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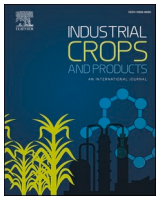

# The morphology, inflorescence yield, and secondary metabolite accumulation in hemp type *Cannabis sativa* can be influenced by the R:FR ratio or the amount of short wavelength radiation in a spectrum

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ABSTRACT

The effect of light spectra on the quality of multiple crops has been established, however, the scientific evidence related to light quality, cannabis (*Cannabis sativa*) morphology, and the secondary metabolite accumulation in the female inflorescences is still sparse. *C. sativa* inflorescences harvested for pharmaceutical purposes are primarily cultivated in controlled environments for their secondary metabolite compounds, such as cannabinoids, terpenes, and flavonoids. Indoor cultivation allows precise control over the environmental parameters, including light, which impact the inflorescence yield and quality. The effect of long (far-red) and short wavelength (blue, UV-A, UV-B) radiation on the morphology, inflorescence yield, and floral cannabinoid and terpene concentrations in a cannabidiol (CBD) dominant hemp type *C. sativa* genotype, FINOLA, was studied in two experiments. In the first experiment, two treatments, LOW R:FR (R:FR ratio of 1) and HIGH R:FR (R:FR ratio of 11), were compared. The second experiment included four treatments with varying blue, UV-A, and UV-B radiation content (CONTROL, BLUE, UVA, and UVB). LOW R:FR ratio treatment increased plant height and decreased inflorescence yield. HIGH R:FR ratio treatment increased CBD, tetrahydrocannabivarin acid (THCVA), and cannabigerolic acid (CBGA), and the sum of measured terpene concentrations compared with the LOW R:FR ratio treatment. Short wavelength radiation treatments did not impact inflorescence yield or plant morphology. BLUE and UVB treatments increased the cannabinoid, THCVA, concentration, but no difference in the sum of measured cannabinoid concentrations was observed between the treatments. UVB treatment increased the monoterpene, myrcene, concentration, but had no impact on the sum of measured terpenes concentration. In conclusion, the morphology, yield, and secondary metabolite accumulation in *C. sativa* can be influenced by altering the R:FR ratio or the amount of short wavelength radiation in a spectrum.

## 1. Introduction

*Cannabis sativa* is a short-day plant cultivated for fiber, food, medicinal, and recreational purposes. The plant can be grouped into hemp and drug type varieties, depending on the end-use purpose and chemical composition of the inflorescences. For the pharmaceutical purposes, cannabis is cultivated for its unique secondary metabolites, cannabinoids, terpenes, and flavonoids. Cannabinoids and terpenes are produced and accumulated inside glandular trichomes, which cover the whole plant, especially on the unfertilized female inflorescences, making the inflorescences the most valuable plant part for the pharmaceutical industry. From the 113 identified cannabinoids, the most studied and well-known are $\Delta^9$-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG) (reviewed e.g. by Desaulniers Brousseau et al., 2021) due to their biological activity in humans. Cannabinoids are primarily present in the plant in their carboxylic acid formats $\Delta^9$-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabigerolic acid (CBGA), which are thermally unstable and can be decarboxylated when exposed to heat or light (Wang et al., 2016). The decarboxylated forms, THC and CBD, are the primary compounds used by the pharmaceutical industry for medicinal purposes (reviewed by Boyaji et al., 2020). More than 120 terpenes have been identified from *C. sativa* (Booth and Bohlmann, 2019). The volatile mono- and sesquiterpenes together with sulfur compounds (Oswald et al., 2021)

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contribute to the flower quality by giving each genotype their distinct fragrance and taste. In addition to cannabinoids and terpenes, more than 20 flavonoids have been identified from the plant, including prenylated flavonoids, cannflavin A, B, and C.

Inflorescence yield quantity and quality can be improved in a closed environment cultivation system, where all environmental parameters and cultivation practices can be controlled. For example, nutrition, planting density, and pruning practices can influence the morphology, inflorescence yield quality and quantity (Bernstein et al., 2019; Shiponi and Bernstein, 2021; Danziger and Bernstein, 2021a; Danziger and Bernstein, 2022). Light is one of the key factors in successful cannabis indoor cultivation; photoperiod, light quality, and light intensity all play a significant role (Magagnini et al., 2018; Danziger and Bernstein, 2021b; Rodriguez-Morrison et al., 2021a; Ahrens et al., 2023). Higher plants can sense specific wavelengths of light ranging from the short wavelength region ultraviolet-B (UV-B) to longer wavelength region far-red through photoreceptors. The photoreceptor mediated responses affect the main morphological and developmental features, and induction of many secondary metabolite biosynthesis. Typically, plants grown under a spectrum rich in short wavelength radiation have thicker leaves, shorter leaf petioles, shorter stems, and increased number of branches (Takemiya et al., 2005; Robson et al., 2015). In contrast, plants grown under a light spectrum rich in far-red photons have an opposite phenotype, a phenomenon known as shade avoidance syndrome, in which plants have elongated leaf petioles and stems and increased apical dominance (Ballaré and Pierik, 2017).

The effects of far-red radiation and low R:FR ratio on plant development and morphology are well documented in multiple species (Brown et al., 1995; Ugarte et al., 2010; Gundel et al., 2014; Kong and Nemali, 2021). However, only a few studies regarding far-red radiation and *C. sativa* have been published. Reichel et al. (2021) cultivated three *C. sativa* varieties under three different light spectra with differing R:FR ratios and concluded that the treatment with the lowest R:FR ratio had the tallest plants and lowest flower yield in all genotypes studied. The study by Magagnini et al. (2018) suggested that blue and UV-A radiation increased the cannabinoid concentrations and hypothesized that the lower cannabinoid concentrations under the high-pressure sodium (HPS) treatment were partially a result of the lower R:FR ratio in the HPS spectrum. In fact, far-red radiation has been shown to down-regulate many secondary metabolite pathways in other species, such as indole glucosinolates and camalexin in *Arabidopsis thaliana* (Cargnel et al., 2014), and anthocyanins in baby leaf lettuce (*Lactuca sativa*) (Li and Kubota, 2009), but no evidence on its impact on cannabinoids, flavonoids, or terpenes in *C. sativa* has been previously published.

First studies suggesting and demonstrating the role of ultraviolet (UV) radiation on cannabinoid accumulation in *C. sativa* were published during the 1980's. Based on the data from flower samples collected from multiple locations, Pate (1983) showed that the floral THC concentration is dependent on the origin of the genetics and the exposure time of the plant to solar UV-B radiation. Later, Lydon et al. (1987) demonstrated a positive correlation between an increasing UV-B dose and THC concentration in leaves and flowers. On the other hand, a more recent paper concluded that additional UV-B dose decreased the flower yield and cannabinoid concentration of one tested genotype and had no effect on the other (Rodriguez-Morrison et al., 2021b).

As the early research focused on the effect of UV-B radiation on cannabinoid concentrations (Pate, 1983; Lydon et al., 1987), responses to UV-A have been less investigated and results are contradictory. In one of the first cannabis and UV-A related studies, UV-A together with blue radiation was suggested to induce higher cannabinoid levels in the female inflorescences (Magagnini et al., 2018). Similar findings were reported by Jenkins (2021), who concluded that additional red and blue (450 and 660 nm) or UV-A (390 nm) radiation increased the THC concentration in two out of the three tested genotypes, and that blue radiation increased the concentration of majority of the tested terpenes, but that UV-A decreased terpene concentrations in all three varieties. Danziger and Bernstein (2021b) reported that a high blue to red (B:R) ratio increased the CBGA concentrations of all three tested genotypes over the lower B:R ratio treatment, while the effect of B:R ratio on CBDA concentration was genotype dependent. These studies support the earlier findings of especially blue radiation having enhancing effect on plant secondary metabolism, which has been reported widely with multiple species (Li and Kubota, 2009; Suyono et al., 2015; Kim et al., 2021). Blue radiation has been shown to increase terpene concentrations in species such as peppermint (*Mentha* x *piperita*) (Ueda et al., 2021) and tea (*Camellia sinensis*) (Fu et al., 2015). On the contrary, Westmoreland et al. (2021) concluded that the fraction of blue radiation in a spectrum did not have an impact on the cannabinoid levels, while the effect of the light spectrum on terpenes was not examined.

The mechanisms of the effect of radiation with specific wavelengths on terpene and cannabinoid biosynthesis in *C. sativa* are not yet completely understood. However, the relation of short wavelength radiation with cannabinoids and monoterpenes can be explained by looking at the biosynthetic pathways of these compound families (reviewed in Desaulniers Brousseau et al., 2021). Cannabinoids and monoterpenes share a partially overlapping biosynthesis pathway and a common precursor molecule, geranyl diphosphate (GPP). In other species, such as peppermint and aquatic mint (*Mentha aquatica*), UV-B and blue radiation have been demonstrated to upregulate isopentenyl-diphosphate delta-isomerase (IPPi) and geranyl pyrophosphate synthase (GPPS) (Dolzhenko et al., 2010; Nazari et al., 2017), the enzymes forming the precursor molecule, GPP (Desaulniers Brousseau et al., 2021).

Through a two-phase experiment, the aim was firstly to demonstrate the role of R:FR ratio in the accumulation of secondary metabolites in the inflorescence, and its impact on the morphology. The hypothesis was that lowered R:FR ratio would negatively impact the inflorescence yield formation and secondary metabolite accumulation in the inflorescences and leaves. Secondly, the specific role of blue, UV-A, and UV-B radiation on floral cannabinoid, terpene, and flavonoid concentrations and leaf flavonoid accumulation during the growth period were examined. Hypotheses were, that depletion of short wavelengths from the spectrum would increase stem elongation, addition of UV-A and UV-B radiation would increase the secondary metabolite concentrations in the inflorescences over the BLUE treatment, additional blue radiation would increase the secondary metabolite concentrations over the CONTROL treatment, and that UV-B radiation would have a negative impact on inflorescence yield. Further, the aim was to experiment whether the leaf adaxial flavonoid concentration could be used as a prediction tool to estimate the secondary metabolite concentrations in the inflorescences. The hypothesis was, that the leaf adaxial flavonoid concentration at harvest time would correlate with some of the individual floral cannabinoid or terpene concentrations or the sum of measured floral cannabinoid and terpene concentrations. Optional measurement practices to predict the floral cannabinoid and terpene content, or the inflorescence maturity, without laborious and destructive laboratory analysis, would add cost efficiency and yield uniformity compared with the current practices.

## 2. Materials and methods

Two separate experiments were conducted; experiment A between 13.7.–21.9.2021 and experiment B between 1.4.–18.6.2021, in similar conditions in a research greenhouse at the University of Helsinki, Finland. The effect of light spectrum on growth, yield, and leaf and flower chemical composition of *C. sativa* was studied in experiment A with two different R:FR ratios and in experiment B with differing amount of blue, UV-A and UV-B radiation in the spectra.

### 2.1. Plant material and growing conditions

The seeds of *C. sativa* cv. FINOLA (Finola Oy, Kuopio, Finland) were

sown in peat (AirBoost 640 R8421, Kekkilä-BVB, Vantaa, Finland) and watered thoroughly with tap water. Light was applied 3 days after sowing (DAS) at 200 μmol $m^{-2}$ $s^{-1}$ (Solray385, Valoya RX600, Valoya Oy, Helsinki, Finland) at soil level. Light intensity was measured with a spectrometer (UPRTek MK350S, Zhunan township, Taiwan). Seedlings were potted into 6 cm diameter pots filled with peat (AirBoost 640 R8421, Kekkilä-BVB, Vantaa, Finland) and light intensity raised to 300 μmol $m^{-2}$ $s^{-1}$ at canopy level 8 DAS. Plants were potted into 12 cm diameter round pots, transferred under light treatments, and light intensity at canopy level raised to 400–450 μmol $m^{-2}$ $s^{-1}$ 22 and 28 DAS in experiment A and B, respectively. Light spectrum used during the vegetative phase prior to light treatments is the same spectrum that was used in experiment B as UVA treatment, the spectrum graph is presented in supplemental material (S2).

In experiment A, fertilized water was given twice a day through a drip irrigation system (electrical conductivity (EC) 1.8 mS/cm, (Kukka-superex N-P-K 10–5–25, Kekkilä-BVB, Vantaa, Finland, (N 220, P 105, K 575, Mg 60, S 80, B 0.7, Cu 0.7, Fe 6, Mn 2.3, Mo 0.1, Zn 0.7 mg $L^{-1}$)) from 28 DAS onwards until harvest.

In experiment B, between 13 and 28 DAS plants were irrigated with fertilized water (EC 1.0–1.5 mS/cm); a fertilizing mix (Vihannes-superex N-P-K 9–5–31, Kekkilä-BVB, Vantaa Finland) and additional nitrate and calcium (Calcinit, N 15.5%, Ca 19%, Yara International ASA, Oslo, Norway) were diluted to water in a ratio of 2:1 (N 130, P 40, K 230, Mg 20, S 27, B 0.24, Cu 0.1, Fe 1.8, Mn 0.7, Mo 0.04, Zn 0.2, Ca 76. mg $L^{-1}$), and 29 DAS each pot was fertilized with 4 g of slow-releasing fertilizer (N-P-K 16–2.2–14.1, Osmocote, Substral, Scotts Celaflor GmbH, Mainz, Germany, total of N 640, P 88, K 564 mg, (the product does not contain other nutrients)) and drip-irrigated with clean tap-water daily until harvest. Plants did not show any signs of nutrition related deficiency symptoms during the experiments. Photoperiod in experiment A during vegetative period was 18 h and during flowering period 12 h, while in experiment B the photoperiod was 18 h during the vegetative and flowering period.

Ambient temperature and relative humidity were automatically controlled by the greenhouse control system (Priva, De Lier, The Netherlands); temperature was set to 24/22 °C day/night and relative humidity to 60%/50% day/night. The air $CO_2$ concentration was 386 ± 14 ppm when lights were on.

### 2.2. Experimental design and light treatments

The experiments were organized in a randomized complete block design with three replicate blocks containing each light treatment once per block. Plants were cultivated on three benches (blocks), each 1.2 × 4.6 m (exp. A) and 1.8 × 6 m (exp. B) in size. Benches were divided into smaller plots, 1.2 × 1.2 m each, which were separated from each other with black-and-white plastic curtains white side facing out to prevent light contamination between treatments. In experiment A, each replicate plot had 16 plants, i.e. 48 plants in total per treatment. In experiment B, each replicate plot had 20 plants, i.e. 60 plants per light treatment in total.

Experiments were conducted in a greenhouse compartment where all replicates could be grown simultaneously in similar conditions within an experiment to avoid the need for replication in time. The greenhouse wall material was polycarbonate, and the roof material was glass, however, blackout curtains to all walls and roof were deployed continuously throughout the experiments to exclude outdoor radiation and maintain stable photoperiods. Experiment A included two light treatments; 1) High R:FR ratio treatment (HIGH R:FR) with a white LED spectrum (Solray385, RX600, Valoya Oy, Helsinki, Finland), with a R:FR ratio of 11 (R:FR calculated according to Sellaro et al., 2010) and 2) Low R:FR ratio treatment (LOW R:FR) with the same white spectrum as in treatment HIGH R:FR and additional far-red LED fixtures with a peak wavelength at ~730 nm (C65, Valoya Oy, Finland) resulting in a R:FR ratio of 1.

Experiment B included four light treatments, where a white light spectrum containing wavelengths between 380 and 780 nm (Solray385, RX600, Valoya Oy, Helsinki, Finland) was modified by using filters and additional UV-B fixtures to create light treatments with different portions of blue, UV-A and UV-B radiation: 1) control treatment (named as CONTROL in graphs and later in the text) with the white LED spectrum and a filter excluding radiation below ~500 nm (#312 CANARY, Rosco, Stamford, USA) 2), blue radiation treatment (BLUE), with the same white LED spectrum and a filter excluding radiation below 400 nm (#3114 tough UV filter, Rosco, Stamford, USA), 3) UVA treatment (UVA) with the white LED spectrum 4) UVB treatment (UVB) with the white LED spectrum and added UVB fixtures producing radiation between 280 and 330 nm (75% UVB and 25% UVA) (MIGRO UVB 310, Partech LED Ltd., Dublin, Ireland). UV-B lights were on for the last 15 min of each hour between 7 and 21, as recommended by the manufacturer, resulting in a total exposure time of 3.5 h per day. Blue peak in treatments BLUE, UVA, and UVB was at ≈ 450 nm, UV-A peak in UVA and UVB treatments at ≈ 385 nm, and UV-B peak in UVB treatment at ≈ 310 nm.

Light intensities (experiments A and B) and R:FR ratios (experiment A) were measured at canopy level weekly with a spectrometer (UPRTek MK350S, Zhunan township, Taiwan) and were adjusted with individual dimmers attached to each luminaire. During flowering, target light intensity was 500 and 650 μmol $m^{-2}$ $s^{-1}$ in experiments A and B, respectively, light intensity was measured between 380 nm and 780 nm.

Spectral photon irradiance was measured from all three replicates at canopy level with an array spectroradiometer (Maya2000 Pro Ocean Optics, Dunedin, Fl, USA; D7-H-SMA cosine diffuser, Bentham Instruments Ltd, Reading, UK). Spectral distributions and photon flux density (PFD) per wavelength area of each light treatment in both experiments are presented Table 1, spectra graphs of the treatments are presented in supplemental material (S1 and S2). Measurements were recorded within the wavelength range from 280 to 900 nm and processed in R (R Core Team, 2017), using the photobiology packages developed for spectral analysis (Aphalo, 2015).

### 2.3. Plant growth and yield measurements

Morphological measurements were conducted on 7 plants per replicate plot i.e. 21 plants per treatment in experiment A and on 15 plants per replicate plot i.e. 45 plants per light treatment in experiment B. Plant height was measured 8 times during experiment A, and 6 times during experiment B. At harvest, 70 DAS and 78 DAS in experiments A and B, respectively, the stem diameter was measured at 3 cm height from the growing media surface with a caliper, after which the plants were cut from the base and leaves were removed. Inflorescences were hand trimmed with scissors by removing all inter-inflorescence leaves according to normal commercial practice. The trimmed inflorescences from the main stem (apical inflorescence) and from the branches (side branch inflorescence) were dried in a plant-drying oven equipped with a fan at 24 °C for 72 h and weighed separately for apical, side branch, and total inflorescence yield. In experiment A, the number of internodes were counted. Stems, and also leaves in experiment A, were dried at 72 °C for 24 h for dry weight measurements. At harvest, plants had been under the light treatments for 48 and 50 days in experiment A and B, respectively.

### 2.4. Sample preparation and extraction of secondary metabolite analysis

Fifteen randomly selected plants from each light treatment, 5 per replicate plot, were analyzed for their cannabinoid, flavonoid, and terpene composition. The apical inflorescences were trimmed and dried for 72 h in 24 °C, after which each inflorescence was ground separately with IKA® A 11 basic analytical mill (IKA-Werke GmbH and Co. KG, Staufen, Germany) for 10 s. From each inflorescence, three sub-samples were analyzed and the mean was used for statistical analysis. With each

**Table 1**
Spectral distribution of light treatments in percentages of total light distribution HIGH R:FR and LOW R:FR in experiment A and CONTROL, BLUE, UVA and UVB in experiment B followed by the PFD in μmol $m^{-2}$ $s^{-1}$ per wavelength area in parenthesis. Wavelength areas were determined as ultraviolet-B (UV-B) = 280–315 nm, ultraviolet A (UV-A) = 315–400 nm, blue = 400–500 nm, red = 600–700 nm, far-red = 700–800 nm, and photosynthetic active radiation (PAR) = 400–700 nm. UV-B and UV-A wavelength areas were defined according to ISO (2007). Blue to green (B:G), blue to red (B:R), and R:FR ratios were calculated according to Sellaro et al. (2010), where blue = 420–490 nm, green = 500–570 nm, red = 650–670 nm, and far-red = 720–740 nm.

| Treatment | Experiment | UVB % | UVA % | Blue % | Green % | Red % | FR % | PAR % | B:G | B:R | R:FR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HIGH R:FR | A | 0.02 (0.03) | 1.09 (5) | 19 (93) | 37 (183) | 40 (201) | 3 (16) | 96 (479) | 0.7 | 0.54 | 11 |
| LOW R:FR | A | 0.01 (0.11) | 1.06 (5) | 14 (67) | 28 (140) | 33 (163) | 25 (124) | 74 (370) | 0.7 | 0.50 | 1 |
| CONTROL | B | 0.01 (0.08) | 0.03 (0.2) | 3 (18) | 42 (270) | 52 (335) | 4 (25) | 96 (623) | 0.1 | 0.04 | 12 |
| BLUE | B | 0.01 (0.04) | 0.28 (1.8) | 18 (119) | 37 (240) | 41 (268) | 3 (20) | 97 (628) | 0.7 | 0.51 | 12 |
| UVA | B | 0.01 (0.04) | 1.28 (8.3) | 18 (117) | 37 (240) | 41 (265) | 3 (20) | 96 (622) | 0.7 | 0.51 | 12 |
| UVB | B | 0.41 (2.69) | 1.94 (12.6) | 16 (106) | 33 (214) | 45 (293) | 3 (22) | 94 (611) | 0.7 | 0.39 | 13 |

sample batch, a quality control sample was included to monitor the consisency of measurements between batches.

The two-step extraction method was modified from the method originally used for hop cone analysis (Bitz et al., 2021). Briefly, milled powder (~100.0 mg) was weighted into a 10 ml volumetric flask and 50 μl of caffeine solution (250 μg $ml^{-1}$ in methanol) was added as a control standard. The flask was then filled to the mark with MeOH – HCOOH (99:1 v/v), sonicated for 30 min, and centrifuged for 10 min at 600 *g*. From the supernatant, an aliquot was filtered through PTFE membrane filter (0.45 μm, Pall Corporation, Port Washington, NY, USA) into an autosampler vial for the high-performance liquid chromatography (HPLC) analysis of cannabinoids and cannflavin A.

Isolation of terpenoids was done from the remaining methanol supernatant by liquid-liquid extraction as follows: 4 ml methanol extract was pipetted into a test tube, 20 μl of bornyl acetate solution (1000 μg $ml^{-1}$ in hexane) and 2 ml of hexane were added and sample was vortexed for 10 s. Next, saturated NaCl solution (1.0 ml) was added, sample was vortexed for 10 s and centrifuged for 10 min at 600 *g* to enhance the separation of the phases, after which the hexane phase was transferred to an autosampler vial for gas chromatography–mass spectrometry (GC-MS) analysis.

Cannabinoid and cannflavin A analyses were performed with Agilent 1100-series high-performance liquid chromatograph equipped with a diode array detector (HPLC-DAD) (Agilent, Santa Clara, CA, USA). The HPLC pumps, autosampler, column oven, and diode array system were operated by the ChemStation computer program. The analytical column was Kinetex C18 Phenomenex C18 (100 × 3.0 mm; 5 μm i.d.; 100 Å, Phenomenex, Torrance, USA) the column oven was set at 35 °C. The mobile phase consisted of 0.05 M phosphate buffer (A) at pH 2.4 and methanol (B) with the following gradient: 5 − 95% B in 30 min; hold at 95% for 15 min and then back to 5% B in 5 min with a post-run time of 10 min. The flow was set at 0.6 ml $min^{-1}$. Chromatograms were recorded at 225, 280, and 350 nm and for the identification purposes the spectra were recorded at 190–600 nm. The wavelength 280 nm was used for quantitation of CBDA, cannabidivarinic acid (CBDVA), CBGA, $\Delta^9$-THCA, tetrahydrocannabivarinic acid (THCVA), cannflavin A, and control standard caffeine by using external standard method. Wavelength 225 nm was used to monitor CBD and THC. Cannabinoid standards (CBDA, CBD, CBDVA, THCA, THCVA, CBGA, Cannabinol (CBN), Cannabidivarin (CBDV), CBG, Cannabichromene (CBC), Cannabicyclol (CBL) and tetrahydrocannabivarin (THCV)) and caffeine were from SigmaAldrich (St. Louis, MO, USA) and cannflavin A from Cayman Chemical Company (Ann Arbor, MI, USA).

Analysis of mono- and sesquiterpenes were made by Clarus 500 GC-MS (Perkin-Elmer, Shelton, CT, USA) using 1 μl injection at splitless mode (split on-time 0.75 min) with double goose-neck liner. Zebron ZB-5MS (30 m x 0.25 mm i.d. x 0.25 μm film thickness, Phenomenex) was used as the analytical column at constant He flow-rate 1.0 ml/min. The oven's starting temperature was 50 °C, temperature was increased to 190 °C after 1 min at 10 °C/min speed, and then to 285 °C at 20 °C/min speed and was hold at 285 °C until the total run time of 30 min was reached. The injector was set at 250 °C and the GC-MS transfer to 270 °C. MSD was employed at scan mode 50– 450 m/e.

The main cannabinoid, mono-, and sesquiterpenes were identified based on their mass spectra using NIST Mass Spectral Library, pure reference compounds, and literature. The following terpenes were quantified: α-pinene, β-pinene, myrcene, limonene, ocimene, terpinolene, β-caryophyllene, bergamotene, β-farnesene, α-humulene, β-selinene, and α-selinene. The first five compounds were quantified using the calibration curve of myrcene, the next four using caryophyllene, and the last three using α-humulene. Bornyl acetate was used as an internal standard. Terpene standards (myrcene, caryophyllene, α-humulene) were from SigmaAldrich (St. Louis, MO, USA). The floral cannabinoid, terpene, and flavonoid concentrations were measured as %/dry weight of dried flower sample.

### 2.5. Optical measurement of leaf flavonoids

Leaf flavonoid measurements were taken to examine the relationship between leaf flavonoid concentrations and the inflorescence cannabinoid, terpene, and flavonoid concentrations. Measurements were taken regularly starting from the day when light treatments were applied; in experiment A measurements were conducted between 23 and 70 DAS every 5–7 days (total of 8 measuring times) and in experiment B between 27 and 76 DAS every 3–5 days (total of 11 measuring times). Measurements were taken from the adaxial epidermis of fully unfolded and expanded leaves from the upper part of the plant each measuring time. Two measurements per plant were taken from 9 and 15 plants per replicate plot, totaling to 54 and 90 measurements per light treatment per measuring time in experiments A and B, respectively. Measurements were conducted with Dualex Scientific optical leafclip meter (FORCE-A, Orsay, France), which measures light absorbance at λ = 375 nm; the absorbance values correlate with the leaf total flavonoid concentration (Julkunen-Tiitto et al., 2015).

### 2.6. Data analysis

All data were analyzed with IBM SPSS Statistics 29 (IBM, New York, USA) using the mixed effects ANOVA model in which light treatment was determined as fixed factor and replicate block as random effect. Data analysis was done according to Rogers et al. (2021), where individual plants are not considered as replicates, but the mean of each replicate block was used for the ANOVA. Therefore, in all analyses in both experiments n = 3. Means in the tables and figures represent the mean of the three replicate blocks per light treatment ± SE. From each replicate block, morphological data (Table 2) was collected from 7 and 15 plants in experiment A and B, respectively, and from 5 plants per replicate block for cannabinoid, terpene, and cannflavin A analysis in both experiments. From each of the five plants, three sub-samples were prepared and analyzed for their cannabinoid, terpene and cannflavin A concentrations. The mean of the three sub-samples per inflorescence was calculated and used in the statistical analysis. Leaf flavonoid concentration was measured from 9 and 15 plants per replicate block in experiment A and B, respectively. From the 9 and 15 plants, two

**Table 2**
Effect of light spectrum (treatment) on apical inflorescence dry weight, side branch inflorescences' dry weight, total inflorescence yield per plant, stem dry weight, stem diameter, number of internodes, and leaves dry weight per plant in *Cannabis sativa* 'FINOLA'. Values are means of three replicates followed by ± SE. Means in the same column per experiment with different letters differ significantly according to the Tukey test (α = 0.05). Nc = Data not collected.

| Treatment | Apical inflorescence dry weight (g) | Side branch inflorescences dry weight (g) | Total inflorescence dry weight (g) | Stem dry weight (g) | Stem diameter (mm) | Number of Internodes | Leaves dry weight (g) |
|---|---|---|---|---|---|---|---|
| HIGH R:FR | 2.5 ± 0.1[a] | 8.0 ± 0.4[a] | 10.4 ± 0.5[a] | 8.7 ± 0.8 | 10.3 ± 0.3 | 11.2 ± 0.1[a] | 14.1 ± 0.7[a] |
| LOW R:FR | 1.4 ± 0.1[b] | 1.9 ± 0.4[b] | 3.3 ± 0.5[b] | 7.9 ± 0.3 | 8.9 ± 0.3 | 10.2 ± 0.1[b] | 7.2 ± 0.7[b] |
| p-value | < 0.001 | 0.009 | 0.004 | 0.331 | 0.169 | 0.017 | 0.056 |
| CONTROL | 2.0 ± 0.1 | 7.5 ± 0.4 | 9.5 ± 0.4 | 8.7 ± 0.5 | 6.6 ± 0.1 | Nc | Nc |
| BLUE | 2.2 ± 0.1 | 8.1 ± 0.3 | 10.2 ± 0.4 | 9.2 ± 0.4 | 6.6 ± 0.1 | Nc | Nc |
| UVA | 2.2 ± 0.1 | 8.0 ± 0.4 | 10.3 ± 0.3 | 8.6 ± 0.4 | 6.5 ± 0.1 | Nc | Nc |
| UVB | 2.3 ± 0.1 | 8.2 ± 0.4 | 10.3 ± 0.4 | 7.4 ± 0.3 | 6.4 ± 0.1 | Nc | Nc |
| p-value | 0.800 | 0.793 | 0.537 | 0.060 | 0.810 | - | - |

measurements were taken each time and the mean of the two measurements was used in the statistical analysis. In ANOVA, the significance level was set to 5%. If ANOVA showed a significance between treatments, a Tukey's post-hoc test was used to evaluate the differences between treatment means ($p \leq 0.05$).

Correlation analyses were conducted using the replicate means of each experiment. In experiment A, there were two treatments and three replicates, thus n = 6, while in experiment B, the number of treatments and replicates were 4 and 3, respectively, thus n = 12. Correlation analyses were performed with IBM SPSS Statistics 29 (IBM, New York, USA), using the Pearson's correlation test and a 2-tailed significance level of 5%.

## 3. Results

### 3.1. The effect of light spectrum on flower yield and plant morphology

High R:FR ratio increased branching, demonstrated as increased number of internodes (Table 2) and the appearance of the plants under high R:FR ratio was bushier compared with the plants grown under the


**Fig. 1.** A) *Cannabis sativa* 'FINOLA' plants cultivated under HIGH R:FR (left) and LOW R:FR treatment (right). White line behind each plant represents the height of one meter. Picture was taken 56 days after sowing when plants had been under the light treatments for 35 days. B) Trimmed *C. sativa* 'FINOLA' apical inflorescences from HIGH R:FR treatment (left) and from LOW R:FR treatment (right). Yellow ruler shows measurement units in cm. Picture was taken at the end of the experiment, 70 days from sowing, when plants had been under the light treatments for 48 days. C) 'FINOLA' plants cultivated under four light treatments; from the left: CONTROL, BLUE, UVA, and UVB. White line behind each plant represents the height of one meter. Picture was taken 70 days after sowing when plants had been under the light treatments for 43 days.

low R:FR treatment (Fig. 1A). Under HIGH R:FR treatment the apical and side branch inflorescences were denser and thicker compared with the LOW R:FR treatment, resulting in a higher total inflorescence yield per plant (Fig. 1B, Table 2). A difference in plant height between HIGH R:FR and LOW R:FR treatments was recorded 13 days after the plants had been transferred under the light treatments and remained throughout the experiment (Fig. 2A). Although the plants under the LOW R:FR treatment were significantly taller, no effect on stem dry weight or stem diameter was observed at the end of the experiment (Table 2). The addition of blue, UV-A, or UV-B radiation did not impact the weights of the apical or side branch inflorescences, the total inflorescence yield, or plant height compared with the CONTROL treatment (Table 2, Figs. 1C and 2B).

### 3.2. Floral cannabinoid and flavonoid concentrations

The concentrations CBD, THCVA, and CBGA were higher in treatment HIGH R:FR compared with LOW R:FR treatment (Table 3) while no differences between treatments in the floral flavonoid, cannflavin A, concentrations were detected in experiment A. In experiment B, the addition of blue and UV-B radiation increased THCVA concentration compared with other treatments (Table 3). The addition of blue, UV-A, or UV-B radiation did not have an impact on other cannabinoids, cannflavin A, or the sum of measured cannabinoid concentration in experiment B (Table 3).

### 3.3. Floral terpene concentrations

In experiment A, the total terpene concentration containing all measured mono- and sesquiterpenes, was 57% higher under the HIGH R:FR treatment (3.8% ± 0.2 /dry weight of dried flower sample) compared with the LOW R:FR treatment (2.4% ± 0.2) ($p = 0.005$). The total monoterpene concentration, including α-pinene, β-pinene, myrcene, and limonene, was 73% higher in HIGH R:FR treatment compared with LOW R:FR treatment and all measured individual monoterpenes had higher concentrations under HIGH R:FR compared with LOW R:FR (Table 4). From individual sesquiterpenes, only β-farnesene had higher concentration under HIGH R:FR treatment compared with LOW R:FR treatment, and no treatment effect on total sesquiterpene concentration (containing caryophyllene, β-farnesene, α-humulene, β-selinene, and α-selinene) was detected in experiment A (Table 5).

The addition of UV-B radiation increased the monoterpene, myrcene, concentration almost 3-fold compared with CONTROL and UVA treatments (Table 4). However, the addition of blue, UV-A, or UV-B radiation did not have an impact on the total terpene ($p = 0.891$), total monoterpene (including α-pinene, β-pinene, myrcene, limonene, ocimene, and terpinolene), total sesquiterpene concentrations (containing caryophyllene, bergamotene, β-farnesene, α-humulene, β-selinene, and α-selinene) or individual sesquiterpene concentrations (Table 5). The total terpene concentration under CONTROL, BLUE, UVA and UVB treatments were 1.2% ± 0.2%, 1.4% ± 0.2, 1.2% ± 0.2%, and 1.3% ± 0.2, respectively.

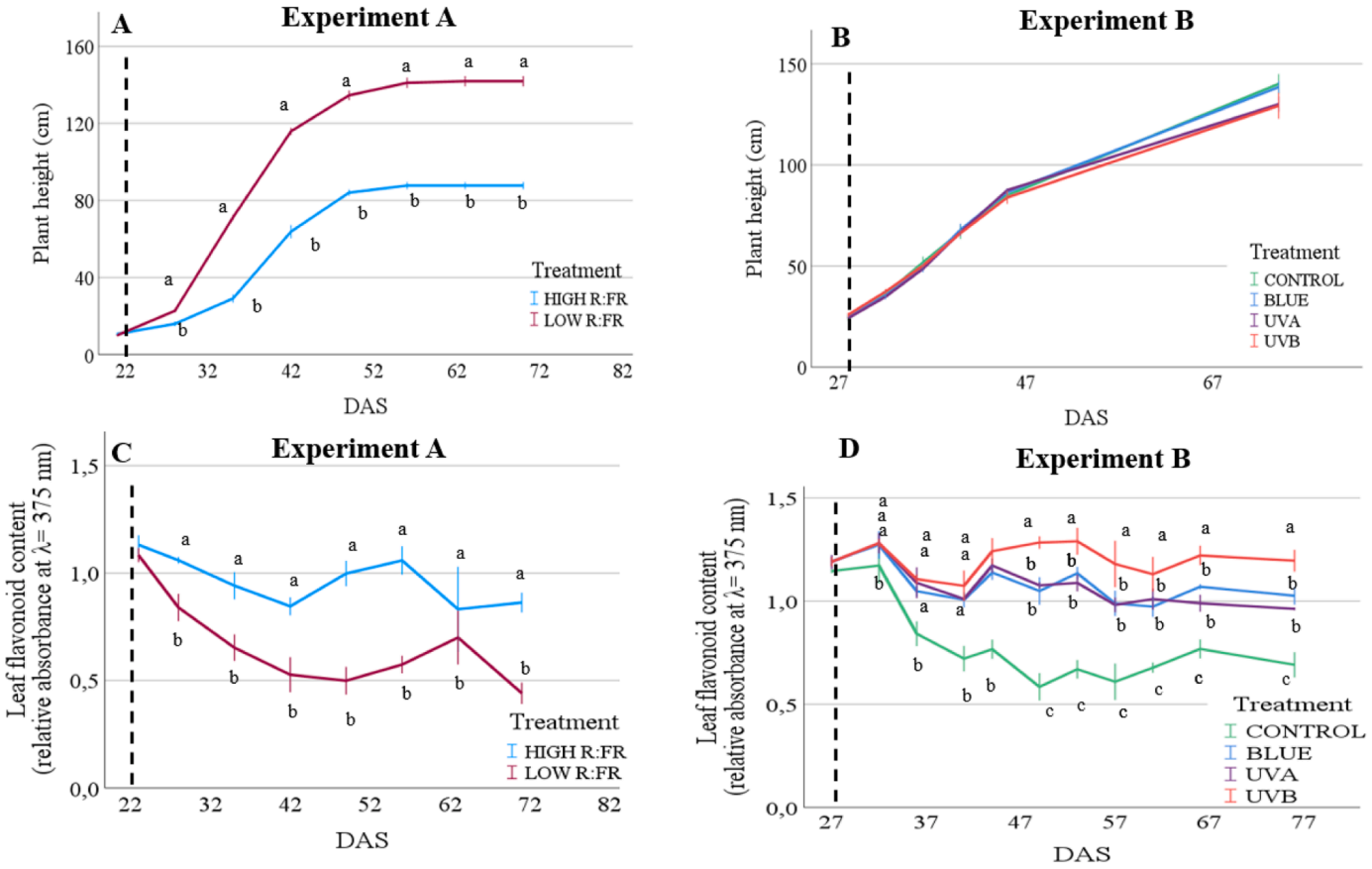


**Fig. 2.** A) Effect of light spectrum on *C. sativa* 'FNOLA' height under treatments HIGH R:FR and LOW R:FR and B) CONTROL, BLUE, UVA, and UVB. Values are means ± SE of three replicates, with 7 and 15 plants each, in experiment A and B, respectively. C) Effect of light spectrum on *C. sativa* 'FINOLA' leaf phenolic content under light treatments HIGH R:FR and LOW R:FR and C) CONTROL, BLUE, UVA, and UVB. Values are means ± SE of three treatment replicates. Different letters within each experiment and measuring day indicate significant differences between light treatments based on Tukey's honestly significant difference test (α = 0.05). DAS = days after sowing. Dashed line represents the starting date of the light treatments.

**Table 3**
Effect of light spectrum (treatment) on different cannabinoid (CBDA, CBD, CBDVA, THCA, THCVA, and CBGA), flavonoid (cannflavin A), and sum of measured cannabinoid concentrations (% of inflorescence dry weight) in two separate experiments. Values are means of three replicates followed by ± SE. Means in the same column per experiment with different letters differ significantly according to the Tukey test (α = 0.05). Nd = compound not detected.

| Treatment | CBDA % | CBD % | CBDVA % | THCA % | THCVA % | CBGA % | Sum of cannabinoids % | Cannflavin A % |
|---|---|---|---|---|---|---|---|---|
| HIGH R:FR | 10.1 ± 0.3 | 0.094 ± 0.005[a] | 0.23 ± 0.02 | 0.49 ± 0.02 | 0.083 ± 0.005[a] | 0.040 ± 0.004[b] | 11.7 ± 0.6 | 1.09 ± 0.06 |
| LOW R:FR | 8.4 ± 0.3 | 0.059 ± 0.004[b] | 0.14 ± 0.02 | 0.39 ± 0.02 | 0.050 ± 0.005[b] | 0.023 ± 0.003[a] | 9.8 ± 0.6 | 0.88 ± 0.06 |
| p-value | 0.059 | 0.013 | 0.127 | 0.079 | 0.012 | 0.007 | 0.054 | 0.186 |
| CONTROL | 2.8 ± 0.2 | 0.28 ± 0.03 | 0.20 ± 0.03 | 0.6 ± 0.1 | 0.018 ± 0.003[a] | Nd | 3.9 ± 0.5 | 0.015 ± 0.003 |
| BLUE | 4.0 ± 0.2 | 0.33 ± 0.03 | 0.36 ± 0.05 | 0.4 ± 0.1 | 0.039 ± 0.003[c] | Nd | 5.1 ± 0.5 | 0.032 ± 0.002 |
| UVA | 3.3 ± 0.2 | 0.27 ± 0.03 | 0.30 ± 0.03 | 0.2 ± 0.0 | 0.020 ± 0.003[a] | Nd | 4.0 ± 0.4 | 0.021 ± 0.002 |
| UVB | 3.2 ± 0.1 | 0.22 ± 0.01 | 0.26 ± 0.03 | 0.7 ± 0.2 | 0.029 ± 0.003[b] | Nd | 4.5 ± 0.4 | 0.021 ± 0.002 |
| p-value | 0.119 | 0.555 | 0.219 | 0.444 | 0.024 | - | 0.140 | 0.081 |

**Table 4**
Effect of light spectrum (treatment) on different monoterpene (α-pinene, β-pinene, myrcene, limonene, ocimene, and terpinolene concentrations in *Cannabis sativa* 'FINOLA' inflorescences grown under light treatments HIGH R:FR and LOW R:FR in experiment A and treatments CONTROL, BLUE, UVA, and UVB in experiment B. Values are means of three replicates followed by ± SE. Means in the same column per experiment with different letters are significantly different according to the Tukey test (α = 0.05). Nd = Compound not detected.

| Treatment | α-pinene % | β-pinene % | Myrcene % | Limonene % | Ocimene % | Terpinolene % | Sum of monoterpenes % |
|---|---|---|---|---|---|---|---|
| HIGH R:FR | 0.7 ± 0.04[a] | 0.23 ± 0.02[a] | 1.9 ± 0.1[a] | 0.3 ± 0.02[a] | Nd | Nd | 2.7 ± 0.2[a] |
| LOW R:FR | 0.5 ± 0.04[b] | 0.16 ± 0.02[b] | 1.0 ± 0.1[b] | 0.2 ± 0.02[b] | Nd | Nd | 1.6 ± 0.2[b] |
| p-value | 0.035 | 0.015 | 0.031 | 0.016 | - | - | 0.013 |
| CONTROL | 0.39 ± 0.04 | 0.08 ± 0.01 | 0.10 ± 0.04[a] | 0.03 ± 0.00 | 0.05 ± 0.01 | 0.06 ± 0.01 | 1.2 ± 0.2 |
| BLUE | 0.41 ± 0.04 | 0.07 ± 0.01 | 0.16 ± 0.04[ab] | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.12 ± 0.02 | 1.4 ± 0.2 |
| UVA | 0.30 ± 0.04 | 0.07 ± 0.01 | 0.10 ± 0.04[a] | 0.04 ± 0.01 | 0.12 ± 0.02 | 0.10 ± 0.02 | 1.2 ± 0.2 |
| UVB | 0.31 ± 0.03 | 0.08 ± 0.01 | 0.27 ± 0.04[b] | 0.03 ± 0.00 | 0.19 ± 0.03 | 0.13 ± 0.02 | 1.3 ± 0.2 |
| p-value | 0.758 | 0.738 | 0.047 | 0.344 | 0.055 | 0.659 | 0.819 |

**Table 5**
Effect of light spectrum (treatment) on different sesquiterpene (caryophyllene, bergamotene, β-farnesene, α-humulene, β-selinene, α-selinene) concentrations in *Cannabis sativa* 'FINOLA' inflorescences grown under light treatments HIGH R:FR and LOW R:FR in experiment A and treatments CONTROL, BLUE, UVA, and UVB in experiment B. Values are means of three replicates followed by ± SE. Means in the same column per experiment with different letters are significantly different according to the Tukey test (α = 0.05). Nd = Compound not detected.

| Treatment | caryophyllene % | Bergamotene % | β-farnesene % | α-humulene % | β-selinene % | α-selinene % | Sum of sesquiterpenes % |
|---|---|---|---|---|---|---|---|
| HIGH R:FR | 0.67 ± 0.04 | Nd | 0.08 ± 0.008[a] | 0.24 ± 0.02 | 0.02 ± 0.001 | 0.05 ± 0.004 | 1.02 ± 0.06 |
| LOW R:FR | 0.52 ± 0. 07 | Nd | 0.04 ± 0.007[b] | 0.19 ± 0.02 | 0.01 ± 0.001 | 0.03 ± 0.004 | 0.81 ± 0.06 |
| p-value | 0.234 | - | 0.006 | 0.177 | 0.071 | 0.092 | 0.313 |
| CONTROL | 0.26 ± 0.03 | 0.020 ± 0.002 | 0.031 ± 0.003 | 0.058 ± 0.006 | 0.038 ± 0.003 | 0.032 ± 0.003 | 0.42 ± 0.08 |
| BLUE | 0.32 ± 0.03 | 0.021 ± 0.002 | 0.033 ± 0.003 | 0.069 ± 0.006 | 0.028 ± 0.003 | 0.022 ± 0.003 | 0.47 ± 0.07 |
| UVA | 0.29 ± 0.03 | 0.018 ± 0.002 | 0.028 ± 0.003 | 0.070 ± 0.006 | 0.024 ± 0.004 | 0.018 ± 0.003 | 0.45 ± 0.08 |
| UVB | 0.26 ± 0.03 | 0.013 ± 0.002 | 0.021 ± 0.003 | 0.059 ± 0.006 | 0.022 ± 0.004 | 0.017 ± 0.003 | 0.38 ± 0.08 |
| p-value | 0.730 | 0.612 | 0.520 | 0.816 | 0.542 | 0.497 | 0.781 |

### 3.4. Leaf epidermal flavonoid concentration

Leaf flavonoid concentration was impacted by light spectrum in both experiments. In experiment A, after five days under the light treatments, plants grown under the high R:FR ratio had 26% higher leaf flavonoid concentration compared with plants grown under low R:FR ratio (Fig. 2C). At the end of the measuring period, when plants had been under the light treatments for 48 days (70 DAS), the difference between HIGH R:FR and LOW R:FR treatments in leaf flavonoid concentration was 96% (Fig. 2C).

UV-B radiation increased the leaf flavonoid concentration; difference to the UVA treatment was recorded once the plants had been under the light treatments for 16 days (44 DAS) and the effect lasted until the end of the experiment (Fig. 2D). At the final measuring day, when plants had been under the light treatments for 38 days (66 DAS), plants under the UVB treatment had 58%, 23% and 14% higher leaf flavonoid concentration compared with CONTROL, UVA, and BLUE treatments, respectively. Depletion of short wavelength radiation decreased leaf flavonoid concentration already after 7 days under the light treatments (Fig. 2D). After 38 days under the light treatments (66 DAS), the addition of blue radiation had increased the leaf flavonoid concentration by 28% compared with the CONTROL treatment while no difference between the BLUE and UVA treatments was recorded throughout the experiment (Fig. 2D).

The leaf flavonoid concentration on the final measuring day (70 DAS in experiment A and 76 DAS in experiment B), correlated positively with the total terpene, CBD, CBGA, and THCVA concentrations measured from the inflorescences at the end of the experiment in experiment A (Table 6). However, no significant correlations were found between leaf flavonoid concentrations and secondary metabolite concentrations measured from the inflorescences in experiment B (Table 6).

## 4. Discussion

### 4.1. Low R:FR ratio induces shade avoidance response in C sativa

Low R:FR ratio treatment increased stem elongation, reduced the number of internodes, and decreased the total inflorescence yield. All

**Table 6**
Pearson's correlation coefficients (r) in experiments A and B between the leaf adaxial flavonoid concentration (light absorbance at λ = 375 nm) and the sum of measured inflorescence monoterpene, sesquiterpene, and all measured terpenes, cannflavin A, CBD, CBDA, CBDVA, CBGA, THCA, THCVA, and the sum of measured cannabinoids. In experiment A, n = 6, and in experiment B, n = 12. Significant ($p < 0.05$) correlations are followed by the p-value. Nc = data not collected.

| | Experiment A r | Experiment B r |
|---|---|---|
| Sum of measured monoterpenes (%) | 0.756 | 0.226 |
| Sum of measured sesquiterpenes (%) | 0.655 | -0.088 |
| Sum of measured terpenes (%) | 0.855 *p = 0.030* | 0.167 |
| Cannflavin A (%) | 0.445 | 0.252 |
| CBD (%) | 0.826 *p = 0.043* | -0.179 |
| CBDA (%) | 0.780 | 0.388 |
| CBDVA (%) | 0.744 | 0.244 |
| CBGA (%) | 0.829 *p = 0.041* | Nc |
| THCA (%) | 0.746 | 0.031 |
| THCVA (%) | 0.833 *p = 0.040* | 0.393 |
| Sum of measured cannabinoids (%) | 0.804 | 0.340 |

the above listed traits are included in the shade avoidance syndrome responses, a phenomenon widely reported with multiple species (Ballaré and Pierik, 2017; Brown et al., 1995; Li and Kubota, 2009) and through this experiment also now with *C. sativa*. Magagnini et al. (2018) hypothesized that in addition to the higher blue fraction in the tested LED spectra in their experiments, also the differences in the R:FR ratio may have contributed to the morphological differences between the LED spectra and HPS treatment. In their experiments, plants grown under HPS (R:FR 2.8) were taller compared with the two tested LED spectra (R:FR 6.07 and 10.05). Additionally, Reichel et al. (2021) reported that the R:FR ratio of the three tested spectra may have contributed to the differences in plant height and yield accumulation. However, before this study, definite conclusions of the role of R:FR ratio on cannabis morphology could not been previously made since the tested spectra in both experiments (Magagnini, and Reichel et al., 2018, 2021) differed significantly also in other areas of the spectrum than solely the R:FR ratio. Although, it is possible, that in addition to the R:FR ratios, the difference in PAR levels between the two treatments (PPFD 370 vs. 479) has influenced plant morphology and yield results in this study, as *C. sativa* yield is known to correlate positively with increasing PAR level (Rodriguez-Morrison et al., 2021a; Llewellyn et al., 2022).

### 4.2. No effect on yield with UV-B radiation

There has been an increasing interest towards UV-A and UV-B and their possible benefits in cannabis cultivation. However, the recent studies have reported contradictious results regarding the effect of UV radiation on cannabis yield; Llewellyn et al. (2022) concluded that additional UV-A or UV-B radiation did not have an impact on the flower yield whereas Rodriguez-Morrison et al. (2021b) showed a negative correlation between the UV-B dose and inflorescence yield in one out of the two tested varieties. In this study, UV-B dose was applied for 15 min at a time fourteen times per day, resulting in a total exposure time of 3.5 h, and UVB treatment did not have an impact on the flower yield, thus contradicting with the hypothesis of UV-B radiation having a negative impact on flower yield. In the studies by Rodriguez-Morrison et al. (2021b) and Llewellyn et al. (2022) UV radiation was deployed without breaks for 3.5 h and 5.5 h, respectively. UV-B is known to cause stress responses and possible yield reductions in other species (Hidema and Kumagai, 2006); however, the results of this study indicate, that when applied in smaller portions, UV-B related yield reductions in *C. sativa* could be avoided. It is also possible, that the plant's tolerance to UV radiation is dependent on the genetics; the study by Rodriguez-Morrison et al. (2021b) showed that while the total inflorescence yield decreased with increasing UV-B dosage in genotype 'Low tide', no effect was detected in genotype 'Breaking wave'.

Both Llewellyn et al. (2022) and Rodriguez-Morrison et al. (2021b) reported a decrease in stem elongation with increasing UV-B dose. A similar response has been reported with blue light by Danziger and Bernstein (2021b), who concluded that the portion of blue radiation in a spectrum correlated negatively with plant height. In this study, the plants grown under the UVB treatment were not significantly shorter compared with other treatments. In addition, contradicting the hypothesis, the exclusion of blue wavelengths (CONTROL) in this study did not lead to an increase in plant height compared with the BLUE treatment. Omitting of blue wavelengths has been reported to have similar effects on plant morphology as low R:FR ratio, responses described as shade avoidance syndrome (Keuskamp et al., 2012). A possible explanation for the reaction to the spectra in this study could be the high photosynthetic photon flux density (PPFD) used in the experiment. Plant response to blue radiation can be more pronounced in low PPFD compared with high PPFD, as demonstrated with basil (*Ocimum basilicum* L.) (Larsen et al., 2020). In addition, Llewellyn et al. (2022) suggest that plants adapted to UV light in earlier stage of growing are less susceptible to UV related growth abnormalities and yield losses which were reported in the study by Rodriguez-Morrison et al. (2021b).

### 4.3. Cannabinoid and terpene concentrations decrease under low R:FR ratio

The effect of R:FR ratio on secondary metabolite concentrations of cannabis is sparsely reported and only a handful of studies have made conclusions of their findings based on the R:FR ratio. Magagnini et al. (2018) hypothesized, that the lower cannabinoid concentrations under the HPS light could partially be a result of the lower R:FR ratio of the HPS spectrum. A similar discussion was presented by Reichel et al. (2022), who hypothesized that higher phytochrome interacting factor (PIF) activity, induced by lower R:FR ratio, would be a key factor regulating the plant's secondary metabolism. The researchers suggested that high R:FR ratio and high proportion of red photons would lower the PIF activity and promote the plastidial methylerythritol pathway (MEP) resulting in higher monoterpene and cannabinoid concentrations. However, as the spectra used in the experiments by Magagnini et al. (2018) and Reichel et al. (2022) differed also in other regions than far-red, they could not answer the question of far-red and its impact on secondary metabolites completely. This study supports, and for the first time provides evidence, for the hypothesis of lowered R:FR ratio having a negative impact on the secondary metabolites. HIGH R:FR ratio treatment increased the concentrations of all measured monoterpenes and some cannabinoid concentrations compared with the LOW R:FR ratio treatment (Tables 4, 5, and 6). In the HIGH R:FR ratio treatment, also the concentration sum of the measured cannabinoids tended to be higher compared with the LOW R:FR treatment (the statistical analysis showed a p-value above the threshold, $p = 0.054$), indicating that further research should be conducted to fully understand the impact of long wavelength radiation on the total cannabinoid accumulation. The results in this study can be considered spectrum dependent, rather than caused by the difference in PAR level between the two treatments (PPFD 370 vs. 479), as light intensity has not been shown to impact the cannabinoid concentrations and have no or minor impact on terpenes according to recent studies (Rodriguez-Morrison et al., 2021a; Llewellyn et al., 2022).

### 4.4. Short wavelength radiation increases the concentration of some cannabinoids and monoterpenes but has no impact on sesquiterpenes

Lydon et al. (1987) reported an increase in THC concentration with increasing UV-B dose, a result which has not been successfully replicated in the more recent experiments (Rodriguez-Morrison et al., 2021b; Llewellyn et al., 2022). According to Rodriguez-Morrison et al. (2021b) the increasing UV-B dose had no effect on cannabinoid concentration in

genotype 'Breaking wave' and decreased the concentration in genotype 'Low tide'. Llewellyn et al. (2022) reported that there were no differences in the floral cannabinoid concentrations between the UV and non-UV treatments, but an increase in the THC concentration under UVA+UVB treatment was found in the small leaves between inflorescences, i.e., the sugar leaves.

In contrast to the UV-B studies, recent studies by Magagnini et. al. (2018) and Jenkins (2021) have reported UV-A and blue radiation to increase cannabinoid concentrations. In the study by Magagnini et al. (2018), plants grown under two LED spectra containing proportionally higher blue and UV-A radiation had significantly higher cannabinoid concentrations (THC, CBD, CBG) compared with a high-pressure sodium (HPS) light source. The researchers suggested that UV-A and blue radiation may synergistically induce higher cannabinoid concentrations via accelerated synthesis of CBGA, the precursor molecule of THCA and CBDA. However, the spectra used in the experiment did not allow them to answer the question whether the response was a UV-A or blue radiation mediated, as the spectra differed in other regions of the spectrum as well. Similarly, Jenkins (2021) reported higher cannabinoid concentrations in two out of the three tested varieties when UV-A or blue + red radiation was deployed simultaneously with a white background light during the last two weeks of cultivation. In the study by Danziger and Bernstein (2021b) three genotypes were cultivated under four different light treatments; HPS, white LED, red + blue LED in ratio of 1:1 and red + blue LED in ratio of 4:1. The researchers concluded that the concentration of CBGA increased with increasing blue portion whereas other cannabinoids (CBDA, THCA, and CBCA) were less affected by light treatments and that the response was genotype dependent. However, the experimental design and statistical analyses in the above mentioned three studies (Magagnini et al., 2018; Danziger and Bernstein, 2021b; Jenkins, 2021) were not described in such detail, that clear comparisons to this study would be possible.

In this experiment, partially against the hypothesis, an increase in the dominant cannabinoids, CBDA or THCA, was not detected with increased blue, UV-A, or UV-B radiation. This study could not therefore reproduce the results reported by Lydon et al. (1987), Magagnini et al. (2018), or Jenkins (2021), where short wavelength radiation increased the dominant cannabinoid concentrations. Instead, these findings support the results recently reported by Llewellyn et al. (2022) and Rodriguez-Morrison et al. (2021b), who did not find a positive effect of UV-B on the dominant cannabinoid concentration in the inflorescence. It is possible that the response is dependent on the genetics and the short wavelength radiation (blue or UV) treatment application method, i.e., the duration, intensity, and timing of the applied light treatment. Lewellyn et al. (2022) also hypothesized that the interplay between UV and photosynthetically active radiation (PAR) could play a role in the magnitude of UV responses; the higher the PAR irradiance in relation to UV, the milder the UV mediated responses would be. In this study the light intensity was 650 μmol $m^{-2}$ $s^{-1}$ of which 0.4% was in UV-B region (2.7 μmol $m^{-2}$ $s^{-1}$). Cannabis can withstand and utilize higher light intensities, up to 1800 μmol $m^{-2}$ $s^{-1}$. In all the above discussed experiments, the blue or UV radiation had been given simultaneously with a background light, possibly decreasing the effect of UV treatment.

Although no treatment effect on the dominant cannabinoids was found in this experiment, the concentration of the cannabinoid, THCVA, increased with added blue and UV-B radiation, partially supporting the hypothesis of short wavelength radiation having a positive impact on floral cannabinoid concentration. The result is relevant for the pharmaceutical industry, as THCV has potential in obesity and diabetes type 1 management (Abioye et al., 2020). This result indicates that some cannabinoids respond to light conditions, and that the cannabinoid profile can be manipulated by using specific light spectra during the flowering period.

Since monoterpenes and cannabinoids share a partially overlapping biosynthesis pathway and have a common precursor molecule, GPP, which has been shown to increase under short wavelength radiation (Desaulniers Brousseau et al., 2021), it can be hypothesized that the concentrations of both compound families would increase or decrease in a similar way under a similar light spectrum. This experiment supports the hypothesis of an increasing effect of short wavelength radiation on cannabinoid and terpene concentrations, since the monoterpene, myrcene, increased with UV-B radiation and the cannabinoid, THCV, increased with blue and UV-B radiation. In addition, the monoterpene, ocimene, tended to increase with increasing amount of short wavelength radiation in the spectrum ($p = 0.055$). However, against the hypothesis, UV-A did not have an impact on the secondary metabolite concentrations.

While some of the monoterpenes increased with added UV-B or blue radiation, all six tested sesquiterpenes were unaffected by the short wavelength radiation treatments. The difference between mono- and sesquiterpenes' response to short wavelength radiation could be explained by the different enzymes involved in their biosynthesis pathways. While in the monoterpene biosynthesis pathway several enzymes are upregulated by UV and blue radiation (Desaulniers Brousseau et al., 2021), the same enzymes are not found in the sesquiterpene biosynthesis pathway (reviewed by Desaulniers Brousseau et al., 2021), indicating that the sesquiterpene pathway is not photoinduced.

### 4.5. Leaf flavonoid concentration as a monitoring tool for floral cannabinoid and terpene concentrations

A precise laboratory level cannabinoid and terpene analysis is laborious, expensive, and requires destruction of the inflorescence, which makes it unappealing to growers. Optional measurement practices to predict the floral cannabinoid and terpene content, without destructive laboratory analysis, would add consistency and uniformity to flower yield quality when a crop could be analysed and monitored prior to harvest and selling. The aim was to test whether the leaf adaxial flavonoid concentration could be used as a prediction tool to estimate the secondary metabolite concentrations in the inflorescences. The leaf adaxial flavonoid concentration increased with short wavelength radiation, whereas the absence of blue radiation and lowered R:FR ratio decreased the flavonoid concentration throughout the measuring period. Similar results have been reported with pea (*Pisum sativum*) and rapeseed (*Brassica napus*) (Siipola et al., 2015; Gerhardt et al., 2008), where higher leaf flavonoid concentrations were detected under a spectrum containing UV-B compared with treatments without UV-B. In addition, Gerhardt et al. (2008) reported the decreasing effect of far-red radiation on leaf flavonoid concentrations.

When examining whether the leaf flavonoid concentration would reflect the cannabinoid or terpene concentrations in the inflorescences, the means of the leaf flavonoid concentration from last measurement time were compared with the means of floral secondary metabolite concentrations at harvest time with a correlation analysis. The hypothesis that the leaf adaxial flavonoid concentration at harvest would correlate positively with the floral cannabinoid and terpene concentrations was supported by experiment A but not by experiment B. In experiment A, the leaf flavonoid concentration correlated positively with the sum of measured monoterpenes, CBD, CBGA, and THCVA concentrations while in experiment B no significant correlations were found. Therefore, this study does not fully support the hypothesis and provides only a robust starting point for a tool for estimating the correct harvest time and the total cannabinoid and terpene concentrations through leaf flavonoid concentrations. To obtain a comprehensive data set for trustworthy conclusions, maturing inflorescences should be collected repeatedly throughout the cultivation period at the same time as leaf flavonoid concentrations are measured.

### 4.6. Using light spectra as a cultivation tool

Using specific light spectra at different cultivation phases could be used as a cultivation tool. Short wavelength radiation can be used to

increase accumulation of specific secondary metabolites in *C. sativa* leaves and inflorescences. Manipulating plant morphology with different R:FR ratios could bring benefits to growers at different stages of cultivation. For example, in a multilayer growing system stem elongation is not a desired trait, but for mother plant production elongation of internodes can be considered positive and facilitate taking of cuttings (Campbell et al., 2019). In other species, a moderate addition of far-red light has increased photosynthesis efficiency and total biomass accumulation (Moon et al., 2023), something which was not examined in this study. In future studies, balance between secondary metabolite and biomass allocation under different light spectra in *C. sativa* should be further investigated.

## 5. Conclusions

Light spectrum influenced the morphology, inflorescence yield, and secondary metabolite accumulation in *C. sativa* leaves and inflorescences. In accordance with the hypothesis, low R:FR ratio increased plant height and decreased secondary metabolite activity in leaves and inflorescences. The leaf adaxial flavonoid concentration was lower throughout the growing period and floral monoterpene and some cannabinoid concentrations were lower at the time of harvest. The hypotheses of the effects of blue, UV-A, and UV-B radiation on stem elongation, yield formation, or secondary metabolites were mostly rejected. Lack of blue light did not increase stem elongation significantly, nor did the addition of blue radiation increase secondary metabolite accumulation in the inflorescences. UV-B radiation did not have a negative impact on the inflorescence yield, however, in accordance with the hypothesis, UV-B radiation increased some of the cannabinoid and terpene concentrations. According to the results, it is possible to manipulate the morphology, inflorescence yield, and yield quality of *C. sativa* with light spectrum.

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## CRediT authorship contribution statement

**Stiina Kotiranta:** Conceptualization, Methodology, Data curation, Writing – original draft. **Pauliina Palonen:** Methodology, Resources, Supervision. **Titta Kotilainen:** Methodology, Writing – review & editing. **Juha-Matti Pihlava:** Resources, Writing – review & editing.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stiina Kotiranta reports equipment, drugs, or supplies was provided by Valoya Oy. Stiina Kotiranta reports a relationship with Valoya Oy that includes: employment. Stiina Kotiranta was an employee of Valoya Oy at the time the experiments were conducted. The work relationship ended in September 2022.

## Data Availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2023.117772.

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