Improving Cannabis Bud Quality and Yield with Subcanopy Lighting

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Abstract. The influence of light spectral quality on cannabis (Cannabis sativa L.) development is not well defined. It stands to reason that tailoring light quality to the specific needs of cannabis may increase bud quality, consistency, and yield. In this study, C. sativa L. 'WP:Med (Wappa)' plants were grown with either no supplemental subcanopy lighting (SCL) (control), or with red/blue ("Red-Blue") or red-green-blue ("RGB") supplemental SCL. Both Red-Blue and RGB SCL significantly increased yield and concentration of total Δ^9 -tetrahydrocannabinol (Δ^9 -THC) in bud tissue from the lower plant canopy. In the lower canopy, RGB SCL significantly increased concentrations of α -pinine and borneol, whereas both Red-Blue and RGB SCL increased concentrations of cis-nerolidol compared with the control treatment. In the upper canopy, concentrations of α-pinine, limonene, myrcene, and linalool were significantly greater with RGB SCL than the control, and cis-nerolidol concentration was significantly greater in both Red-Blue and RGB SCL treated plants relative to the control. Red-Blue SCL yielded a consistently more stable metabolome profile between the upper and lower canopy than RGB or control treated plants, which had significant variation in cannabigerolic acid (CBGA) concentrations between the upper and lower canopies. Overall, both Red-Blue and RGB SCL treatments significantly increased yield more than the control treatment, RGB SCL had the greatest impact on modifying terpene content, and Red-Blue produced a more homogenous bud cannabinoid and terpene profile throughout the canopy. These findings will help to inform growers in selecting a production light quality to best help them meet their specific production goals.

The production and consumption of drugtype cannabis (C. sativa L.) has seen increased acceptance and legalization in North America in recent years (ArcView Market Research, 2017). "Drug-type" cannabis, as opposed to "hemp" or "fiber-type," is characterized by high concentrations of Δ^9 tetrahydrocannabinol-9-carboxylic acid (Δ^9 -THCA) and relatively low concentrations of cannabidiolic acid (CBDA) (van Bakel et al., 2011; Vollner et al., 1986). "Drug-type" cannabis will henceforth be referred to in this

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study more simply as cannabis. Like any other cash crop, producers seek to maximize yield, while also optimizing or otherwise standardizing quality.

Floral bud tissue is of primary interest when attempting to maximize cannabis yield. Floral bud has a relatively high density of glandular trichomes rich in cannabinoids and terpenes that are of medicinal and recreational interest (Happyana et al., 2013). There are relatively few peer-reviewed studies on optimizing environmental parameters for bud yield, and commercial cannabis producers are typically guarded with their production strategies. Nonetheless, one could assume that producers are using the typical production strategies of high light intensities and CO₂ concentrations in an effort to achieve higher yields. The specifics of optimal light qualities and CO₂ concentrations are known to vary with species, cultivars, and production strategies (Blom et al., 2016; Critten, 1991; Fu et al., 2012; Ilić et al., 2012; Li et al., 2017; Nemali and van Iersel, 2004). Given the paucity of scientifically peer-reviewed cannabis production data, it is likely that producers have not yet determined the optimal light (quality and quantity) and CO2 inputs for their specific cultivars and production methods (e.g., indoor), but are supplying reasonable levels based on black-market production information for what would be optimal in similar species.

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

Most commercial cannabis production occurs in greenhouses or growth chambers with supplemental or sole source electric lighting. respectively (Knight et al., 2010; Potter and Duncombe, 2012; Vanhove et al., 2011, 2012). Many horticultural lighting companies looking to capitalize on the cannabis boom are now offering lighting systems that claim to optimize cannabis production. Some companies offer data supporting their claims, although these data are rarely replicated, reviewed, or published in a peer-reviewed journal. Although the influence of spectral quality on plant development is well documented in the scientific literature (Beaman et al., 2009; Chang et al., 2009; Goins and Yorio, 2000; Lefsrud et al., 2008; Loughrin and Kasperbauer, 2001; Yorio et al., 2001), none yet, to our knowledge, demonstrate the influence of spectral quality on cannabinoid and/or terpene profiles in cannabis. Notably, many recent studies have demonstrated relationships between light quality, intensity, and secondary metabolism in a variety of species including St. John's wort (Mosaleeyanon et al., 2005), mint (Kim et al., 2017), perilla (Lu et al., 2017), lettuce (Miyagi et al., 2017; Son et al., 2017), and Cyclocarya paliurus (Liu et al., 2018). The cannabis secondary metabolome may be comparatively more sensitive to its light environment.

To directly investigate the impacts of lighting on cannabis bud yield and quality, supplemental light-emitting diode (LED) bars of two different spectra were deployed below the cannabis canopy in a commercial production environment. Supplemental SCL, as opposed to overhead lighting, was used in this case because it required minimal modifications of infrastructure in the production room, did not add any bulky hardware around plants that would make general plant husbandry

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cumbersome, and has been proven in the past to be a viable strategy for manipulating plant development (Jiang et al., 2017; Stasiak et al., 1998). The objectives of this study were to evaluate bud yield, and cannabinoid and terpene contents when plants were grown with no SCL (control), Red-Blue SCL, or RGB SCL. Two crop cycles are presented; the results of the first crop cycle had variability in metabolome that informed changes to data collection and analysis for the second crop cycle.

Materials and Methods

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

Supplemental lighting. Plants were exposed to three different supplemental SCL spectra during their reproductive stage of development. The Red-Blue and RGB spectra are illustrated in Fig. 1, whereas the control has no supplemental SCL. An acknowledged limitation of this experiment is that the "control" treated plants experienced a reduced total photosynthetic photon flux density (*PPFD*) than the other two treatments. The study has been performed in this way to accommodate two somewhat competing objectives: measuring the value of added subcanopy light, and measuring changes in metabolome as a product of spectral quality. The Red-Blue and RGB spectra were produced with 244 cm long LED bars provided by Intravision Light Systems (Toronto, ON, Canada).

Plant preparation: propagation and vegetative growth. Two hundred C. sativa L. 'WP:Med (Wappa)' plants were clonally propagated via cuttings taken from mother plants maintained under "mother room" environmental conditions detailed in Table 1. During propagation of the first crop cycle, cut stems were dipped in EZ Gro Root Gel 0.20% indole-3-butyric acid (IBA) (EZ Gro, Kingston, ON, Canada). During propagation of the second crop cycle, cut stems were dipped in EZ Gro Root Liquid 1.00% IBA and 0.50% 1-naphthaleneacetic acid (NAA). Cuttings were established in PRO-MIX RG600 soil (Premier Tech, Rivière-du-Loup, QC, Canada) and Jiffy 7 Peat Pellets (Jiffy Products of America Inc., Lorain, OH, Canada) in the first and second crop cycles, respectively. Mother plants were originally established from seed obtained from Paradise Seeds (Amsterdam, The Netherlands).

The cuttings were placed in a Conviron ATC60 Multi-Tier Arabidopsis Growth Chamber (Conviron, Winnipeg, MB, Canada), henceforth described as the "propagation chamber," for 18-days, during which time the environmental conditions were adjusted according to the schedule outlined in Table 1. After establishment, the rooted cuttings were transplanted into 10.2 cm (4.0") square pots (JVK vcc10us; JVK, St. Catherines, ON, Canada) and transferred to a vegetative

plant production room for 20 d during which time the environmental conditions were adjusted according to the schedule outlined in Table 1. After completing the vegetative production phase, 140 of the original 200 plants were selected for homogeneity and transplanted to 11.4-L (3-gallon) pots (NS C1200; Nursery Supplies, Ancaster, ON, Canada) containing a custom organic growth substrate (Premier Tech Promix TD Custom Blend Organic HP-CC MYC BIO Perlite C028733RG586; Premier Tech, Rivière-du-Loup, QC, Canada). The pots were then transferred to 'Bloom Room 2' for 56 d during which time the environmental conditions were adjusted according to the schedule outlined in Table 1. Plants were fertigated as needed via drippers with EZ-Gro Organic Grow 4-3-2 during vegetation, and a solution of EZ-Gro Organic Bloom 4-3-2, Organa Add 2-0-0, EZ-Gro Calmag 0-0-0, and Ez-Gro Enzymatic Complex 0-0-0 during bloom (EZ-Gro, Kingston, ON, Canada).

Layout and production with SCL. In the bloom room, the 140 plants were evenly divided between four benches and arranged in a 5×7 grid on each bench. The first, third, and fifth rows of plants on each bench were exposed to one of the three SCL treatments,

which were randomized within each replicate bench. The rows with SCL had two upwardfacing 244-cm long LED lamps of the Red-Blue or RGB spectra irradiating the plant canopy from below. The lamps were positioned 15 cm to either side of the plant stem and raised 2 cm off the soil surface (Fig. 2). Rows two and four acted as "barrier" rows, blocking light contamination between treatments. For most of the time that plants were exposed to SCL treatments (days 8-56; Table 1), overhead lighting had a PPFD setpoint of 500 µmol·m⁻²·s⁻¹ at the top of the canopy, and the treatments provided 95 \pm 5 μ mol·m⁻²·s⁻¹ at the bottom of the canopy, measured 20 cm from the SCL. All light measurements were taken using an Ocean Optics USB2000 + Spectroradiometer (Ocean Optics, Largo, FL). Subcanopy lighting treatment PPFDs were calibrated on a laboratory bench before installation in the production room: for a given SCL treatment, the two blades were placed face-up on the bench, spaced 32 cm apart. The tip of the fiberoptic cable attached to the spectroradiometer was positioned 20 cm above the blades pointing downward, and was horizontally positioned halfway between the two blades. Some light leakage between treatments was unavoidable

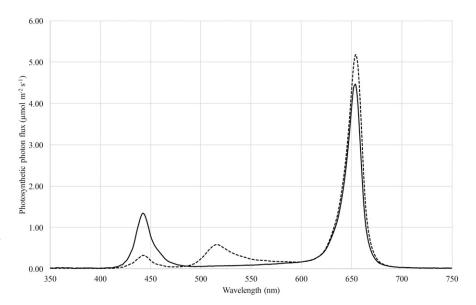


Fig. 1. Supplemental subcanopy light spectra. Red-blue subcanopy lighting (solid line) and Red-Green-Blue subcanopy lighting (dashed line).

Table 1. Controlled environment chamber schedules for the various production phases of cannabis.

	Controlled environment chamber/stage of production						
	Mother room	Propagation	Vegetative	Bloom 22 Days 1–21: 65			
Temperature (°C)	24	24	24				
Humidity (%)	56	Days 1-7: 100	Days 1-5: 80				
		Days 8-10: 90	Days 6-16: 75	Days 22-31: 63			
		Days 11-13: 85	Days 17-20: 70	Days 32-44: 60			
		Days 14-18: 80		Days 45-56: 55			
Carbon dioxide (ppm)	800	800	800	800			
Photosynthetic photon flux	400	Days 1-9: 50	Days 1-3: 100	Days 1-7: 400			
density (µmol·m ⁻² ·s ⁻¹)		Days 10-13: 80	Days 4-5: 200	-			
		Days 14-18: 100	Days 6-10: 300	Days 8-56: 500			
			Days 11-20: 400				
Photoperiod (h/24 h)	12	18	18	12			

as the barrier row plants did not offer complete light occlusion and installation of solid light barriers would have confounded the environmental conditions by obstructing air flow. The amount of light contamination between rows was less than 5 μ mol·m⁻²·s⁻¹ at the bottom of the canopy, which was less than 1% of the total light the treatment rows were exposed to, and \approx 5% the *PPFD* of the treatment itself. Given the operational restrictions and objectives of the study, this level of light leakage was considered acceptable.

Harvest and analysis. After 56 d, the middle five plants of each treatment row were harvested. During the first production run, each treatment row was batched. In the second production run, the bud tissue was separated into upper (upper two thirds of shoot) and lower (lower third) canopy buds. Plants were harvested by cutting the shoot at soil level. Plant height was measured with a meter stick and the intact shoots were photographed using a Samsung Galaxy S7 smartphone (Samsung Canada, Mississauga, ON, Canada). The leaves, stems, and buds were separated and fresh weights of each were collected using a Rice Lake 480plus-2A scale (Rice Lake Weighing Systems, Rice Lake, WI). Stems and leaves were completely dried over 6 weeks in an 18 °C drying room in brown paper bags (#20; Kraft, Montreal, QC, Canada). The buds were spread on mesh drying racks and dehydrated over 4 weeks in an 18 °C drying room to a moisture content of $12.0 \pm 2.0\%$ according to ABcann standard operating procedures. Five grams of dehydrated bud tissue from each experimental unit (batched sample for production run one), or upper and lower canopy for production run two, were sent to RPC Science and Engineering (Fredericton, NB, Canada) for cannabinoid and terpene analysis.

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

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

$$[Total \Delta^9-THC] = [\Delta^9-THCA]$$
$$* (0.877) + [\Delta^9-THC]$$

compensating for the loss of molecular weight as Δ^9 -THCA would be decarboxylated to the psychoactive Δ^9 -THC. The rational is the same for "total CBD" and "total cannabigerol (CBG)."

Statistical analysis. Means comparing SCL treatments were compared using least-squares regression and Tukey's multiple comparison analyses using SAS JMP 13.2.0 (SAS, Cary, NC). "Crop Cycle" and "Block" were treated as random effects in yield analysis. "Block" was treated as a random effect in analysis of cannabinoid and terpene concentrations and in analysis of canopy position.

Means comparing canopy positions were compared using Student's *t* test.

Results and Discussion

Yield. Red-Blue and RGB SCL treatments significantly increased dry bud yield in the

second crop cycle, whereas only RGB SCL significantly increased yield in the first crop cycle (Fig. 3). This was expected due an overall greater amount of light being delivered to the plants in these treatments relative to the control treatment (Peat, 1970; Ralph and Gademann, 2005; Stasiak et al.,

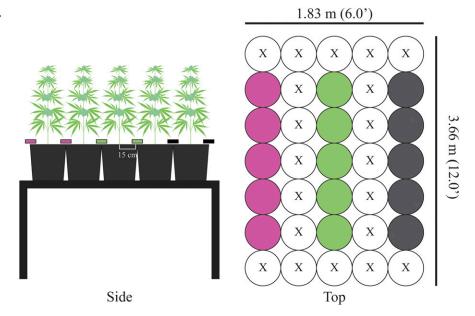


Fig. 2. Left: side view of LED lamp spacing on a bench of plants. Red-Blue, Red-Green-Blue, and control subcanopy lighting treatments are illustrated, respectively, by the pink, green, and black rectangles. Right: Top view. Treatment rows are indicated by the colored circles. Circles marked with an "X" were discarded.

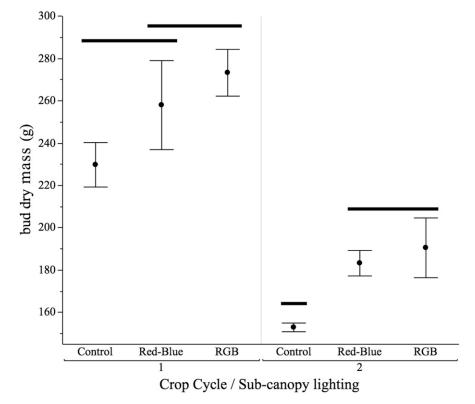


Fig. 3. Total dry bud yields of five plants per treatment per crop cycle when grown with no subcanopy light (control), Red-Blue, or Red-Green-Blue (RGB) subcanopy light. Vertical bars indicate standard error. Horizontal disconnected bars indicate significant differences between treatments using Tukey's multiple comparisons test, $\alpha=0.05$.

1998). Furthermore, the additional light energy was being delivered to leaves that would have otherwise been shaded by upper canopy leaves. Regardless, it is notable that Red-Blue and RGB SCL treatments increased yield by 19.8% and 24.5%, respectively, relative to the control in the second crop cycle, which is a disproportional yield enhancement with the RGB treatment given that the SCL only contributed an additional 19% greater *PPFD* measured at midcanopy than the control treatment. By contrast, in the first crop cycle the RGB SCL treatment increased yield by only 18.9% relative to the control treatment. Below the point of light saturation and limitations of water, CO₂, or nutrition, increasing the intensity of light generally increases photosynthesis, and ultimately yield, proportionally (Stasiak et al., 1998). The disproportionate increase in yield via SCL may be explained by improved light distribution and penetration into the lower canopy than what would be available by simply increasing the overhead *PPFD*. The difference in yield enhancement between crop cycles one and two may be explained by the second crop cycle having more vegetative tissue in the lower canopy; in the first crop cycle, leaves and branches were pruned from the bottom 20 cm of the stem, which is a common practice (gyping) in cannabis production. Gyping was omitted from the second crop cycle with the rationale that lower-canopy leaves, which normally would receive relatively little light and therefore

contribute very little to whole-plant photosynthesis and biomass production, may be of greater photosynthetic value when growing with SCL. Future studies with lights positioned above and to the sides of the plant canopy are planned to address this further.

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

In both crop cycles, both Red-Blue and RGB SCL treatments significantly increased the ratio of bud to nonbud tissue (Fig. 4). This is desirable for production; a canopy less dense with leafy tissue will have better air circulation, and bud will be more accessible for monitoring.

Metabolome. Subcanopy lighting had a local stimulatory effect on Δ^9 -THC and select terpenes in bud tissue harvested from the lower canopy (Table 2). In the first crop cycle, no significant differences were found in measured cannabinoid and terpene concentrations from the pooled bud tissue samples (Table 2). Cannabinoid and terpene concentration variability was considerably

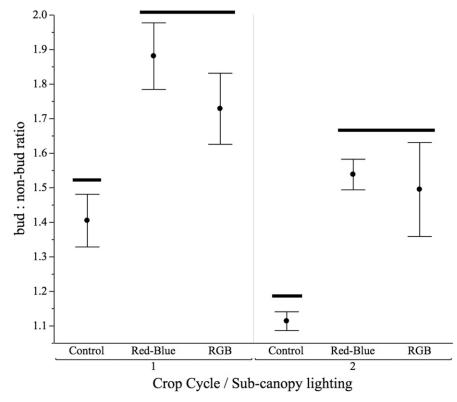


Fig. 4. Ratio of bud to nonbud (stem and leaf) tissue. Plants were grown with no subcanopy light (control), Red-Blue, or Red-Green-Blue (RGB) subcanopy light. Vertical bars indicate standard error. Horizontal disconnected bars indicate significant differences between treatments using Tukey's multiple comparisons test. $\alpha = 0.05$.

higher among the Red-Blue and RGB SCL treatments than the control SCL treatment (Table 2). It was theorized that the observed variability was the result of vertical stratification of secondary metabolite production because of the presence of the SCL. It has been established in other crops that light quality affects secondary metabolism (Chang, 2015; Chang et al., 2009; John et al., 2008; Loughrin and Kasperbauer, 2001) although evidence for such localized effects (within canopy stratification) is not as widely available. To identify potential variability in cannabinoid and terpene concentrations because of canopy position, bud samples from the upper and lower canopy were analyzed separately in the second crop cycle.

Cannabinoid concentrations from the lower canopy of crop cycle two are presented in Table 2. Lower canopy concentrations of Δ^9 -THC, total Δ^9 -THC, and their biosynthetic precursor Δ^9 -THCA were significantly increased under RGB and Red-Blue SCL treatments compared with the control. Concentrations of CBDA, total CBD, CBG, total CBG, and CBGA were not significantly different between treatments.

In the lower canopy, RGB SCL significantly increased concentrations of alphapinine and borneol, and both Red-Blue and RGB SCL significantly increased concentrations of *cis*-nerolidol compared with control SCL (Table 2). The other measured terpenes did not differ at $\alpha=0.05$, although the general patterns suggest similar overall tendencies which may be borne out in further studies with tighter between chamber error control and a greater number of replications.

In the upper canopy of crop cycle two, there were no significant differences in cannabinoid concentrations between treatments (Table 2); however, there were detectable differences in terpene profiles (Table 2). Alpha-pinene, limonene, myrcene, and linal-ool were present at significantly higher concentrations in the RGB SCL treatment than in the control treatment, whereas *cis*-nerolidol concentration was significantly higher in both Red-Blue and RGB SCL than in the control (Table 2).

Comparing cannabinoid and terpene concentrations in the upper and lower canopy of crop cycle two, the control and RGB SCL treatments had significantly more CBGA and total CBG in the upper canopy than in the lower canopy (Fig. 5). The control SCL upper canopy had significantly more transocimene in the upper canopy than the lower canopy (Fig. 6). The Red-Blue SCL yielded the most consistent cannabinoid and terpene concentrations throughout the upper and lower canopy (Figs. 5 and 6).

Cannabinoid and terpene biosynthesis. Careful consideration of the biosynthetic pathways for cannabinoid and terpene biosynthesis offers a possible explanation for the differences observed between SCL treatments. Figure 7 provides a simple outline of some of the major steps involved in cannabinoid and terpene biosynthesis relevant to this study. Geranyl pyrophosphate (GPP) is condensed from dimethylallyl pyrophosphate

Table 2. Cannabinoid and terpene content in dehydrated cannabis bud tissues. In crop cycle 1, 5.0 g of dehydrated bud tissue was randomly sampled from all bud tissue in a given treatment and replication; in crop cycle 2, bud tissue was similarly sampled, but buds were distinctly sampled from the upper and lower plant canopy. Different letters indicate significant differences between treatments in a given sample set using Tukey's multiple comparisons test, $\alpha = 0.05$. Asterisks in place of values in crop cycle 1 indicate unmeasured compounds.

Compound				Crop cycle 2					
	Crop cycle 1			Lower canopy			Upper canopy		
	Control	Red-Blue	RGB	Control	Red-Blue	RGB	Control	Red-Blue	RGB
Cannabinoids (% mass)									
Δ^9 -THCA	19.83 a	20.45 a	20.33 a	23.37 a	24.33 b	24.27 b	23.73 a	24.7 a	25.00 a
Δ^9 –THC	0.27 a	0.30 a	0.28 a	0.52 a	0.61 b	0.70 b	0.55 a	0.62 a	0.77 a
Total Δ^9 –THC	17.65 a	18.23 a	18.10 a	21.00 a	21.93 b	22.00 b	21.37 a	22.27 a	22.70 a
CBDA	<0.05 a	<0.05 a	<0.05 a	<0.05 a	<0.05 a	<0.05 a	<0.05 a	<0.05 a	<0.05 a
Total CBD	<0.05 a	<0.05 a	<0.05 a	<0.05 a	<0.05 a	<0.05 a	<0.05 a	<0.05 a	<0.05 a
CBGA	0.72 a	0.70 a	0.63 a	0.88 a	0.92 a	0.91 a	1.04 a	1.03 a	1.08 a
CBG	0.08 a	0.07 a	0.06 a	0.11 a	0.10 a	0.11 a	0.10 a	0.11 a	0.11 a
Total CBG	0.71 a	0.68 a	0.61 a	0.88 a	0.92 a	0.91 a	1.02 a	1.01 a	1.06 a
Terpenes (% mass)									
3-carene	*	*	*	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a
Alpha-pinine	<0.18 a	<0.18 a	<0.18 a	0.09 a	0.09 ab	0.14 b	0.08 a	0.10 ab	0.11 b
Alpha-bisabolol	*	*	*	0.03 a	0.03 a	0.04 a	0.03 a	0.03 a	0.04 a
Beta-pinine	<0.18 a	<0.18 a	<0.18 a	0.05 a	0.06 a	0.08 a	0.05 a	0.06 a	0.06 a
Borneol	*	*	*	0.01 a	0.02 ab	0.02 b	0.01 a	0.02 a	0.02 a
Caryophyllene	0.44 a	0.46 a	0.48 a	0.29 a	0.32 a	0.43 a	0.31 a	0.33 a	0.36 a
Cis-nerolidol	*	*	*	<0.01 a	0.17 b	0.02 b	<0.01 a	0.02 b	0.02 b
Cis-ocimene	*	*	*	<0.01 a	0.02 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a
Eucalyptol	*	*	*	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a
Fenchol	*	*	*	<0.01 a	<0.01 a	<0.01 a	<0.01 a	0.01 a	0.01 a
Guaiol	*	*	*	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a
Humulene	0.16 a	0.18 a	0.17 a	0.11 a	0.12 a	0.15 a	0.12 a	0.13 a	0.14 a
Limonene	0.06 a	0.07 a	0.07 a	0.05 a	0.05 a	0.07 a	0.05 a	0.06 ab	0.07 b
Linalool	0.08 a	0.09 a	0.08 a	0.07 a	0.09 a	0.12 a	0.07 a	0.09 ab	0.10 b
Myrcene	0.66 a	0.76 a	0.72 a	0.87 a	1.02 a	1.21 a	1.05 a	1.13 ab	1.25 b
Sabinene	*	*	*	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a
Terpineol	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a	0.01 a	<0.01 a	<0.01 a	<0.01 a
Terpinolene	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a
Trans-nerolidol	*	*	*	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 a
Trans-ocimene	*	*	*	0.05 a	0.06 a	0.08 a	0.06 a	0.07 a	0.07 a
Valencene	*	*	*	<0.01 a	<0.01 a	<0.01 a	<0.01 a	0.01 a	<0.01 a

RGB = Red-Green-Blue; Δ^9 -THCA = Δ^9 -tetrahydrocannabinol-9-carboxylic acid; Δ^9 -THC = Δ^9 -tetrahydrocannabinol; CBDA = cannabidiolic acid; CBD = cannabidiol; CBGA = cannabigerolic acid; CBG = cannabigerol.

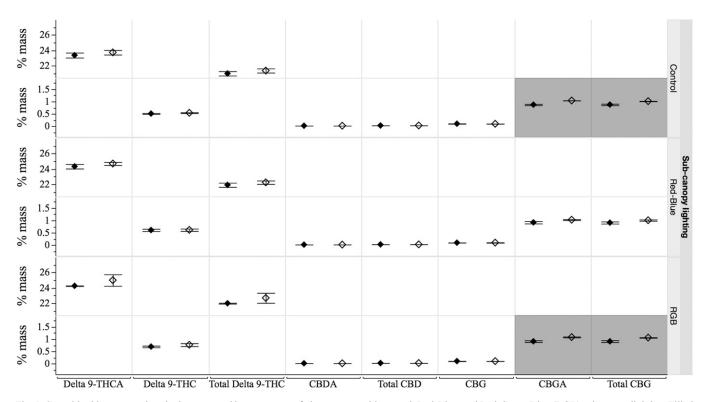


Fig. 5. Cannabinoid concentrations in the upper and lower canopy of plants grown with control, Red-Blue, and Red-Green-Blue (RGB) subcanopy lighting. Filled diamonds indicate lower canopy; empty diamonds indicate upper canopy. Vertical bars indicate standard error. Shaded cells indicate a significant difference between canopy positions using Student's *t* test, α = 0.05. Delta 9-THCA = Delta 9-tetrahydrocannabinol-9-carboxylic acid; Delta 9-THC = Delta 9-tetrahydrocannabinol; CBDA = cannabidiolic acid; CBD = cannabidiol; CBG = cannabigerol; CBGA = cannabigerolic acid.

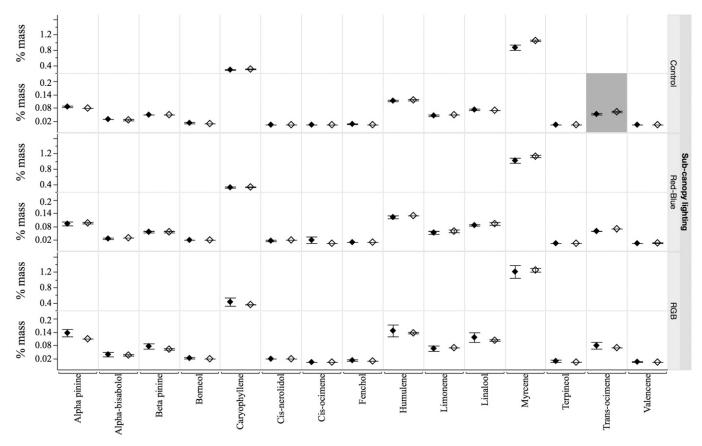


Fig. 6. Terpene concentrations in the upper and lower canopy of plants grown with control, Red-Blue, and Red-Green-Blue (RGB) subcanopy lighting. Filled diamonds indicate lower canopy; empty diamonds indicate upper canopy. Vertical bars indicate standard error. Shaded cells indicate a significant difference between canopy positions using Student's *t* test, α = 0.05.

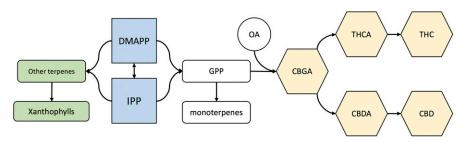


Fig. 7. Simplified overview of terpene and cannabinoid biosynthesis. Dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) (shaded blue) are the common precursors to light stress–mitigating terpenes and xanthophylls (shaded green) and to cannabinoids (shaded yellow). Condensation of geranyl pyrophosphate (GPP) with olivetolic acid (OA) yields cannabigerolic acid (CBGA). Various synthases cyclize CBGA to subsequent cannabinoids such as Δ°-tetrahydrocannabinol-9-carboxylic acid (Δ°-THCA) and cannabidiolic acid (CBDA). Δ°-THCA and CBDA are decarboxylated to Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), respectively.

(DMAPP) and isopentenyl pyrophosphate (IPP), and is the basic subunit of all monoterpenes in higher plants (Banthorpe et al., 1972; Croteau and Purkett, 1989). In the synthesis of cannabinoids, GPP and olivetolic acid (OA) are combined via GPP-OA transferase to produce CBGA (Fellermeier and Zenk, 1998; Fellermeier et al., 2001). Various synthases then convert CBGA to derivatives such as Δ^9 -THCA and CBDA (Taura et al., 1995, 1996). All terpenes in this study are also downstream products of DMAPP and IPP, including several carotenoids and xanthophylls that mitigate damage from harmful

radiation (Croteau and Purkett, 1989; Demmig-Adams and Adams, 1996; Dorothea, 2008; Lichtenthaler, 1987).

Perhaps by supplementing additional light, particularly a spectrum relatively rich in green light that is normally absorbed by some terpenes (Miller et al., 1935; Zur et al., 2000), the plants up-regulated terpene biosynthesis to manage that environmental condition. In so doing, IPP and DMAPP precursors were also up-regulated to supply the demand for these terpenes. A greater pool of IPP and DMAPP would also theoretically be available for the production of GPP to be condensed with OA to

produce CBGA, and Δ9-THCA and CBDA in turn. Conversion from CBGA to Δ^9 -THCA via THCA synthase may have happened with a high enough efficiency that most extra CBGA produced was converted to Δ^9 -THCA, accounting for the lack of increase in observed CBGA concentrations. By contrast, CBDA synthase may have an extremely low activity in this cannabis variety, so even in the presence of an increased CBGA pool, there is no increase in the amount of CBDA produced. This explanation is conjecture given that, to the best of our knowledge, there are no studies directly measuring IPP or DMAPP concentrations under varying light qualities. The secondary metabolism in cannabis is complex and requires a great deal more study before we will have a satisfactory explanation for this observation.

Conclusions

Results suggest that supplemental SCL can increase bud yield and modify cannabinoid and terpene profiles. The increase in bud yield is likely a product of greater *PPFD* compared with production with overhead lighting, alone. Red-Blue SCL yielded a more consistent metabolite profile throughout the canopy, and RGB SCL had the greatest impact on up-regulating metabolites. Future studies could expand on this research by deploying light qualities richer in the green

region of the spectrum, modifying the overhead light spectrum, and deploying LED arrays within the canopy, as opposed to below. It would be of academic value in future studies to quantify IPP and DMAPP pools to better understand the influence of spectral quality on terpene and cannabinoid biosynthesis.

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