Cannabis yield increased proportionally with light intensity, but additional ultraviolet radiation did not affect yield or cannabinoid content

David Llewellyn[1], Scott Golem[2], Elizabeth Foley[2], Steven Dinka[2], A. Maxwell P. Jones[3], and Youbin Zheng[1]*

[1]School of Environmental Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

[2]HEXO Corp., 67 Sinclair Boulevard, Brantford, Ontario, Canada, N3S 7X6

[3]Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

*Corresponding author: yzheng@uoguelph.ca

Abstract

Cannabis (*Cannabis Sativa* L.) is now legally produced in many regions worldwide. Cannabis flourishes under high light intensities (LI); making it an expensive commodity to grow in controlled environments, despite its exceptionally high market value. It is commonly believed that cannabis secondary metabolite levels may be enhanced both by increasing LI and by exposing crops to ultraviolet radiation (UV). However, there is sparse scientific evidence to guide cultivators. Therefore, the impact of LI and UV on yield and quality must be elucidated to enable cultivators to optimize their lighting protocols. We explored the effects of LI, ranging from 350 to 1400 μmol m^{-2} s^{-1} and supplemental UV spectra on cannabis yield and potency. There were no spectrum effects on inflorescence yield, but harvest index under UVA+UVB was reduced slightly (1.6%) vs. the control. Inflorescence yield increased linearly from 19.4 to 57.4 g/plant and harvest index increased from 0.565 to 0.627, as LI increased from 350 to 1400 μmol m^{-2} s^{-1}. Although there were no UV spectrum effects on total equivalent Δ^9-tetrahydrocannabinol (T-THC) content in leaves, the neutral form, THC, was 30% higher in UVA+UVB vs. control. While there were no LI effects on inflorescence T-THC content, the content of the acid form (THCA) increased by 20% and total terpenes content decreased by 20% as LI increased from 350 to 1400 μmol m^{-2} s^{-1}. High LI can substantially increase cannabis yield and quality, but we found no commercially-relevant benefits of adding supplemental UV radiation to indoor cannabis production.

Abbreviations

APPFD, average integrated photosynthetic photon flux density; BSWF, biological spectral weighting function for plant growth; CBC, cannabichromene; CBD, cannabidiol; CBN, cannabinol; DW$_f$, dry weight of inflorescence; DW$_{nf}$, dry weight of non-floral aboveground tissues; FW$_f$, fresh weight of inflorescence; FW$_{nf}$, fresh weight of non-floral aboveground tissues; HI, harvest index; LI, light intensity; PAR,

photosynthetically active radiation; PPFD, photosynthetic photon flux density; THC, Δ^9-tetrahydrocannabinol; THCA, Δ^9-tetrahydrocannabinolic acid; T-THC, total Δ^9-tetrahydrocannabinol; UV, ultraviolet (100-400 nm); UVA, ultraviolet-A (315-400 nm); UVB, ultraviolet-B (280-315 nm)

Introduction

Drug-type *Cannabis sativa* L. (hereafter: cannabis) is one of the highest-value crops that are commercially grown in indoor environments, where electric lighting is the principal source of photosynthetically active radiation (PAR; 400-700 nm). Given cannabis' high market value and exceptional tolerance of high light intensity (LI) (Chandra et al. 2008), canopy level photosynthetic photon flux densities (PPFD) can be several-fold higher than many other indoor-grown commodities (Bilodeau et al. 2019). In order to optimize profitability, the concomitant premiums in energy and lighting (and related) infrastructure costs must be carefully considered as offsets to any potential increases in yield and quality that may be achieved under higher LI. A major component of this balancing process is the development of LI response models of cannabis yield and quality, over a broad range of PPFDs. Investigating LI ranges beyond the commercially relevant levels enables the interpolation of LI response models within the commercial context. This approach has a higher degree of confidence than extrapolation of (linear) models that only have LI ranges that do not surpass commercially relevant boundaries (e.g., Potter and Duncombe 2012).

The efficient production of marketable biomass (i.e., mature, unfertilized female inflorescences) is clearly of foremost importance in cannabis production. However, indoor-grown cannabis is a highly specialized crop, in that the major product of interest is not simple biomass but the secondary metabolites. These are predominantly cannabinoids and terpenes, which are chiefly associated with the inflorescence tissues from unfertilized female plants (hereafter: inflorescence) (Potter 2009, Small 2017, Livingston et al. 2020). In modern drug-type cannabis genotypes, these secondary metabolites can comprise $\geq 25\%$ of the total biomass in mature inflorescence tissues and this is one of the primary metrics determining marketability (Dujourdy and Besacier 2017, Jikomes and Zoorob 2018, Cash et al. 2020).

Cannabinoids are normally present in living cannabis tissue in their acid forms, which are naturally decarboxylated into the neutral forms over time, or through application of heat or light (Pascifico et al. 2007). Given the highest bioactivity of the neutral cannabinoids, potencies are often (mathematically) converted and referred to according to their neutral forms. For example, Δ^9-tetrahydrocannabinol (THC) is the neutral (and most psychoactive) form of Δ^9-tetrahydrocannabinolic acid (THCA) and the potential psychoactive potency of the THCA content in a tissue sample can be calculated based on the relative mass of the acid moiety that is lost from the molecule during the decarboxylation process. Therefore, total equivalent THC content (T-THC, mg g^{-1}) is calculated as: T-THC = (THCA $\times$ 0.877) + THC.

Cannabinoids may have photoprotective roles in cannabis ecology, with some (sparse) scientific evidence that light stress from either high LI or spectral manipulations can alter the metabolomic composition (see: Magagnini et al. 2018 for a review). Further, it has been posited that the ultraviolet (UV) absorption properties of some cannabinoids may represent an ecological justification (i.e., as photoprotective elements) as to why high concentrations of some cannabinoids are associated with inflorescence tissues. In particular, some older studies have alluded to possible links between UV radiation exposure and THC content (Fairbairn and Liebmann 1973, Pate 1983, Lydon et al. 1987) in indoor-grown cannabis. However, no mechanism for upregulating THC vs. other cannabinoids under UV exposure has been elucidated (Pate 1994). Further, UV absorption of THC does not confer a clear ecological advantage relative to other major cannabinoids, which have similar [e.g., cannabidiol (CBD)] or greater [e.g., cannabichromene (CBC) and cannabinol (CBN)] UV absorption than THC (Pate 1994, Hazekamp et al. 2005, De Backer et al. 2009). Despite a lack of contemporary published scientific studies on the effects of UV exposure on cannabinoid content (Magagnini et al. 2018), there is a popular belief in the cannabis industry that UV radiation exposure can substantially enhance cannabinoid content – particularly THC – in inflorescence tissues in modern cannabis genotypes. Distinguishing the UV-radiation effects on THC content as it relates to typical potency levels (for a given genotype) may also be an important consideration, since the inflorescence THC content may be many times higher in current drug-type vs. older cannabis genotypes (Dujourdy and Besacier 2017). This may suggest that other factors (e.g., genetics) may play a more significant role in moderating inflorescence THC content than environmental stresses such as UV radiation.

The strongest links between UV radiation and cannabinoid content relate to ultraviolet-B (UVB, 280-315 nm), however radiation from the ultraviolet-A (UVA, 315-400 nm) and shorter wavelength blue (400-500 nm) wavebands have also been implicated in moderating the cannabis inflorescence chemical composition (Magagnini et al. 2018, Bilodeau et at. 2019) and mediating cellular repair provoked by UVB damage (Krizek 2004), sometimes called photoreactivation (Gill et al. 2015).

The objectives of this study were to investigate the impacts of broad range of LI and the exposure to UVA and UVB radiation on the yield and quality of mature female inflorescences in a high-THC cannabis genotype. Two parallel, concurrent experiments were conducted in an indoor environment where light emitting diodes (LEDs) were the sole source of PAR and UV radiation treatments were provided using LED and fluorescent lighting technologies.

Materials and Methods

The experiment was conducted in a commercial cannabis greenhouse in Southern Ontario, Canada. Three enclosures were used (5.9 × 4.1 × 2.7 m), each consisting of two benches (5.9 × 1.8 m) that were separated by 0.5 m and encompassed with "panda film" (Vivosun, City of Industry, CA, USA), black side facing inwards, to block natural light and minimize light contamination between treatments. Each enclosure contained five 0.54 m^2 plots, with a lateral separation of ≥ 0.65 m between plots. Air temperature and relative humidity (RH) were recorded every 300 s using data loggers (HOBO MX2301A; Onset Computer Corporation, Bourne, MA, USA) located at light fixture level in each enclosure. Across the three enclosures, the daytime temperature and RH were (mean ± SD) 26 ± 1.2°C and 40 ± 6.9%, respectively, and nighttime temperature and RH were 22 ± 1.9°C and 47 ± 3.9%, respectively. No supplemental CO_2 was provided during this trial.

Lighting treatments. Pairs of LED bars (Toplight-Targeted Spectrum; LumiGrow, Emeryville, CA, USA) were spaced 0.4 m apart, on-center, over each plot. One plot in each enclosure had an additional pair of Toplight LED bars, evenly spaced between the first pair of LED bars, to facilitate higher light intensities in this plot. These fixtures were comprised of a combination of blue, phosphor-converted white (5000 K), and red LEDs. The blue and red LEDs had peak wavelengths [± half-width at half maximum (HWHM)] of 445 ± 8.5 nm and 665 ± 8.0 nm, respectively. The native spectrum (Fig. 1A) of the Toplight-Targeted Spectrum fixtures (i.e., when all LED channels are operated at maximum intensity) was maintained in all cases where dimming was used to reduce intensity. The UVA spectrum (Fig. 1B) was provided by custom-made LED bars (0.05 × 0.6 m, Yunustech, Mississauga, ON, Canada) which had a peak wavelength (± HWHM) of 385 ± 5.5 nm. In one plot in each enclosure, two UVA LED bars were centered 0.24 m apart, at the same height of the LED Toplight fixtures. The UVA+UVB spectrum (Fig. 1C) was provided by a broad-band fluorescent lighting technology (SolarSystem UVB, California LightWorks, Canoga Park, CA, USA). The dominant peak in the spectrum spans from ≈ 275 to 380 nm, with additional narrow-band peaks at 312, 365, 404, 435, 545, and 579 nm. On a photon-flux basis, the spectrum has a ratio of UVB (280-315 nm) to UVA (315-400 nm) of 1.07 (i.e., almost equal photon flux levels of UVB and UVA). When the Biological Spectral Weighting Function (BSWF; Flint and Caldwell, 2003) is applied to the UVA+UVB spectrum, the photon flux ratio of raw to BSWF UV (280-400 nm) is 0.49. Further, after conversion of the spectrum with the BSWF, the ratio of radiant to photon flux is 0.38 (J μmol^{-1}). A single SolarSystem UVB fixture was centered over one plot in each enclosure, positioned at the same height as the LED Toplight fixtures.

The experiment was arranged as a randomized complete block design (RCBD) with three LI and two UV-spectrum treatments and three concurrent replications (blocks). In each block, the three intensity treatment

plots targeted canopy-level PPFDs of 600, 800, and 1000 µmol m^{-2} s^{-1}, respectively. The UV-spectrum treatments had targeted canopy-level PPFDs of 600 µmol m^{-2} s^{-1} plus an additional 50 and 3.0 µmol m^{-2} s^{-1} of UV photon flux (280 to 400 n,) for the UVA and UVA+UVB treatments, respectively. The Toplight fixtures in all plots, plus the UVA fixtures in the UVA treatments had a photoperiod of 12 h (06:30 HR to 18:30 HR). The SolarSystem UVB fixtures in UVA+UVB treatment plots had a 5-h photoperiod (13:30 HR to 18:30 HR) and were only operated during the last 20 d of the trial. The light spectra were evaluated using a radiometrically-calibrated spectrometer (XR-Flame-S; Ocean Optics, Dunedin, FL, USA) coupled to a CC3 cosine-corrector attached to a 1.9 m × 400 µm UV-Vis optical fibre. The PAR-Spec subroutine in SpectraSuite (Ocean Optics) was used for spectrum characterizations. Intensities of the Toplight LEDs were modified using the lighting control software (smartPAR; LumiGrow) to achieve the prescribed intensity and spectra. The UV intensity in the UVA treatment was adjusted using analog dimmers. The UV intensity in the UVA+UVB treatment was adjusted using neutral density screen affixed to underside of the SolarSystem UVB fixture.

Following spectrum characterization, the canopy-level PPFD of each plant was measured and recorded, twice weekly, using a handheld light sensor (LI-180; LI-COR Biosciences, Lincoln, NE, USA) and the light fixture hang-heights were adjusted accordingly to ensure the targeted PPFD levels were maintained as the plants grew. Although the initial layout of the experiment was a RCBD, the component of the trial was conducted as a gradient design (Jones-Baumgardt et al., 2019) with each plant treated as an experimental unit and assigned a light level, average PPFD (APPFD) reflective of their individual accumulated light histories. The APPFD value for each individual plant was obtained by computing the light integrals between each PPFD measurement period (i.e. 3-4 d), summing these integrals over the entire 45-d trial to determine a total light integral (TLI, mol m^{-2}), and then determining the APPFD by dividing TLI by the total number of seconds of lighting during the entire trial.

Plant cultivation. Uniform rooted cuttings of the clonal cannabis genotype 'Meridian' were transplanted into rockwool cubes (0.15 × 0.15 × 0.15 m; Grodan, Milton, ON, Canada) and grown using the Toplight-Targeted Spectrum (LumiGrow) fixture, as described above. Transplants were grown for 21 d under PPFD of ≈ 600 µmol m^{-2} s^{-1} and were maintained in a vegetative state using a 16-h photoperiod (06:30 HR to 22:30 HR). The transplants selected for the trial had their apical meristems removed and were trimmed to uniform height and number of nodes. In each plot, the trimmed transplants were arranged in two rows of three, with adjacent plants spaced 30 cm apart, on center (i.e., planting density of ≈ 12 plants/m^2). The photoperiod was immediately reduced to 12-h to induce flowering, and plants were grown for an additional 45-d. Throughout the vegetative and flowering stages, plants were drip-irrigated twice daily at 2 L hr^{-1} for 540 s, such that each plant received ≈ 0.6 L d^{-1}. The nutrient solution was comprised of Dutch

Nutrients Gro A and Gro B (Homegrown Hydroponics, Toronto, ON, Canada) at a rate of 5 mL L^{-1} in rainwater, resulting in an EC of $\approx$ 1.75 dS m^{-1} and pH of $\approx$ 5.6.

Harvest and post-harvest measurements. At harvest, the stems were cut at growing medium level and the inflorescences were hand-trimmed from each plant. The fresh weight of inflorescence (FW$_f$) and non-floral (FW$_{nf}$) aboveground tissues (i.e., stems and leaves) were recorded using a precision digital balance scale (AX622N/E Adventure Precision Balance; OHAUS Corporation, Parsippany, NJ, USA). The separated aboveground tissues from one randomly selected plant in each plot were oven dried to constant weight at 65 ^{o}C and re-weighed (AX622N/E Adventure Precision Balance; OHAUS Corporation) to determine water content of the respective tissues. After determining there were no treatment effects on tissue water content, the dry weight of inflorescence (DW$_f$) and non-floral (DW$_{nf}$) aboveground tissues were calculated for each plant using the average water content for the respective tissues. Harvest index for each plant was calculated using the following formula: HI = (DW$_f$ / (DW$_f$ + DW$_{nf}$). The entire inflorescence tissue from one randomly selected plant in each LI treatment plot were spread out in a single layer on perforated drying trays and air dried at (mean $\pm$ SD) 19 $\pm$ 1.8°C and 51 $\pm$ 9.2 RH% for 5 d (i.e., final moisture content of $\approx$ 10-15%). Once dry, the inflorescence material from each plant was homogenized and composite $\approx$ 5 g samples were collected and submitted to a 3rd-party lab (RPC; Fredericton, NB, Canada) for analysis of cannabinoid and terpene content. In addition, the foliar tissues that were trimmed from the upper inflorescences (commonly called "sugar leaves" in the cannabis production industry) from two randomly selected plants from each control, UVA and, UVA+UVB treatment plot were spread out in a single layer on perforated drying trays and air dried for 5 d. Approximately 1 g of dried sugar leaves from each sample were submitted to the internal lab (HEXO; Gatineau, QC, Canada) for analysis of cannabinoid content (18 samples total).

Data processing. The LI responses were analyzed using linear regression ($P \leq 0.05$) in Prism (GraphPad Software, San Diego, CA, USA) with APPFD as the independent variable. Where appropriate, for parameters that exhibited treatment effects (i.e., non-zero slopes), the linear models were compared ($P \leq$ 0.05) with higher-order models (e.g., linear, quadratic, logarithmic, and asymptotic) to determine the best-fit models. For parameters that lacked LI treatment effects, experiment means ($\pm$ SE) were presented. The light spectrum responses were analyzed in JMP (version 10; SAS Institute Inc., Cary, NC) using Tukey's honestly significant difference test ($P \leq 0.05$).

Results

The APPFD calculated for each plant in the LI treatment plots resulted in a range of APPFDs from 350 to 1400 μmol m^{-2} s^{-1}. For all parameters where LI treatment effects were found, linear models best fit the

parameter responses to increasing LI. Every 100 µmol m^{-2} s^{-1} increase in APPFD resulted in commensurate 3.6 and 1.8 g/plant increases in inflorescence and non-floral aboveground biomass (i.e., stems and leaves), respectively (Fig. 2A). The disparity in the slopes of these models yielded a 10% increase in HI as APPFD increased from 350 to 1400 µmol m^{-2} s^{-1} (Fig. 2B). While there were no PPFD treatment effects on T-THC content in the inflorescence, THCA content increased by 2% (i.e., from 196 to 201 mg g^{-1}) as APPFD increased from 350 to 1400 µmol m^{-2} s^{-1} (Table 1). Typical for this genotype, CBD was not detected in any samples. Terpinolene content was reduced by ≈ 30% as APPFD increased from 350 to 1400 µmol m^{-2} s^{-1} while caryophyllene and humulene content both increased by ≈ 40% over the same APPFD range (Table 1).

There were no spectrum treatment effects on inflorescence and non-floral aboveground biomass, however harvest index (HI) under UVA+UVB was reduced slightly (1.6%) vs. the control (Table 2). While there were no spectrum treatment effects on T-THC content in 'sugar' leaves, the THC content was 30% higher in UVA+UVB vs. control (Table 3). The beta-pinene content in dry inflorescence was 6% lower in UVA+UVB vs. control (Table 4). There were no other spectrum treatment effects on biomass parameters or cannabinoid and terpene content in either inflorescence or foliar tissues.

Discussion

Two of the dominant phytogenic factors that affect profitability in commercial drug-type cannabis production are marketable yield (i.e., mature, unfertilized female inflorescences) and the secondary metabolite content (e.g., content of cannabinoids and terpenes) in these marketable tissues. A primary objective of this study was to explore proof of concept for the potential for UV radiation treatments for increasing cannabinoid content, particularly THC, in a modern indoor-grown cannabis genotype.

The genotype used in this study was typical of Type-I (i.e., drug-type) cannabis (de Meijer et al. 1992); with > 20% THC (i.e., > 200 mg g^{-1}) and no detectable CBD in the inflorescence tissue (Table 1). A low amount of cannabigerol (CBG, the chemical precursor to THC and CBD) was detected. The ratio of T-THC to total equivalent CBG (T-CBG) was ≈ 18, possibly indicating the potential to increase the T-THC content by ≈ 5% (i.e., from ≈ 200 to 210 mg g^{-1}). There was also no cannabinol (CBN) – a natural THC breakdown product – detected; which, along with the high ratio of T-THC to T-CBG, indicated that the plants were near peak maturity at harvest (Aizpurua-Olaizola et al. 2016).

Yield is proportional to light intensity. Many (interrelated) environmental parameters can be optimized in order to maximize yields, including temperature, humidity, CO_2 concentration, and fertility. However, in indoor cultivation environments, LI is one of the most prominent and expensive input parameters under

growers' complete control (Mills 2012). The optimum LI in a given production scenario will depend on many economic factors, but the responses of modern cannabis genotypes' yield and potency to LI are key input factors that can only be elucidated experimentally. The linear yield response to LI (Fig. 2A), over a range of LIs that exceed normal production levels (Potter and Duncombe 2012), confers a relatively reliable and easy to interpret basic model for how cannabis yield responds to increases (or decreases) in LI. Aside from the general shape of the model (i.e., a positively sloped line), the intercept, which relates to a crop's fundamental capacity to convert PAR into biomass, and the slope, which relates to the crop's phenotypic plasticity to respond to changes in environmental inputs, will of course vary by genotype and production environment. Nevertheless, using the present study's genotype as a proxy, one can estimate the payoff associated with increases in LI. For example, a 100 µmol m^{-2} s^{-1} increase in APPFD resulted in 40.2 g m^{-2} of additional yield (3.6 g $plant^{-1}$ × 6 plants / 0.54 m^2 $plot^{-1}$). The total light integral (TLI) for 100 µmol m^{-2} s^{-1} of canopy level PAR over 45 d is 195 mol m^{-2}, resulting in a light use efficiency of 0.21g mol^{-1}. The energy cost for additional lighting relies heavily on fixture efficacy, light distribution, and local cost of electricity. Modern LED fixtures have efficacy values exceeding 2.5 µmol J^{-1} (i.e., 9 mol kWh^{-1}) (Design Lights Consortium, Qualified Product List, https://www.designlights.org/horticultural-lighting/search/, accessed on 11 February 2021). If electricity cost was 0.10 \$CAD/kWh, the estimated energy cost in this scenario would be approximately 0.054 \$CAD/g or approximately 2 g kWh^{-1}. This estimate is twice the suggested energy efficiency of cannabis production that was estimated from scientific studies in past decades (EMCDDA 2012). The difference may be attributed to the much higher efficacy ascribed to modern LED vs. conventional high intensity discharge technologies (e.g., HPS) (Kusuma et al. 2020) and the inclusion of the TLI during vegetative stage in their calculations. At 2 g kWh^{-1} and a current wholesale price for dried flower of 4.80 \$CAD g^{-1} (Cannabis Benchmarks, https://www.cannabisbenchmarks.com/report-category/canada/, accessed 25 Jan. 2021), the added electricity cost comprises only about 1.1% of the wholesale price, and therefore may make economic sense.

In addition to higher yields, the proportion of marketable aboveground biomass also rose with increasing LI (Fig 2B). This has been observed in other species, but the rate of increase in cannabis HI was approximately 4-fold higher than in indoor-grown wheat (Bugbee and Monje 1992) over a similar LI range. This serves as further evidence of cannabis' enormous phenotypic plasticity in response to LI. Higher HI could enhance harvest efficiency by reducing the non-marketable proportion of the biomass, that needs to be removed at harvest and disposed (EMCDDA 2012). Further, since inflorescence tissues have substantially higher cannabinoid potencies than other aboveground tissues (Richins et al. 2018), plants that produce proportionally higher inflorescence biomass under higher LI may also increase overall cannabinoid yield. While this was not evaluated in the present study, it is also likely that the increased HI

is associated with larger inflorescences and increased floral density, which is generally valued by the industry.

Light intensity did not substantively affect chemical composition. The only LI treatment effect on cannabinoid content was THCA but the slope was very shallow (i.e., only ≈ 2% increase as APPFD increased from 350 to 1400 μmol m^{-2} s^{-1}), and did not translate to increases in T-THC content (Table 1). The lack of LI effects on cannabinoid content was consistent with other studies (Vanhove et al. 2011, Potter and Duncombe 2012), although their LI ranges were much narrower. In contrast to cannabinoids, the LI treatment effects on terpene content were more nuanced (Table 1). The content of two minor terpenes increased with increasing LI, while terpinolene (the dominant terpene in this genotype) content decreased with increasing LI; conferring a combined ≈ 20% decrease in total terpene content as APPFD increased from 350 to 1400 μmol m^{-2} s^{-1}. In contrast, Namdar et al. (2018) found that inflorescence cannabinoid and terpene content increased with proximity to the light source, which the authors attributed to the localized LI of the sampled tissues. However, this was not tested experimentally and could have also been due to gradients in other exogenous (e.g., temperature) or endogenous (e.g., biomass allocation and variable inflorescence maturation) factors related to branch position (Hemphill et al. 1980, Diggle 1995).

Increasing canopy-level LI increased had variable effects on terpene composition, which may affect aroma and flavor, but no effect on cannabinoid content. Overall, both cannabinoid and terpene yield (i.e., g/plant) increased concurrently with increasing inflorescence DW, which may be important for processing cannabis extracts. While many factors are involved in evaluating profitability of adopting a specific production practice, raising canopy-level LI may be an economically feasible way to increase inflorescence and secondary metabolite yield – but not potency – in indoor cannabis production.

Secondary metabolite content was unaffected by UV radiation. UV radiation can invoke both eustress and distress responses in plants, depending on many intrinsic (e.g., genotype and ontological stage) and extrinsic factors (e.g., UV intensity, duration, and spectrum, PAR intensity). The major modes of action of UV radiation on plants are through photoreceptor-mediated responses (e.g., UVR8) and the production of reactive oxygen species (ROS), which can cause cellular damage and influence gene expression (Jansen et al. 1998, Hideg et al. 2013). One common response to UV exposure is the upregulation of the production of photoprotective compounds, particularly in epidermal regions, to diminish UV penetration deeper into plant tissues (Frohnmeyer and Staiger 2003, Huché-Thélier et al. 2016). In indoor cannabis production,

9

the primary goal of exposing flowering plants to UV radiation is to elicit the production of cannabinoids, which have UV-photoprotective properties (Hazekamp et al. 2005), on and around inflorescence tissues.

Since there were no yield reductions, there was little evidence of spectrum-induced distress in either UV treatment. While we did not quantify trichome density, we noted that there appeared to be higher trichome density on the sugar leaves in the UVA+UBV vs. UVA and control treatments. This observed increase in trichome density may explain the $\approx 30\%$ higher foliar THC content and trends (not statistically significant at $P \leq 0.05$) of $\approx 10\%$ higher THCA and T-THC content in the UVA+UBV treatment vs. control (Table 3). However, since foliar cannabinoid content was much lower ($\approx 10X$) than inflorescence tissues due to lower trichome density (Small 2017), these tissues are of relatively low value in commercial indoor cannabis production and are normally discarded (Potter 2013). Therefore, from a production perspective, the minor increases in foliar cannabinoid potency under UV exposure were probably not commercially relevant. In contrast to the foliage, the only spectrum treatment effect on the inflorescence secondary cannabinoid and terpene composition was a $\approx 15\%$ reduction in beta pinene content in the UVA vs. control treatment (Table 4). Since none of the cannabinoid levels were affected by the UV treatments, the eustress levels of UV radiation did not have substantial effects on the potency of the cannabis genotype used in this investigation. It is possible that the relatively low cannabinoid content of genotypes used in prior studies (e.g., Pate 1983, Lydon et al. 1987) had conferred a relatively greater potential for stress-induced cannabinoid upregulation than in modern genotypes that have much higher cannabinoid content. Further, within a given genotype's overall genetic potential for producing various cannabinoids, there maybe a higher likelihood for a plant to upregulate the production of one metabolite over another if both are normally present at reasonable proportions. Therefore, in contrast with the genotype in the present study, a genotype with a characteristically more balanced ratio of THC to CBD may show higher plasticity in modifying the cannabinoid metabolome under UV eustress conditions.

These results do not mean that that UV radiation cannot be used to manipulate cannabis potency, since there are myriad combinations of genotype, spectrum, and dose paradigms yet to be studied. Further, we did not evaluate all aspects of cannabis quality, including: the composition of other groups of biologically active compounds (e.g., flavonoids) or organoleptic quality. However, in this 'proof of concept' study – and under the selected experimental conditions (e.g., UV treatments and Type-I genotype) – UV radiation had no commercially-relevant benefit to either cannabis yield or quality.

Conclusion

Cannabis proliferates at very high canopy level LIs in indoor production. The linearly-increasing yield response to the broad range of LI levels (up to 1400 µmol m^{-2} s^{-1}) in this trial clearly shows the benefits to

maximizing canopy-level PPFD within the economical constraints imposed by other production logistics (including input costs). While increased yield did not have a major impact on cannabinoid composition, it did result in lower terpene content and further study is needed to assess the impact this has on product quality. Conversely, we saw no commercially-relevant benefits to exposing cannabis plants to UV radiation. Given the myriad potential UV exposure algorithms (i.e., combinations of spectrum, intensity, and temporal application strategies) more research is needed to determine whether UV treatments may be a commercially-relevant production tool and elucidate appropriate treatment protocols for commercial applications.

Author Contributions

All authors contributed to the experimental design. DL, SG, LF, and SD performed the experiment, and collected data. DL analyzed the data and wrote the manuscript draft. YZ, SG, AMPJ, LF, and SD revised the manuscript. All authors approved the final manuscript.
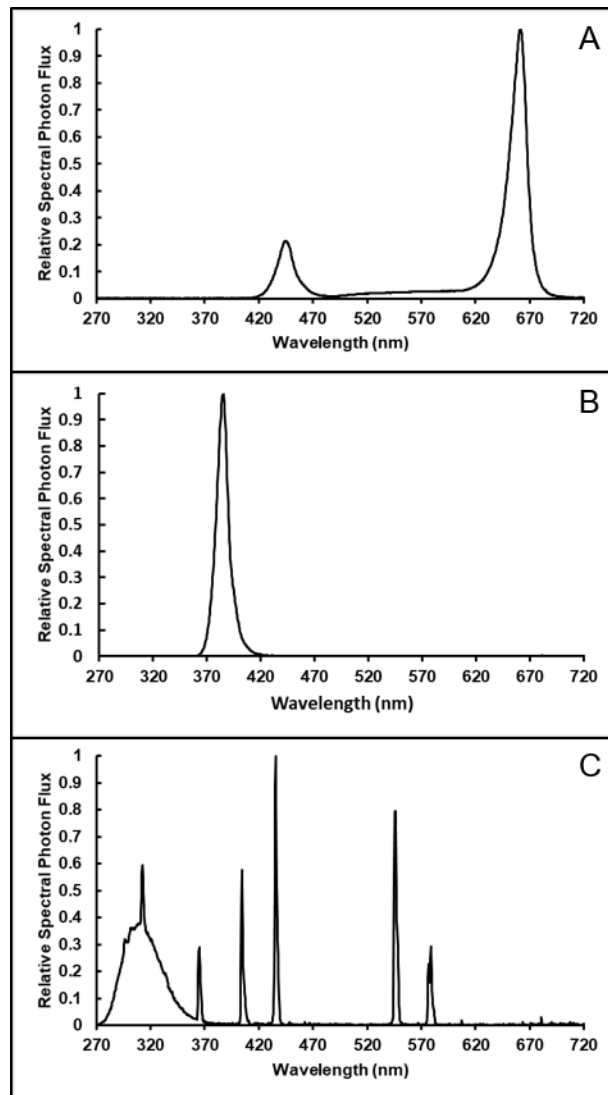
Acknowledgements

Figure 1. Spectrum distributions of: (A) Lumigrow Toplight LED source of photosynthetically active radiation; used in all UV spectrum and intensity treatments, (B) 385 nm peak UVA LEDs; used in UVA treatment (photon flux density of 50 μmol m^{-2} s^{-1}), and (C) broad-band fluorescent UV; used in the UVA+UVB treatment (photon flux density of 3 μmol m^{-2} s^{-1}).
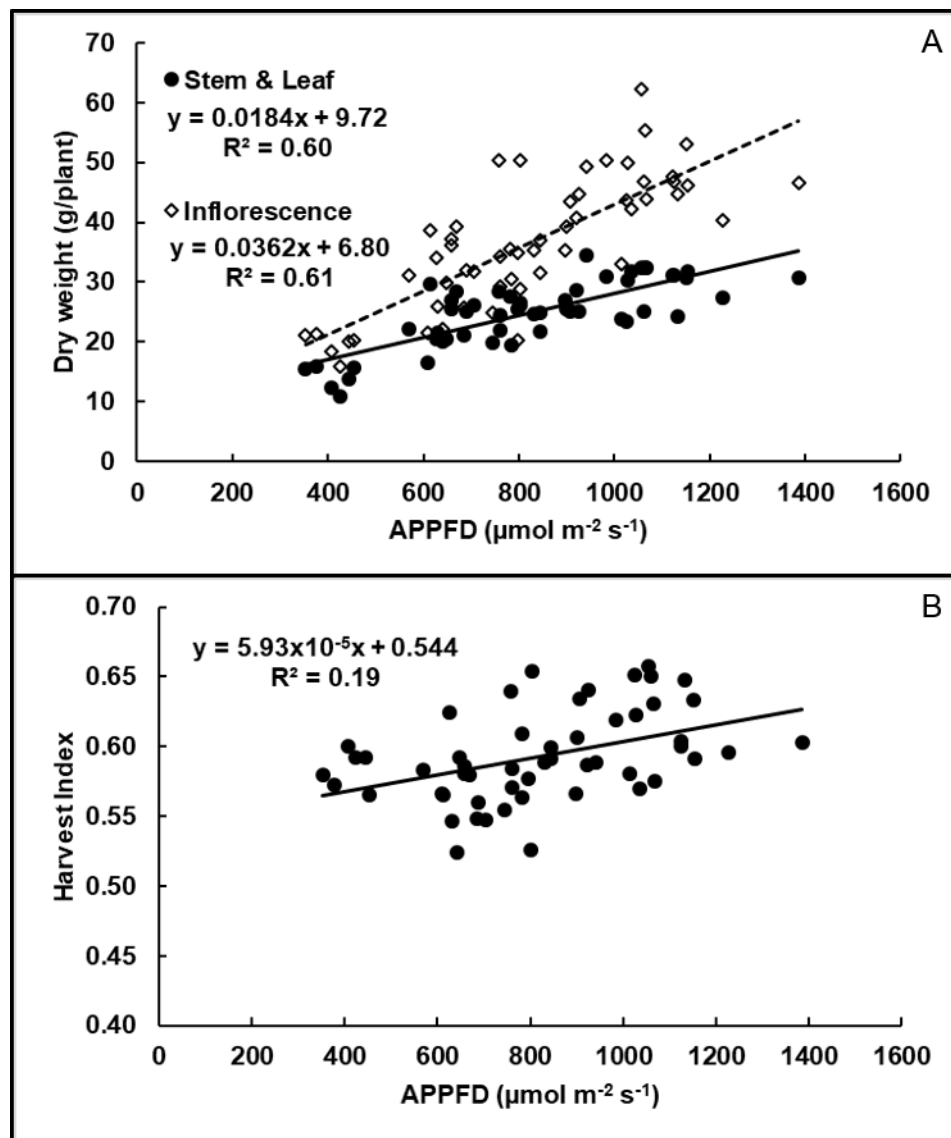
Figure 2. Dry weight of inflorescence and aboveground non-inflorescence tissues (A) and harvest index (B) of *Cannabis sativa* 'Meridian' plants grown under light emitting diodes (LEDs) for 45 d under average photosynthetic photon densities (APPFD) ranging from 350 to 1400 μmol m^{-2} s^{-1} for 12 h d^{-1}. Each datum is a single plant.

Table 1. Secondary metabolite contents of dry composite inflorescence samples of *Cannabis sativa* 'Meridian' plants grown under light emitting diodes (LEDs) for 45 d under average photosynthetic photon densities (APPFD) ranging 350 to 1400 µmol m^{-2} s^{-1} for 12 h d^{-1}.

Secondary metabolite	Content in dry inflorescence (mg g^{-1})
Δ^9-tetrahydrocannabinol (THC)	6.75 ± 0.32^z
Δ^9-tetrahydrocannabinolic Acid (THCA)	Y = 0.00426*X + 195 (0.46)
Total equivalent Δ^9-tetrahydrocannabinol (T-THC)	211 ± 4.6
Cannabigerol (CBG)	0.579 ± 0.077
Cannabigerolic acid (CBGA)	12.2 ± 0.69
Total equivalent cannabigerol (TCBG)	11.3 ± 0.65
Cannabichromene (CBC)	BDL^y
Cannabidiol (CBD)	BDL
Cannabidiolic acid (CBDA)	BDL
Total equivalent cannabidiol (TCBD)	BDL
Cannabinol (CBN)	BDL
Terpinolene	Y = -0.00238*X + 8.86 (0.75)
Myrcene	2.63 ± 0.087
Limonene	1.98 ± 0.079
Beta pinene	1.30 ± 0.034
Trans-ocimene	1.22 ± 0.046
Alpha pinene	0.921 ± 0.023
Caryophyllene	Y = 0.000691*X + 0.890 (0.78)
Terpineol	0.744 ± 0.020
3-carene	0.340 ± 0.00050
Fenchol	0.340 ± 0.019
Humulene	Y = 0.000246*X + 0.231 (0.62)
Sabinene	0.139 ± 0.017
Alpha-bisabolol	BDL
Borneol	BDL
Cis-nerolidol	BDL
Cis-ocimene	BDL
Eucalyptol	BDL
Guaiol	BDL
Linalool	BDL
Valencene	BDL
Trans-nerolidol	BDL
Total terpenes	Y = -0.00314*X + 21.2 (0.56)

^{z}mean ± SE (n = 9) or linear model and R^2 (in brackets)
^{y}below detection limit

Table 2. Dry weight (DW; g/plant) of inflorescence and aboveground non-inflorescence tissues and harvest index of *Cannabis sativa* 'Meridian' plants grown under three lighting treatments: (1) 12 h d^{-1} of 600 µmol m^2 s^{-1} of photosynthetically active radiation for 45 d (Control), (2) 12 h d^{-1} of 600 µmol m^{-2} s^{-1} of PAR plus an additional 12 h d^{-1} of 50 µmol m^{-2} s^{-1} of ultraviolet-A radiation from LEDs (385nm peak) for 45 d (UVA), and (3) 12 h d^{-1} of 600 µmol m^{-2} s^{-1} of PAR for 45 d plus an additional 3 µmol m^{-2} s^{-1} of an equal ratio of ultraviolet-A and ultraviolet-B radiation for 5 h d^{-1} for the 20 days prior to harvest (UVA+UVB).

Harvest parameter	Control	UVA	UVA+UVB
Inflorescence DW	20.4 ± 1.1 a[z]	22.2 ± 0.94 a	22.4 ± 0.91 a
Aboveground non-inflorescence DW	27.6 ± 1.4 a	31.3 ± 1.2 a	29.3 ± 1.4 a
Harvest index	0.575 ± 0.0056 a	0.584 ± 0.0073 ab	0.566 ± 0.0055 b

[z]means (± SE, n = 3) followed by the same letter are not different at P ≤ 0.05 according to Tukey's honestly significant difference test

Table 3. Dry foliar ('sugar leaves') content of secondary metabolites of *Cannabis sativa* 'Meridian' plants grown under three lighting treatments: (1) 12 h d^{-1} of 600 µmol m^{-2} s^{-1} of photosynthetically active radiation for 45 d (Control), (2) 12 h d^{-1} of 600 µmol m^{-2} s^{-1} of PAR plus an additional 12 h d^{-1} of 50 µmol m^{-2} s^{-1} of ultraviolet-A radiation from LEDs (385nm peak) for 45 d (UVA), and (3) 12 h d^{-1} of 600 µmol m^{-2} s^{-1} of PAR for 45 d plus an additional 3 µmol m^{-2} s^{-1} of an equal ratio of ultraviolet-A and ultraviolet-B radiation for 5 h d^{-1} for the 20 days prior to harvest (UVA+UVB).

Cannabinoid	Content in dry 'sugar' leaves (mg g^{-1})		
	Control	UVA	UVA+UVB
Cannabichromene (CBC)	0.193 ± 0.013	0.185 ± 0.026	0.197 ± 0.011
Cannabidiol (CBD)	BDL[z]	BDL	BDL
Cannabidiolic acid (CBDA)	BDL	BDL	BDL
Total equivalent cannabidiol (TCBD)	BDL	BDL	BDL
Cannabigerol (CBG)	BDL	BDL	BDL
Cannabigerolic acid (CBGA)	1.12 ± 0.17	1.27 ± 0.11	1.30 ± 1.1
Total equivalent cannabigerol (TCBG)	0.983 ± 0.14	1.12 ± 0.098	1.14 ± 0.062
Cannabinol (CBN)	BDL	BDL	BDL
Δ^9-tetrahydrocannabinol (THC)	2.84 ± 0.20 b[y]	2.81 ± 0.12 b	3.70 ± 0.14 a
Δ^9-tetrahydrocannabinolic Acid (THCA)	29.8 ± 3.2	29.2 ± 1.6	32.4 ± 1.2
Total equivalent Δ^9-tetrahydrocannabinol (T-THC)	29.0 ± 2.9	28.4 ± 1.5	32.1 ± 1.1

[z]below detection limit
[y]means (± SE, n = 3) followed by the same letter are not different at P ≤ 0.05 according to Tukey's honestly significant difference test

Table 4. Secondary metabolite contents of dry inflorescence of *Cannabis sativa* 'Meridian' plants grown under three lighting treatments: (1) 12 h d^{-1} of 600 µmol m^{-2} s^{-1} of photosynthetically active radiation for 45 d (Control), (2) 12 h d^{-1} of 600 µmol m^{-2} s^{-1} of PAR plus an additional 12 h d^{-1} of 50 µmol m^{-2} s^{-1} of ultraviolet-A radiation from LEDs (385nm peak) for 45 d (UVA), and (3) 12 h d^{-1} of 600 µmol m^{-2} s^{-1} of PAR for 45 d plus an additional 3 µmol m^{-2} s^{-1} of an equal ratio of ultraviolet-A and ultraviolet-B radiation for 5 h d^{-1} for the 20 days prior to harvest (UVA+UVB).

Secondary metabolite	Content in dry inflorescence (mg g^{-1})		
	Control	UVA	UVA+UVB
Δ^9-tetrahydrocannabinol (THC)	7.49 ± 0.25[z]	7.53 ± 0.43	7.54 ± 0.68
Δ^9-tetrahydrocannabinolic Acid (THCA)	225 ± 1.9	216 ± 5.1	222 ± 6.7
Total equivalent Δ^9-tetrahydrocannabinol (T-THC)	205 ± 2.0	197 ± 5.1	202 ± 6.7
Cannabigerol (CBG)	0.607 ± 0.078	0.607 ± 0.078	0.490 ± 0.16
Cannabigerolic acid (CBGA)	10.9 ± 0.26	10.9 ± 0.42	11.2 ± 0.40
Total equivalent cannabigerol (TCBG)	10.2 ± 0.15	10.1 ± 0.39	10.2 ± 0.30
Cannabichromene (CBC)	BDL[y]	BDL	BDL
Cannabidiol (CBD)	BDL	BDL	BDL
Cannabidiolic acid (CBDA)	BDL	BDL	BDL
Total equivalent cannabidiol (TCBD)	BDL	BDL	BDL
Cannabinol (CBN)	BDL	BDL	BDL
Terpinolene	7.34 ± 0.51	7.00 ± 0.24	6.45 ± 0.27
Myrcene	2.84 ± 0.17	2.54 ± 0.051	2.41 ± 0.14
Limonene	2.16 ± 0.13	2.08 ± 0.071	1.92 ± 0.067
Beta pinene	1.32 ± 0.015 a[x]	1.14 ± 0.033 b	1.24 ± 0.035 ab
Trans-ocimene	1.29 ± 0.078	1.32 ± 0.057	1.13 ± 0.022
Alpha pinene	0.947 ± 0.014	0.833 ± 0.012	0.867 ± 0.026
Caryophyllene	1.29 ± 0.014	1.40 ± 0.056	1.32 ± 0.044
Terpineol	0.797 ± 0.025	0.760 ± 0.022	0.713 ± 0.022
3-carene	0.340 ± 0.012	0.303 ± 0.024	0.340 ± 0.012
Fenchol[w]	0.340	0.340	0.340
Humulene	0.377 ± 0.021	0.377 ± 0.021	0.377 ± 0.042
Sabinene	0.110 ± 0.013	0.110 ± 0.013	0.150 ± 0.027
Alpha-bisabolol	BDL	BDL	BDL
Borneol	BDL	BDL	BDL
Cis-nerolidol	BDL	BDL	BDL
Cis-ocimene	BDL	BDL	BDL
Eucalyptol	BDL	BDL	BDL
Guaiol	BDL	BDL	BDL
Linalool	BDL	BDL	BDL
Valencene	BDL	BDL	BDL
Trans-nerolidol	BDL	BDL	BDL
Total terpenes	19.3 ± 0.93	18.3 ± 0.52	17.3 ± 0.42

[z]secondary metabolite contents are in terms of mg g^{-1} (± SE, n=3) of oven-dry tissue
[y]below detection limit
[x]means followed by the same letter are not different ($P \leq 0.05$) using Tukey's honestly significant difference test
[w]all samples had the same Frenchol content so no SE is provided

References

Aizpurua-Olaizola O, Soydaner U, Öztürk E, Schibano D, Simsir Y, Navarro P, Etxebarria N, Usobiaga A (2016) Evolution of the cannabinoid and terpene content during the growth of *Cannabis sativa* plants from different chemotypes. J Nat Prod 79: 324-331

Bilodeau S, Wu B-S, Rufyikiri A-S, MacPherson S, Lefsrud M (2019) An update on plant photobiology and implications for cannabis production. Front Plant Sci 10:296

Bugbee B, Salisbury F (1988) Exploring the limits of crop productivity. Plant Physiol 88: 869-878

Cash C, Cunnane K, Fan C, Romero-Sandoval E (2020) Mapping cannabis potency in medical and recreational programs in the United States. PLOS ONE 15(3): e0230167

Chandra S, Lata H, Khan I, Elsohly M (2008). Photosynthetic response of *Cannabis sativa* L. to variations in photosynthetic photon flux densities, temperature and CO_2 conditions. Physiol Mol Biol Plants 14: 299-306

de Backer B, Debrus B, Lebrun P, Theunis L, Dubois N, Decock L, Verstraete A, Hubert P, Charlier C (2009) Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material, J Chromatography 877: 4115-4124

de Meijer E, van der Kamp H, van Eeuwijk F (1992) Characterisation of cannabis accessions with regard to cannabinoid content in relation to other plant characters. Euphytica 62: 187-200

Dujourdy L, Besacier F (2017) A study of cannabis potency in France over a 25 years period (1992-2016), Forensic Sci International 272: 72-80

EMCDDA (2012) Cannabis production and markets in Europe. Luxembourg: Publications Office of the European Union. 10.2810/52425: 1-268.

Fairbairn JW, Liebmann JA (1974). The cannabinoid content of *Cannabis sativa* L. grown in England. J. Pharm. Pharmacol. 26: 413-419

Frohnmeyer H, Staiger D (2003) Ultraviolet-B radiation-mediated responses in plants: Balancing damage and protection. Plant Physiol 133: 1420-1428

Gill SS, Anjum NA, Gill R, Jha M, Tuteja N (2015) DNA damage and repair in plants under ultraviolet and ionizing radiations. Sci World J 250158

Hazekamp A, Peltenburg A, Verpoorte R, Giroud C (2005) Chromatographic and spectroscopic data of cannabinoids from *Cannabis sativa* L., J Liquid Chromatography Related Technol 28: 2361-2382

Hideg E, Jansen M, Strid Å (2013) UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? Trends Plant Sci 18: 107-115

Huché-Thélier L, Crespel L, le Gourrierec J, Morel P, Sakr S, Leduc N (2016) Light signaling and plant responses to blue and UV radiations—Perspectives for applications in horticulture. Enviro Exp Botany 121: 22-38

Jansen MAK, Gaba V, Greenberg BM (1998) Higher plants and UV-B radiation: balancing damage, repair and acclimation, Trends Plant Sci 3: 131-135

Jikomes N, Zoorob M (2018) The cannabinoid content of legal cannabis in Washington state varies systematically across testing facilities and popular consumer products. Sci Rep 8-4519

Krizek DT (2004) Influence of PAR and UV-A in determining plant sensitivity and photomorphogenic responses to UV-B radiation. Photochem Photobiol 79: 307-315

Kusuma P, Pattison PM, Bugbee B (2020) From physics to fixtures to food: current and potential LED efficacy. Hortic Res 7, 56

Livingston SJ, Quilichini TD, Booth JK, Wong DCJ, Rensing KH, Laflamme-Yonkman J, Castellarin SD, Bohlmann J, Page JE, Samuels AL (2020) Cannabis glandular trichomes alter morphology and metabolite content during flower maturation. Plant J 101: 37-56

Lydon J, Teramura AH, Coffman CB (1987) UV-B radiation effects on photosynthesis, growth and cannabinoid production of two *Cannabis sativa* chemotypes. Photochem Photobiol 46: 201-206

Magagnini G, Grassi G, Kotiranta S (2018) The effect of light spectrum on the morphology and cannabinoid content of *Cannabis sativa* L. Med Cannabis Cannabinoids 1: 19-27

Mills E (2012) The carbon footprint of indoor Cannabis production. Energy Polic 46: 58-67

Namdar D, Mazuz M, Ion A, Koltai H (2018). Variation in the compositions of cannabinoid and terpenoids in *Cannabis sativa* derived from inflorescence position along the stem and extraction methods. Ind Crops Prod 113: 376-382

Pacifico D, & Miselli F, Carboni A, Moschella A, Mandolino G (2007) Time course of cannabinoid accumulation and chemotype development during the growth of *Cannabis sativa* L. Euphytica 160: 231-240

Pate DW (1983) Possible role of ultraviolet radiation in evolution of cannabis chemotypes. Econ. Bot 37: 396-405

Pate DW (1994) Chemical ecology of Cannabis. J International Hemp Assoc 1L 32-37

Potter D (2009) The propagation, characterisation and optimisation of *Cannabis sativa* L. as a phytopharmaceutical. Ph.D. dissertation. King's College London.

Potter D (2014). A review of the cultivation and processing of cannabis (*Cannabis sativa* L.) for production of prescription medicines in the UK. Drug Testing Analysis. 6: 10.1002/dta.1531

Potter D, Duncombe P (2012) The effect of electrical lighting power and irradiance on indoor-grown cannabis potency and yield. J Forensic Sci 57: 618-622

Richins RD, Rodriguez-Uribe L, Lowe K, Ferral R, O'Connell MA (2018) Accumulation of bioactive metabolites in cultivated medical Cannabis. PloS one, 13(7), e0201119.

Small E (2017) Cannabis: A complete guide. CRC Press, Boca Raton, FL

Data available on request from the authors.