.6.4 Total terpene production

Results were quantified for a total of 88 terpenes by Phytochemia laboratory (Chicoutimi, QC, Canada). Total terpene content is presented in Figure 20. No differences were observed for total terpene production between the four lighting treatments (p=0.178). The treatment with the most abundant terpene production was LT+I (46.2 mg g^{-1} ± 3.4), followed by HT+I (44.3 mg g^{-1} ± 2.4), LT (43.5 mg g^{-1} ± 2.0) and HT (41.6 mg g^{-1} ± 2.7). A table presenting the 6 major terpenes is presented in Table 3. No significant differences were obtained for α-humulene (p=0.651), β-caryophyllene (p=0.979), limonene (p=0.294) and linalool (p=0.075). The only significant differences that were observed for terpene production were selina-3,7(11)-diene (p=0.021) and selina-4(15),7(11)-diene (p=0.01). After two-way analysis, LED toplights increased the concentration of selina-3,7(11)-diene (p=0.024) and selina-4(15),7(11)-diene (p=0.042) compared to HPS. Similarly, the use of interlights led to a significant increase in linalool (p=0.019), selina-3,7(11)-diene (p=0.008) and selina-4(15),7(11)-diene (p=0.036). Furthermore, a list of the 18 most important terpenes can be found in the Appendix Table A3.

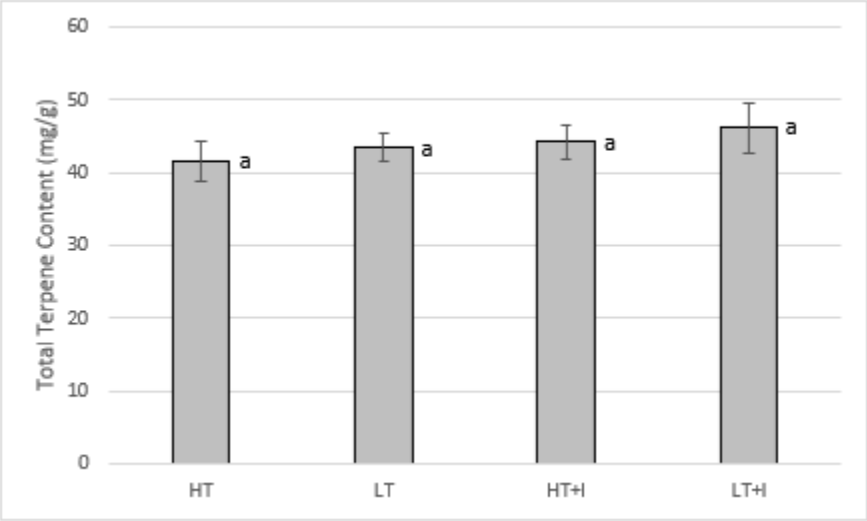

Figure 20. Effect of lighting type on total terpene content. Data is presented as mean ± SD in mg g^{-1}(n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at α = 0.05.

Mean terpene content	HT	LT	HT+I	LT+I
β-Caryophyllene	8.18	8.12	8.40	8.28
	±0.48[a]	±0.79[a]	±1.38[a]	±1.28[a]
α-Humulene	2.20	2.19	2.32	2.31
	±0.12[a]	±0.20[a]	±0.23[a]	±0.26[a]
Limonene	5.90	6.04	6.23	6.86
	±0.74[a]	±0.66[a]	±0.38[a]	±0.68[a]
Linalool	2.05	2.12	2.26	2.40
	±0.06[a]	±0.15[a]	±0.14[a]	±0.22[a]
Selina-4(15),7(11)-diene	1.57	1.95	2.01	2.10
	±0.10[b]	±0.29[a]	±0.17[a]	±0.24[a]
Selina-3,7(11)-diene	2.22	2.91	2.92	2.96
	±0.07[b]	±0.42[ab]	±0.60[a]	±0.23[a]

Table 3. Effect of lighting type on six main terpene content. Data is presented as mean ± SD in mg g^{-1} (n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

Chapter 5. Discussion

This study aimed to investigate the effectiveness of different LED lighting methods on *Cannabis sativa*. The experiment was designed to analyze two variables using four lighting treatments. First, LED toplights were compared to HPS toplights. Second, LED interlighting modules were added to the lower canopy to compare against treatments with solely toplighting. Both variables were evaluated based on their effect on plant height, fresh and dry inflorescence production, and secondary metabolite production specifically cannabinoids and terpenes. Certain significant differences were observed between lighting systems to aid the determination of the optimal lighting strategy for controlled environment cannabis production systems.

5.1 Environmental conditions

Temperature and relative humidity conditions collected from data loggers were stable between both replicates. Significant differences were observed with two-way ANOVAs between LED and HPS toplights. One-way ANOVAs only had a significant difference for relative humidity. This may be due to the spectrum difference between both lighting sources which impacts stomata opening (Li et al., 2023). A slight increase in leaf temperature under the HPS lights might have played a role in changing the relative humidity due to changes in water usage (Nelson & Bugbee, 2015). The average relative humidity values for toplight type varied by 2.1% having minimal effect on the experiment. Temperature differences between treatments were a major concern for the experimental design. The increased heat emittance of HPS lighting could have increased temperatures ultimately leading to increased growth rates. However, the experimental layout used adequate ventilation with both LEDs and HPS in the same room to allow a uniform temperature distribution. The environmental conditions were similar between all four lighting treatments.

5.2 Light intensity

Inflorescence production has been shown to increase linearly with increasing light intensity up to 1800 µmol m^{-2} s^{-1} (Rodriguez-Morisson et al., 2021a). Due to the various lighting fixtures used in this trial, light intensity was monitored to ensure differences were recorded. A known limitation was the increase light intensity emitted from the interlights. Supplemental interlighting accounted for an average of 220 µmol m^{-2} s^{-1} for treatments HT+I and LT+I. Consequently, an increase in inflorescence production was expected for the interlighting treatments. Furthermore,

differences in light levels were observed between HPS and LED toplight treatments. Each plot included either 2 HPS or 3 LED toplight fixtures to achieved comparable light intensities. Further explanation is provided in the limitations section (Section 5.7).

5.3 Plant height

Several factors had an impact on the final plant height observed in this experiment. Treatments exposed to HPS lighting had an increase in plant height. HPS fixtures have greater far-red photons, altering plant morphology by inducing leaf expansion and stem elongation (Franklin and Whitelam, 2005). LED lights contain higher blue photon emittance which has been linked to shorter growth rates in cannabis (Morello et al., 2022). The proportions of far-red and blue spectrum between both lights used explained the difference in height observed. The height differences indicate the significant impact lighting treatments had on the growth rates of cannabis plants. Treatments with interlights resulted in shorter plants than without interlights. The temperature differential between canopies in the different treatments with and without interlights may have impacted plant height. The second defoliation event also had an important influence on the disparities observed. 15 days into the flowering phase, biomass at the bottom of the plants was removed. The height differed between treatments based on the decision to leave an extra 15 cm below the net for treatments with interlights. Conversely, biomass was removed up to the net to mimic an approach employed for treatments without supplemental lighting to reduce underdeveloped inflorescence in the lower canopy. The biomass removal led to an increased stretching effect for plants without interlighting. It is important to note that increased plant height may not necessarily be desirable for indoor cannabis production due to the increased harvest difficulty exhibited by those conditions. In a study conducted by Magagnini et al. (2018), plants grown under HPS were on average 30% taller than LED grown plants. The difference in height wasn't as drastic in this study with an average difference of 11% between HPS and LED toplighting. Observations by Namdar et al. (2019) agreed with these results as plants grown under LED's were more compact due to blue-enriched light.

5.4 Biomass and inflorescence mass

For total biomass, there were no significant differences between the use of LED or HPS toplighting. LED's produced comparable quantities of fresh inflorescence mass as HPS lighting. However, LED lighting significantly improved dry inflorescence production for the whole plant

as well as the upper canopy. It is important to note that higher light intensities (Figure 7) were measured on LED treatments which partly explains this increase in yield. Plants grown under LED had an increase in carbohydrate production since they lost a lower fraction of their total mass during the drying process. These results differ from Magagnini et al. (2018), where LED grown plants had a decrease in inflorescence production. The environmental parameters in this study were maintained at an average temperature of 25.9 ℃ ± 0.4 while in the previous study they were much more variable with an average temperature of 23.7℃ ± 3.6. Cannabis plants have an increased photosynthetic capacity at higher temperatures which may explain the difference between both papers (Chandra et al., 2011). The red:blue ratios also differed between the studies with a higher quantity of red light in the LEDs used in this experiment. CO_2 levels were not reported by Magagnini et al., (2018). A study by Westmoreland et al. (2021) obtained lower yields with LED treatments which appear to correlate to the percentage of blue photons. Similarly, Namdar et al (2019) had a decline of 40% in total flower mass for plants grown under LEDs. Different LEDs were used in these studies which may explain the discrepancies observed with yield. New LED technologies have improved in design and efficiency to achieve higher inflorescence production than HPS lighting. In a study by Danzinger and Bernstein (2021a), one of the balanced chemotypes tested under LEDs with a ratio of 1:1 and 1:4 (blue:red) significantly increased inflorescence production compared to HPS. Although it wasn't the case for the chemotypes of high THCA and high CBDA tested in the study (Danzinger and Bernstein, 2021a)

Total fresh biomass, fresh inflorescence and dry inflorescence mass were increased with the use of interlighting. Interlighting treatments had no significant differences in upper canopy inflorescence production compared to treatments without interlights (Figure 11). The main benefit of implementing interlights was an increase in lower canopy inflorescence (Figure 11). HT+I had 17.3 % more yield than control (HT). Meanwhile, LT+I increased yield by 26.9 % compared to control. Supplemental lighting has shown to increase inflorescence production in other cannabis studies. The results in this study coincide with the increase in yield of 18.9-24.5 % from subcanopy lighting in Hawley et al., (2018). The increase in inflorescence mass was similar even though subcanopy lighting strategies were used. In this study, higher PPFD levels were achieved in local environments that would normally be shaded without overhead lighting solely. Less biomass was removed from the second defoliation which may have impacted the total amount of dry

inflorescence produced. The biomass was removed before any substantial inflorescence growth took place therefore allowing plants without interlights to focus on UC inflorescence production.

Interlighting LEDs increased fresh stem mass by providing higher light intensities in the lower canopy segment of the plants. Similar results were obtained with the amount of dry trim produced. Part of the reason for this increase can be explained by the reduction in defoliation (Figure A4) for these specific treatments.

Dry inflorescence was separated by size based on marketability of flowers where large inflorescence is sold as dried flower and small inflorescence for pre-rolled joints (Figure 12). LED toplights had a significantly higher proportion of large flowers (3% increase). Plants grown under LEDs were shorter in height which could have contributed to an increase in compactness and size of individual flowers. Interlighting had no significant impact on inflorescence size. The use of interlighting helped achieve a similar ratio of large to small flowers in the LC as in the UC.

Light use efficiency (LUE) was significantly increased with HPS lighting since the lights emitted 11% less photons as LED lighting. The addition of a third LED light explained the 11% increase in PPFD for LED treatment whereas inflorescence production only increased by 6%. Consequently, HPS lighting was more efficient at converting emitted photons into inflorescence for the environmental conditions used in this study. Climate parameters were steered towards the HPS since leaf burn became an issue at higher temperatures which would have been more suitable for LED production. *Cannabis sativa* respiration rates had a two-fold increase when grown from an environment with 20℃ to 40℃ indicating higher photosynthetic rates are achievable at higher temperatures (Chandra et al., 2011). The LED treatments would have benefited from a higher temperature setpoint. The previous calculation of LUE does not consider the photon efficacy of the light source. LED treatments significantly increased power use efficiency (PUE) by increasing inflorescence production for every kWh consumed ($p \leq 0.001$). LED toplights alone (treatment LT) had comparable PUE values to both HPS treatments with one-way ANOVAs ($p=0.123$, $p=0.934$). Interestingly, the best treatment from this study was the combination of both LED toplights and interlights (treatment LT+I) which significantly increased PUE compared to all other treatments ($p=0.005$, $p \leq 0.001$, $p=0.002$). In a study by Westmoreland et al. (2021), yield per square meter was 4.6% lower with white and red LEDs compared to HPS although they observed a 27% increase when analyzing on a per dollar of electricity basis. The use of interlights proved itself to be an

efficient strategy to improve PUE compared to treatment without interlights ($p \leq 0.001$). The increase in yield offset the added electricity costs associated to implementing supplemental lighting.

Labor requirements were significantly increased for treatments with interlights (Figure A4) even though less biomass was removed (Figure A5). The interlight position increased the defoliation difficulty since they were in the canopy. Large fan leaves needed to be removed since they were reducing light penetration to local environments. The accession used had relatively large fan leaves creating a need for defoliation. The selection of accessions with smaller leaf morphology may reduce these requirements. Future studies on interlighting need to evaluate positioning and plant architecture to optimize the use of this new lighting system. Interlights positioned below the canopy emitting light with an upwards angle may facilitate defoliation events. Row spacing would also need to be studied to avoid photobleaching caused from plants touching the interlights (Figure B6). Possible solutions may include an increase in row spacing or a different plant architecture which would be parallel to the interlights to limit contact.

5.5 Cannabinoids

Cannabinoids samples were partitioned into lower (LC), middle (MC) and upper (UC) canopy. The main cannabinoid of interest, THC, was significantly increased in the upper canopy with the use of LED toplights compared to HPS. The results of this study coincide with several other studies which analyzed the impacts of LED lighting on secondary metabolite production. In a study by Namdar et al. (2019), THCa concentrations were increased with the use of LEDs as well as a 66% increase in total cannabinoids. Similarly, Morello et al. (2022) observed an increase in THC content with plants grown under blue-dominant LEDs as well as Magagnini et al. (2018) observed an increase in THC content with LED toplights. The increase in THC content may be related to the increase blue spectrum of the LED lights compared to HPS. Low red:far red ratios in the HPS lights contributed to shade avoidance responses resulting in taller plants at the expense of a downregulation of certain biosynthetic pathways which are responsible for cannabinoid production (Franklin and Whitelam, 2005). The use of interlighting did not impact UC values since they were positioned in the lower canopy therefore having minimal impact on the upper canopy secondary metabolite production.

No significant differences were observed with LC and MC THC values. Treatments with interlights did achieve the highest mean concentrations. The samples had a larger range of values which resulted in no significance. Conflicting results were observed in a study by Hawley et al. (2018), subcanopy lighting increased lower canopy THC values. Plants were partitioned into upper and lower canopy segments only for one cycle and the samples were obtained from the same location on the plants for treatment with and without subcanopy lighting (Hawley et al., 2018). In this study, samples were collected from a different location depending on the use of interlights, which may explain why similar values were obtained (Figure 5). Defoliation techniques varied during the second defoliation event. Plants without interlights had more biomass removed from LC to mimic a pruning strategy for overhead lighting solely. For this reason, LC samples for interlighting treatments were in the lower canopy whereas samples were in the middle canopy for the treatments without interlights. Even with the different sampling locations, interlights were able to produce lower canopy inflorescence which had a similar secondary metabolite profile as middle canopy of treatments without interlights.

In terms of chemical uniformity, treatments with interlights had a smaller difference between different plant partitions. Both treatments with interlights improved chemical uniformity compared to their counterparts (without interlights). For this study, the use of interlights reduced the gap between UC and LC or MC by 18% for LEDs and 42% for HPS. Chemical uniformity between plant partitions is rarely measured in most studies involving *Cannabis sativa*. Research by Hawley et al. (2018) investigated subcanopy lighting. Canopy partitioning took place during one growing cycle. The control treatment without subcanopy lighting achieved the highest chemical uniformity (0.36%) compared to subcanopy lighting treatments of red-blue (0.37%) and red-green-blue (0.73%). Small differences were observed compared to the percentage of THC difference in this study partly due to the plant height differences between both studies. Larger plants were analyzed in this study, which increased the variation between lower and upper canopy flowers. Danzinger and Bernstein (2021b) analyzed chemical uniformity in large cannabis plants (~2.5 m) which revealed that the spatial standardization of cannabinoid profiles correlated with light penetration to the lower canopy. Further investigation demonstrated that large cannabis plants increase yield at the expense of low chemical uniformity (Danzinger and Bernstein, 2021b). We suggest future investigations should be carried out to expand our understanding on the cannabinoid

differences observed between the upper and lower canopies of cannabis. The variability may be due to differences in the translocation of nutrients between both plant partitions.

In this study, total THC content per plant was most abundant in the LT+I treatment. Both variables considered in the calculation of total THC (inflorescence mass and THC content) were increased with the combination of LED toplight and LED interlight resulting in a significantly superior quantity of total THC per plant. Higher light intensities emitted in the LED treatments are part of the explanation for this observation. These results differ from a study by Morello et al. (2022), where HPS had the highest total THC. The blue LED treatment in that study was used to observe spectrum effects. Such light with only blue spectrum would not be used in cannabis production because it has been shown to suppress inflorescence growth (Morello et al., 2022). In a study by Westmoreland et al. (2021), the increase in blue spectrum was shown to decrease inflorescence production whereas no effects were observed on cannabinoid concentration. The LEDs used in this study contained light higher in the blue spectrum which resulted in increased inflorescence production. When comparing both lights in terms of LUE, we observe that HPS light with lower blue spectrum increased the yield mol^{-1} of light which supports the observations by Westmoreland et al., (2021). In this study, an increase in THC was observed with the use of LED toplights which was not the case for Westmoreland et al., (2021). The far-red spectrum is another important aspect to evaluate in this study. The HPS lights used contained higher quantities of far-red photons. Limited information is available regarding the effects of far-red light on inflorescence and cannabinoid production. The increase of far-red intensities resulted in a decrease in yield for *Cannabis sativa* (Carter, 2022). Similar observations were made in this study since LED lights resulted in an increase yield $plant^{-1}$. Although when looking at LUE, the HPS light increased yield on a gram mol^{-1} basis. Further research into the effects of far-red spectrum on inflorescence and cannabinoid production are needed.

Analysis of grams of THC per kWh (THC PUE) revealed that the best lighting treatment was the combination of LED toplights and LED interlight which led to an increase of 29% compared to the control. Both LED toplight and interlights significantly increased THC production efficiencies ($p \leq 0.001$). These results demonstrate the superior THC production efficiencies LED lighting is capable to achieve. LED surpassed HPS lighting even with certain limitations related to the layout of the fixtures and environmental conditions. LED lighting has a large potential to be

employed in future designs with fewer limitations, to optimize their effect on the production of THC. Important considerations should be employed by growers when choosing the appropriate light type to achieve increased THC production.

Upper canopy (UC) CBG values were increased by 29% with the use of LED toplighting. CBG concentrations were relatively low compared to THC concentrations (1:70). These results coincide with a study by Morello et al. (2022) where blue-dominant LEDs yielded the highest CBG content. Similarly, CBG levels were increased using LED toplighting in a study by Magagnini et al. (2018). Overall, the use of LED lighting resulted in an upregulation of secondary metabolites production pathways resulting in higher concentrations. No differences were observed with MC and LC CBG content. Values ranged between 0.29 to 0.32 % CBG. The addition of interlights had no effect on CBG content.

5.6 Terpenes

No significant differences were observed in total terpene content between all lighting treatments analyzed in this study. Similarly, there was no variation between treatments in major terpene production such as α-humulene ($p=0.651$), β-caryophyllene ($p=0.979$), limonene ($p=0.294$) and linalool ($p=0.075$). Regarding minor terpenes, LED toplights increased the concentration of selina-3,7(11)-diene ($p=0.024$) and selina-4(15),7(11)-diene ($p=0.042$). In Hawley et al., 2018, significant differences were observed in terpene content with the use of red, blue, and green LEDs as a subcanopy lighting strategy. Several of these differences are cases where the concentration found in the samples were minimal therefore a slight increase would result in a significant difference. Namdar et al. (2019) observed an increase in terpene content with plants that were grown vegetatively under blue-enriched LEDs and flowered under HPS compared to flowering under blue-enriched LEDs. Similarly, Morello et al. (2022) found that plants grown vegetatively with LEDs and flowered under HPS or purple which contains a higher proportion of blue led to higher total terpene content. Terpenes quantities are difficult to compare with other studies since terpenes production is accession specific and affected by post harvest methods (Richins et al., 2018; Booth et al., 2020). No standard terpene analysis is yet to be determined for cannabis therefore total quantities can be extremely variable from one study to another. For example, terpene contents observed by Morello et al. (2022) were similar to the results presented in this study with a range between 0.347-8.91 mg g^{-1}. These results are much higher in comparison

to other studies such as Rodriguez-Morrisson et al. (2021) (0.03-0.53 mg g^{-1}) and Namdar et al. (2019) (76-215 μg g^{-1}). Terpenes are responsible for the distinct aromas in cannabis inflorescence (Desaulniers Brousseau et al., 2021). Higher terpene concentrations are desired by cannabis producers to increase the marketability of their product. For this study, only upper canopy terpene content analysis was performed due to sampling cost limitations. Future interlighting studies should compare terpene content between the UC and LC.

5.7 Limitations and future directions

Several limitations were encountered in this trial. First, the experimental design was segregated into small plots using polyethylene film to ensure homogeneous replications (Figure B1). As a result, difficulties in obtaining identical light levels from the two toplight sources was observed. In these circumstances, the light shape emitted by the LED luminaire shape and HPS were different which with the current design resulted in inferior light intensities with the use of 2 LED fixtures therefore one to one replacement with HPS fixtures wasn't feasible. An extra LED fixture was added to the toplight configuration comparing two HPS toplights with three LEDs. The LEDs used in this study had lower output than the HPS, allowing us to add an extra fixture to get as close as possible to the HPS output although not eliminating the light intensity variability completely. Light orientation and spacing within treatments are presented in Figure 3. Light intensity variability between treatments was present due to slightly higher PPFD provided by the LED toplights.

Second, environmental conditions were steered towards the HPS lighting to avoid excessive heat loads on plants. Chandra et al. (2011) discovered that *Cannabis sativa* optimizes photosynthetic activity at higher temperatures which may have been a limiting factor for the LED treatments. Temperature setpoints were monitored rigorously with a setpoint of 24℃ and maintained an average ambient temperature of 25.9℃ ± 0.4. At the beginning of the 1st cycle, temperature was increased for a short period to achieve optimal conditions for LED lights although plants under HPS began to have early stages of tissue death. Temperature setpoints were adjusted to avoid this issue. In the 2nd cycle, temperature setpoints were increased by 1℃ for a 2-week period to account for shorter growth rates and cooler canopy temperatures. The temperature increase triggered a faster plant growth rate supporting previous claims made by Chandra et al. (2011). The setpoint was returned to its original setting after the plants measured a comparable

height to cycle 1. The temperature adjustment in cycle 2 was justified since similar plant height values were observed between both cycles.

Third, a slight fraction of inflorescence positioned in proximity of the interlights in the lower canopy were photobleached resulting in rejected flowers. Photobleaching is a process of prolonged exposure to excessive light which ultimately leads to photoinhibition. This begins with damage to the photosynthetic apparatus which decreases photosynthetic activity. Followed by chlorosis and death of tissues due to excess light (Lingvay et al., 2020). Chlorophyll interacts with oxygen forming reactive oxygen species in the Photosystem II reaction center (Lingvay et al., 2020). The proportion of rejected flowers was negligeable (0.5% of total yield and 5% of the LC inflorescence mass) but required extra labor to remove them. Narrow plant spacing played a role in increasing the number of rejected flowers. Further investigation of interlights should be performed with an investigation on larger plant spacing and different plant architecture based on the interlight positioning to avoid photobleaching and facilitate labor requirements. Furthermore, two different mechanisms of photobleaching were observed in the first cycle of this experiment (Carvalho, 2023). Whitening of the apical meristem was observed only in the first growing cycle. This phenomenon occurred for plants exposed to the highest light intensities on the inside row of the LED treatments. Photobleaching occurred for lower inflorescence that was positioned too close to the interlights. The amount of flower that was photobleached from the interlights represents 0.5% of the total yield (5% of lower canopy inflorescence). Photobleached plant tissues are presented in the appendix B (Figure B2).

Fourth, inflorescence mass was higher by 24 % in the 1st growing cycle compared to the 2nd. Temperature setpoints were identical for both growing cycles although cooler temperatures were observed in the vegetative stage of the second growing cycle. The setpoint was increased by 1°C from the first day of the flowering stage until the fourteenth day. The initial plant height was shorter although plants grew back to comparable heights after the adjustment. The inflorescence produced in the second growing cycle lacked density resulting in a lower final dry yield. Conversely, higher THC content was achieved for mean upper canopy concentrations which increased by 6%. Although minimal changes were observed in mean lower canopy values which had a 1% drop in the second growing cycle.

Fifth, the accession “Kush Mint F2” was chosen due to its high THC content which made it a conservative choice for ROSE Lifescience Inc. Characterized as a indica-dominant accession, this type of plant isn’t ideal for the use of interlighting. Shorter plants were obtained which limited the gap between lower and upper canopies. Future considerations should be employed regarding the use of interlighting with tall sativa accessions.

Last, elevated costs associated to terpene and cannabinoid sampling limited sample numbers. More emphasis should be employed to encourage cheaper laboratory rates for research purposes. Sampling costs create a difficult barrier for researchers to overcome to ensure validity of their results.

Limited research is available in the scientific literature on the novel lighting strategy known as interlighting. This study serves as a starting point on the potential advantages of implementing this new lighting system. Future research needs to evaluate the optimal methods of LED positioning and plant architecture to maximize light capture and facilitate the growing process. There is a need to determine the main causes responsible for the differences observed between plant partitions (upper and lower canopies). Future studies should perform a cost analysis to evaluate the labour needs for commercial applications.

Chapter 6. Conclusion

Electrical lighting plays an important role in the production of inflorescence and secondary metabolites of cannabis. The objective of this study was to evaluate different lighting systems on cannabis and the cannabinoid profile. In this study, significant differences were observed in yield and secondary metabolite production. Regarding our first hypothesis on toplight systems, new LED technologies were able to achieve higher THC concentrations and dry inflorescence mass per plant. Higher photosynthetic photon flux density (PPFD) values were achieved under LEDs which explains the increase in inflorescence production. For the second hypothesis regarding the interlights, an increase in inflorescence production and cannabinoid uniformity was achieved. This could have major implications for the medical cannabis market where uniformity of cannabis products is crucial. In this study, the combination of both LED toplights and interlights resulted in the optimal lighting system. By combining both lighting orientations, increases in inflorescence mass, THC production, power use efficiency and cannabinoid uniformity were achieved. Licensed producers should consider new LED lighting technologies to increase their profitability.

Chapter 7. References

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Appendix A

	Factor 1: Toplight type (HPS vs LED)	Factor 2: Use of interlight (No vs.Yes)	Interaction effect
Temperature	**0.041 HPS**	0.274	0.137
Relative Humidity	**0.02 LED**	1.0	**0.035**
Light Intensity	**≤0.001 HPS**	**≤0.001 YES**	0.453
Canopy Temperature	0.063	0.682	0.837
Plant Height	**≤0.001 HPS**	**0.005 NO**	0.451
Total Fresh Biomass	0.432	**≤0.001 YES**	**0.025**
Fresh Inflorescence Mass	0.502	**≤0.001 YES**	**0.016**
Dry Inflorescence Mass	**0.032 LED**	**≤0.001 YES**	0.166
Dry Upper Inflorescence Mass	**0.029 LED**	0.131	0.28
Harvest Index	**0.023 LED**	0.331	0.881
Light Use Efficiency	**≤0.001 HPS**	0.081	**0.015**
Power Use Efficiency	**≤0.001 LED**	**≤0.001 YES**	0.155
Defoliated Biomass	0.979	**≤0.001 NO**	0.39
Time Requirements	0.897	**≤0.001 YES**	0.459
THC Upper Canopy	**0.039 LED**	0.829	0.782
THC Lower Canopy	0.891	0.242	0.612
Total THC	**≤0.001 LED**	**≤0.001 YES**	0.405
THC PUE	**≤0.001 LED**	**≤0.001 YES**	0.455
CBG Upper Canopy	**≤0.001 LED**	0.691	0.894
CBG Lower Canopy	0.615	0.779	0.695
Total Terpenes	0.184	0.075	0.973
Limonene	0.304	0.138	0.503
Linalool	0.27	**0.019 YES**	0.704
β-Caryophyllene	0.861	0.71	0.947

α-Humulene	0.93	0.226	0.99
Selina-3,7(11)-diene	**0.042 LED**	**0.036 YES**	0.063
Selina-4(15),7(11)-diene	**0.024 LED**	**0.008 YES**	0.141

Table A1. P values obtained from two-way ANOVA's. Significant differences are highlighted in bold and defined with the optimized treatment.

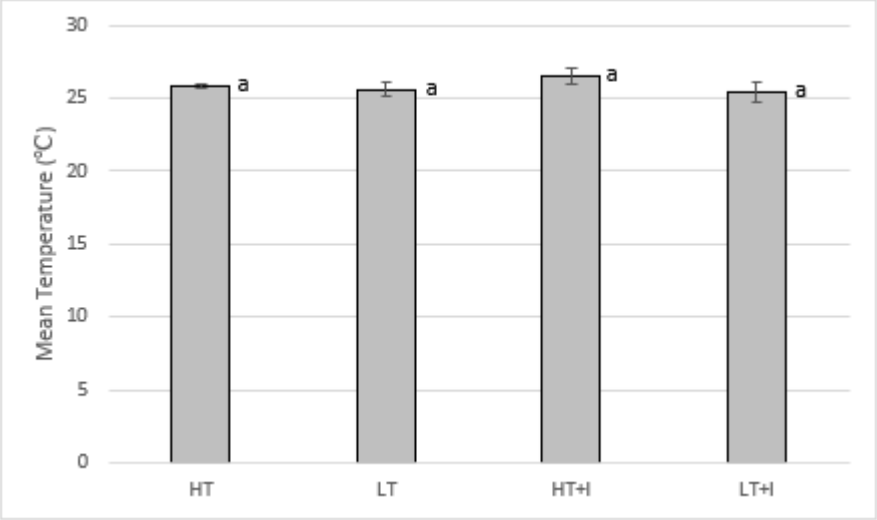

Figure A1. Effect of lighting type on temperature. Data is presented as mean ± SD in ℃ (n=4). Different small letters above means represent significant differences between treatments determined by Tukey HSD test at α = 0.05.

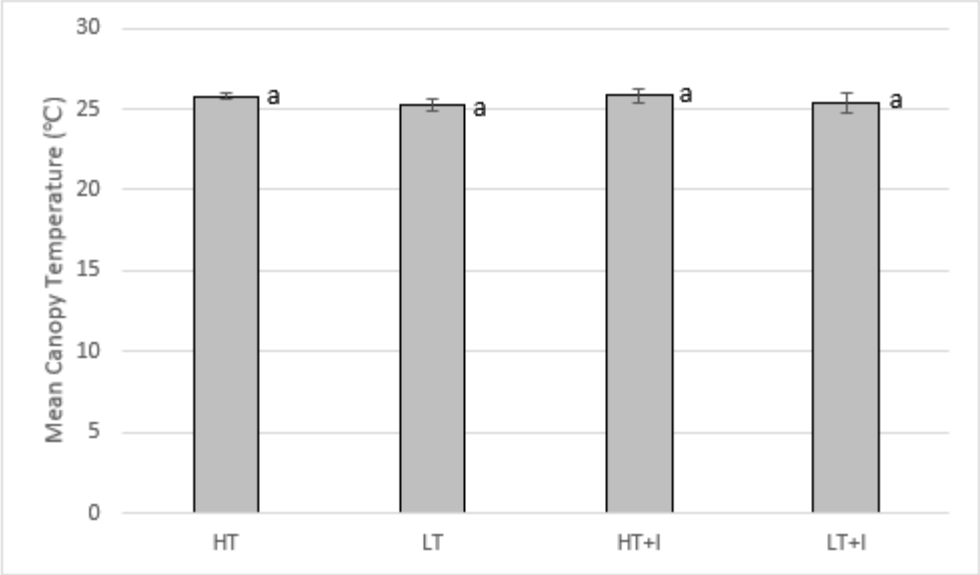

Figure A2. Effect of lighting type on canopy temperatures. Data is presented as mean ± SD in °C (n=4). Different small letters above means represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

	HT	LT	HT+I	LT+I
HT		0.8813	0.0029	0.0001
LT	0.8813		0.0079	0.0002
HT+I	0.0029	0.0079		0.0737
LT+I	0.0001	0.0002	0.0737	

Table A2. Differences between treatments are presented with respective P values for multiple pairwise comparisons of mean dry inflorescence mass per plant.

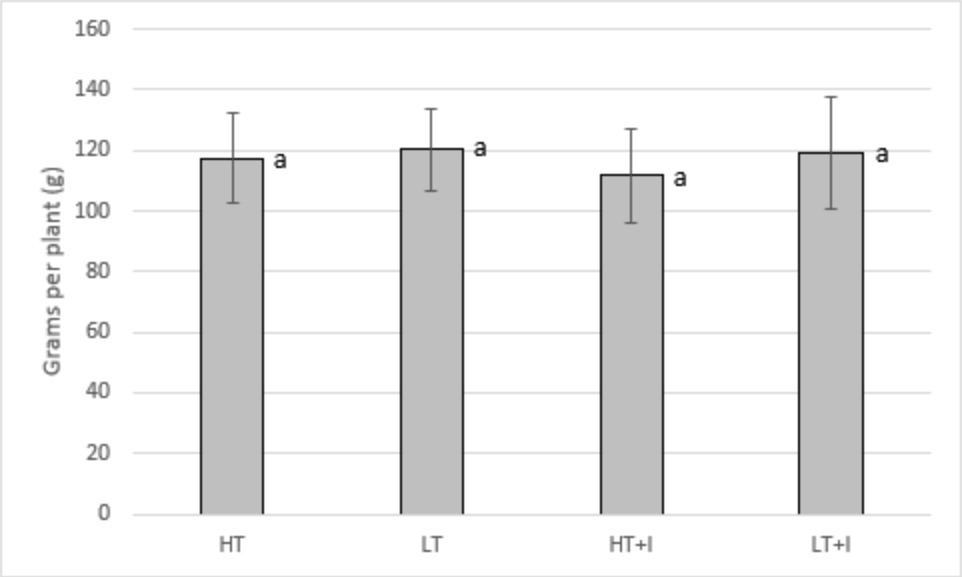

Figure A3. Effect of lighting type on upper canopy dry inflorescence mass. Data is presented as mean ± SD in grams (n=4). Different small letters above means represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

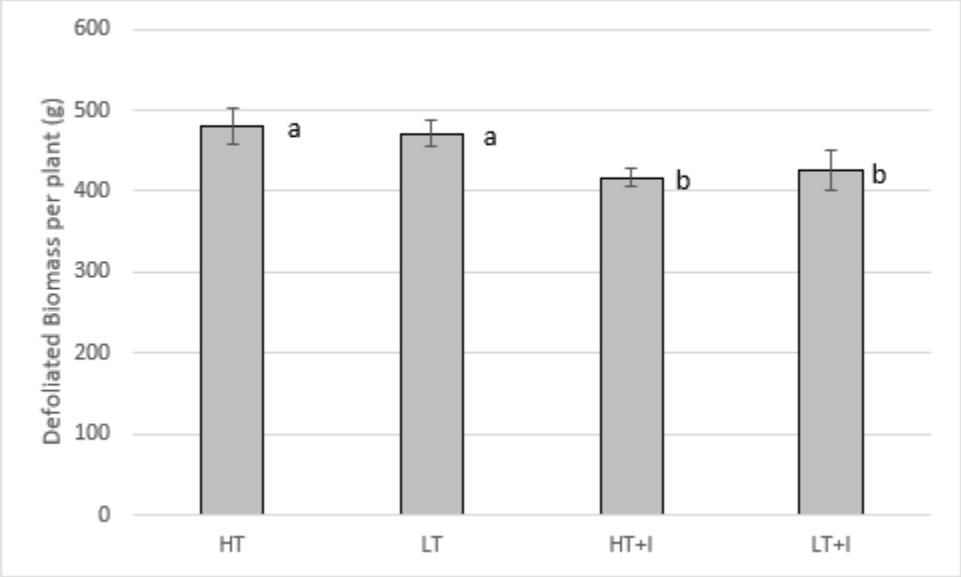

Figure A4. Effect of lighting type on fresh biomass removed per plant during defoliation events. Data is presented as mean ± SD in grams (n=4). Different small letters above means represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

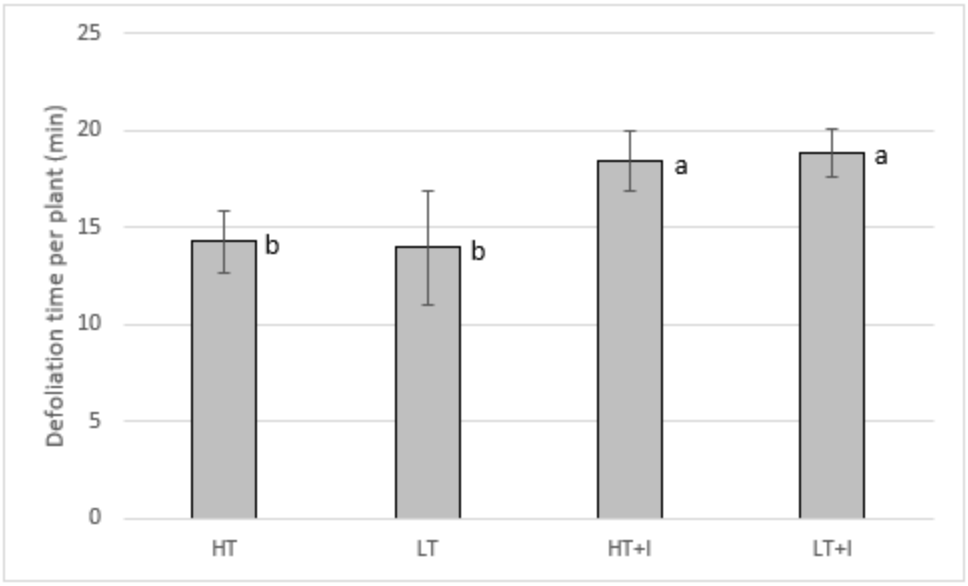

Figure A5. Effect of lighting type on labor requirements per plant for defoliation events. Data is presented as mean ± SD in minutes (n=4). Different small letters above means represent significant differences between treatments determined by Tukey HSD test at α = 0.05.

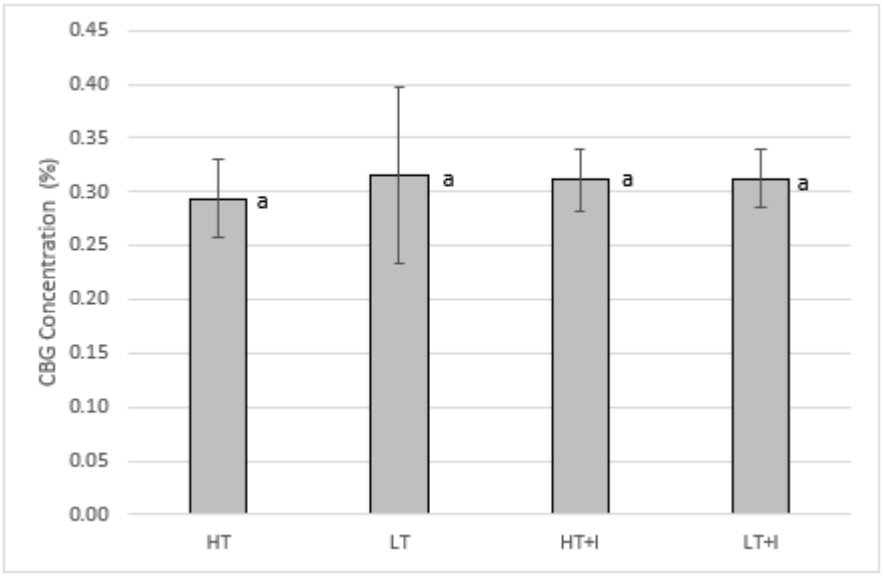

Figure A6. Mean lower canopy CBG content. Anhydrous values are presented. Data is presented as mean ± SD in percentage (n=4). Different small letters above means represent significant differences between treatments determined by Tukey HSD test at α = 0.05.

Mean terpene content	HT	LT	HT+I	LT+I
α-Pinene	0.70 ±0.03[a]	0.71 ±0.05[a]	0.70 ±0.02[a]	0.76 ±0.05[a]
α-Bisabolol	1.09 ±0.08[a]	1.04 ±0.28[a]	1.18 ±0.15[a]	0.94 ±0.57[a]
β-Pinene	1.32 ±0.06[a]	1.32 ±0.11[a]	1.29 ±0.05[a]	1.39 ±0.07[a]
Borneol	0.2 ±0.01[a]	0.2 ±0.02[a]	0.21 ±0.02[a]	0.16 ±0.10[a]
β-Caryophyllene	8.18 ±0.48[a]	8.12 ±0.79[a]	8.40 ±1.38[a]	8.28 ±1.28[a]
y-Elemene	1.91 ±0.14[a]	1.95 ±0.08[a]	2.01 ±0.25[a]	2.09 ±0.19[a]
Germacrene B	4.29 ±0.50[a]	4.45 ±0.50[a]	4.45 ±0.06[a]	4.69 ±0.45[a]
endo-Fenchol	0.77 ±0.04[a]	0.79 ±0.07[a]	0.79 ±0.04[a]	0.85 ±0.06[a]
α-Humulene	2.20 ±0.12[a]	2.19 ±0.20[a]	2.32 ±0.23[a]	2.31 ±0.26[a]
Limonene	5.90 ±0.74[a]	6.04 ±0.66[a]	6.23 ±0.38[a]	6.86 ±0.68[a]
Linalool	2.05 ±0.06[a]	2.12 ±0.15[a]	2.26 ±0.14[a]	2.40 ±0.22[a]
Myrcene	1.23 ±0.48[a]	1.38 ±0.39[a]	1.04 ±0.24[a]	1.22 ±0.41[a]
Selina-4(15),7(11)-diene	1.57 ±0.10[b]	1.95 ±0.29[a]	2.01 ±0.17[a]	2.10 ±0.24[a]
Selina-3,7(11)-diene	2.22 ±0.07[b]	2.91 ±0.42[ab]	2.92 ±0.60[a]	2.96 ±0.23[a]
α-Selinene	0.91 ±0.08[b]	0.97 ±0.04[b]	0.96 ±0.06[b]	1.07 ±0.05[a]
β-Selinene	0.95 ±0.09[a]	1.03 ±0.07[a]	1.02 ±0.05[a]	1.08±0.1[a]
α-Terpineol	0.66 ±0.03[a]	0.68 ±0.05[a]	0.67 ±0.04[a]	0.72 ±0.07[a]
Valencene	0.03 ±0.01[a]	0.05 ±0.05[a]	0.06 ±0.05[a]	0.04 ±0.01[a]

Table A3. Effect of lighting type on terpene content for 18 most important terpenes. Data is presented as mean ± SD in mg g^{-1} (n=4). Different small letters represent significant differences between treatments determined by Tukey HSD test at $\alpha = 0.05$.

Appendix B

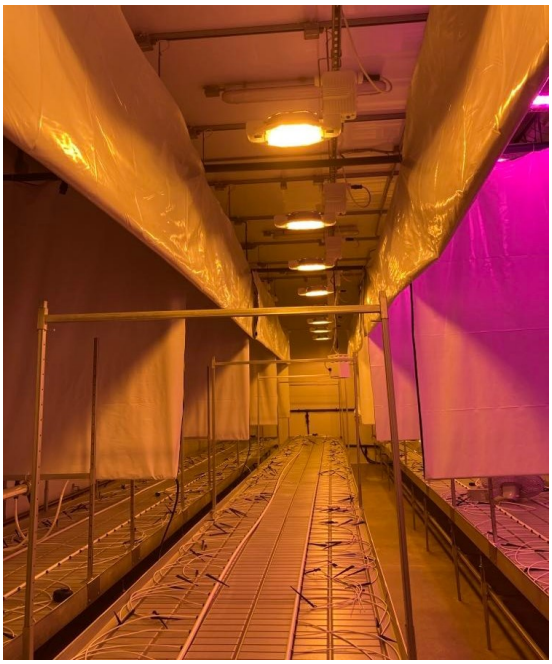

Figure B1. Experimental layout of polyethylene film to separate treatments.

Figure B2. Plant life cycle at 2-week intervals from the transplant until harvest.

Figure B3. Interlight positioning within the plant canopy. Treatment HT+I is pictured on the left and treatment LT+I on the right.

Figure B4. Bucking machine (left) and M108S trimmer (right) used for the harvest process.

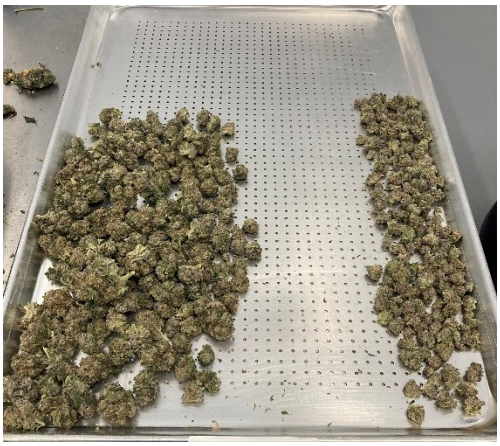

Figure B5. Separation of small and large inflorescence.

Figure B6. Photobleaching occurred on the apical meristems of LED grown plants in cycle 1 (left and center). Photobleached inflorescence in the lower canopy due to interlight proximity (right).