



OPEN ACCESS

EDITED BY

Leo Marcelis,
Wageningen University and Research,
Netherlands

REVIEWED BY

Aušra Brazaitytė,
Lithuanian Research Centre for Agriculture
and Forestry, Lithuania
Hannah M. Rivedal,
Agricultural Research Service (USDA),
United States
Anna Kotton,
University of Agriculture in Krakow, Poland

*CORRESPONDENCE

Ricardo Hernández
✉ rhernan4@ncsu.edu

RECEIVED 16 January 2024

ACCEPTED 06 May 2024

PUBLISHED 07 June 2024

CITATION

Collado CE, Hwang SJ and Hernández R
(2024) Supplemental greenhouse lighting
increased the water use efficiency, crop
growth, and cutting production in *Cannabis
sativa*.

Front. Plant Sci. 15:1371702.

doi: 10.3389/fpls.2024.1371702

COPYRIGHT

© 2024 Collado, Hwang and Hernández. This
is an open-access article distributed under the
terms of the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or reproduction
is permitted which does not comply with
these terms.

Supplemental greenhouse lighting increased the water use efficiency, crop growth, and cutting production in *Cannabis sativa*

Cristian E. Collado¹, Seung Jae Hwang²
and Ricardo Hernández^{1*}

¹Department of Horticultural Science, North Carolina State University, Raleigh, NC, United States,

²Division of Horticultural Sciences, Institute of Agriculture & Life Sciences, Research Institute of Life Sciences, Division of Crop Sciences, Gyeongsang National University, Jinju, Republic of Korea

The expanding cannabis production sector faces economic challenges, intensified by freshwater scarcity in the main US production areas. Greenhouse cultivation harnesses sunlight to reduce production costs, yet the impact of greenhouse light levels on crucial production components, such as plant growth, branching, and water use efficiency (WUE), remains poorly understood. This study aimed to assess the effects of combined sunlight and supplemental lighting on the crop's main production components and leaf gas exchange of *Cannabis sativa* 'Suver Haze' in the vegetative stage. Within a greenhouse, LED lighting provided at intensities of ~150, 300, 500, and 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (18-hour photoperiod), combined with solar radiation, resulted in average daily light integrals of 17.9, 29.8, 39.5, and 51.8 $\text{mol m}^{-2} \text{d}^{-1}$. Increasing light levels linearly increased biomass, leaf area, and the number of branches per plant and square meter, with respective rates of 0.26 g, 32.5 cm^2 , and 0.41 branches per mole of additional light. As anticipated, crop evapotranspiration increased by 1.8-fold with the increase in light intensity yet crop WUE improved by 1.6-fold when comparing the lowest and highest light treatments. Moreover, water requirements per unit of plant biomass decreased from 0.37 to 0.24 liters per gram when lighting increased from ~18 to 52 $\text{mol m}^{-2} \text{d}^{-1}$, marking a 35% reduction in evapotranspiration. These results were supported by increments in leaf photosynthesis and WUE with light enhancement. Furthermore, our findings indicate that even 52 $\text{mol m}^{-2} \text{d}^{-1}$ of supplemental lighting did not saturate any of the crop responses to light and can be economically viable for cannabis nurseries. In conclusion, light supplementation strongly enhanced photosynthesis and plant growth while increasing WUE. Additionally, a comprehensive discussion highlights the shared physiological mechanisms governing WUE in diverse plant species and their potential for water conservation under enhanced lighting conditions.

KEYWORDS

HEMP, stock plant, propagation, water management, water reduction, crop yield, PPFD, DLI

Introduction

The expansion of both medicinal and recreational cannabis sectors within the United States is witnessing persistent growth, attributable to the ongoing legalization efforts across various states (DeCarcner et al., 2021). The cultivation of high-value cannabis crops is predominantly conducted within controlled environments, encompassing indoor sole-source-LED light facilities and greenhouses. While greenhouse cultivation harnesses solar energy to enhance cost-efficiency in production (Vezdos and Martinez, 2021), it confronts the challenge of potentially insufficient natural sunlight levels required to attain targeted crop quality and yield. Furthermore, the escalating demand for water resources poses a pressing concern, as approximately 37% of the freshwater supply in the United States is allocated to irrigation (Dieter et al., 2018). This issue is exacerbated by other factors, including population growth, agricultural demands, complexities in water infrastructure, and recurring drought conditions (GAO, 2014). Furthermore, California, a state deeply affected by water shortages, accounted for 58% of medical and recreational cannabis licenses issued in 2021 (Conway, 2023). Therefore, it is imperative to optimize cannabis production and other crops through the strategic application of supplemental lighting and water-saving approaches. Addressing these multifaceted challenges requires a comprehensive understanding of crop light and water utilization.

Research pertaining to *Cannabis sativa* cultivated under sole-source LED lighting reported a correlation between the daily light integral (DLI) and crop productivity. For instance, cannabis flower yield exhibited a linear increase in response to increasing DLI levels, up to $78 \text{ mol m}^{-2} \text{ d}^{-1}$, which represented the highest DLI investigated under short-day conditions, 12 h (Rodriguez-Morrison et al., 2021). Moreover, when subjected to long day durations, 16 h, the shoot biomass demonstrated a similar positive trend and increased even with $82 \text{ mol m}^{-2} \text{ d}^{-1}$ (Moher et al., 2021). However, it is essential to acknowledge that the dynamics of light within the context of greenhouse production can deviate substantially from those observed in sole-source LED facilities, and those changes may strongly affect plant physiological and crop efficiencies. Greenhouse environments are characterized by diurnal fluctuations in light intensity, a balanced spectrum encompassing blue, green, red, and far-red wavelengths, as well as the introduction of additional radiation from 800 to 2500 nm. The resultant but dynamic spectral combination between sunlight and electrical lighting further confounds the light environment within greenhouses. These dynamic light conditions can influence diverse facets of plant biology, including growth patterns, morphological attributes, and key physiological processes such as photosynthesis, stomatal conductance, transpiration, and energy balance (Pieruschka et al., 2010; Nelson and Bugbee, 2015; Zhen and Bugbee, 2020). Consequently, it is imperative to emphasize the need for comprehensive characterizations of the lighting environment in greenhouse supplemental lighting studies. Moreover, this research enhances the limited body of knowledge concerning the impact of supplemental lighting on cannabis greenhouse production (David Potter, 2009).

Radiation influences water usage in non-stressed plants, primarily by increasing transpiration rates (Whitehead, 1998; Pieruschka et al., 2010; Pokorný, 2019). While it is known that increased radiation levels correspondingly lead to increased water consumption, a more comprehensive approach for assessing water utilization leads to calculating water-use efficiency (WUE). WUE captures the interaction between plant growth parameters, such as yield and biomass, with transpiration or evapotranspiration and can be quantified at the leaf, plant, and crop levels (Jackson et al., 2016; Hatfield and Dold, 2019).

One of the principal strategies employed to enhance crop WUE is the deliberate reduction of water availability within the soil or substrate, a practice that significantly decreases the transpiration rate. Nonetheless, this approach most often reduces plant productivity, given the associated decrease in photosynthetic rates (Hubick and Farquhar, 1989; Lawson and Blatt, 2014; Xing et al., 2022). Remarkably, research has demonstrated that trees originating from diverse botanical families and adapted to varying geographical regions exhibit reduced leaf transpiration when subjected to elevated radiation levels in comparison to conspecifics grown under lower irradiance conditions, even while concurrently displaying heightened rates of photosynthesis (Idris et al., 2019). This suggested that leaves adapted to highlight environments may possess superior WUE compared to their shade-adapted counterparts when both leaf types are subjected to identical radiation regimes. Therefore, one of the main focuses of this investigation was to quantify leaf and crop water-use efficiencies in the context of cannabis cultivation under increased supplemental light conditions.

Cannabis nurseries and flower producers employ extended photoperiods to increase branch proliferation and plant size. This enhances cutting production in nurseries and conditions plants for higher flower yields. Therefore, enhancing biomass and branch count and minimizing cultivation time is vital for cannabis nurseries, ensuring a seamless supply of plants to cannabis flower producers. Prior investigations have yet to elucidate the impact of lighting on branch development (potential cuttings) and the associated morphological and physiological parameters of cannabis vegetative plants in greenhouses.

The primary objective of this research was to investigate the impacts of greenhouse light levels, encompassing solar radiation in conjunction with various supplemental LED light intensities ($\sim 150, 300, 500, \text{ and } 700 \mu\text{mol m}^{-2} \text{ s}^{-1}$), on growth parameters, the number of branches, water utilization (WU), and water-use efficiency (WUE) in a vegetative crop of *Cannabis sativa* L. Additionally, we aimed to assess the effects of these prolonged light treatments on key leaf physiological attributes, specifically photosynthesis (A), stomatal conductance (g_{sw}), and transpiration (E), which are pivotal determinants of both growth and water consumption.

The study involved the examination of two hypotheses. 1) Increments in the cumulative light dosage would elicit an increase in plant growth and in the number of branches. 2) Leaves of cannabis plants cultivated under an elevated DLI would display higher WUE due to increased photosynthetic rates than their counterparts exposed to a lower DLI when leaves were evaluated under high PPFDs.

Materials and methods

Starting plant material and location

Rooted cuttings of *Cannabis sativa* cv Suver Haze (© Oregon CBD) served as the initial material. The cuttings underwent rooting in a greenhouse for 24 days and a photoperiod of 18 hours. The propagation was conducted by a commercial nursery in Broadway, NC, US (Ryes Greenhouses LLC). On October 9, 2020, 240 plug plants (rooted cuttings) were selected to be planted, as well as five additional and representative samples for destructive measurements. The most uniform plants were selected based on morphological similarities (mean \pm SD of five representative samples: number of leaves: 3.6 ± 0.9 , shoot fresh and dry mass: 1.4 ± 0.4 g and 0.26 ± 0.08 g, root dry mass: 18.1 ± 7.2 mg, and leaf area: 37.5 ± 10.5 cm²). The plants were evenly distributed among 12 research plots within a glass greenhouse at the NC State Horticulture Field Lab in Raleigh, NC, USA. Further details can be found in [Supplementary Figure S1](#) of the [Supplementary Material](#) section.

Plot sensing and controlling capabilities

Each production-research plot was equipped with two LED dimmable lighting fixtures to establish and maintain specific light levels, as illustrated in [Supplementary Figure S1](#). Additionally, two soil moisture sensors, two load cells, and a solenoid valve were employed to quantify water evapotranspiration and sustain optimal substrate moisture and nutrition due to the crop requirements at each plot. Furthermore, a fine-wire thermocouple and quantum sensor were utilized to address greenhouse air temperature and solar variations. In total, 72 sensors and 12 solenoids were connected to a datalogger and controller through four sensor multiplexers and a relay driver, with data logged at 5-minute intervals. Hardware specifications are detailed in the [Supplementary Material](#) section.

The treatments

The day after planting, the supplemental lighting was increased from ~ 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to $\sim 150, 300, 500,$ and 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ([Table 1](#)) and maintained for 20 days until harvest. Each light level represents the average of three plots or production areas ([Supplementary Figure S1](#)). However, the treatments consisted of a combination of supplemental LED and solar radiation. Details are presented in [Table 1](#), and the light spectra of average sunlight, as well as the lowest and highest light treatments, are presented in [Table 2](#) and [Supplementary Figure S2](#). The calibration and control of the lights are described in the [Supplementary Material](#) under the title [Supplemental Light Calibration and Control](#).

Greenhouse conditions

The greenhouse air conditions were regulated with forced hot air, a cooling wet pad, two exhaust fans, and a Priva Maximizer control system (De Lier, Netherlands). The average temperature and humidity were $25.4 \pm 1.7^\circ\text{C}$ and $73.3 \pm 10.4\%$ (mean \pm SD) at the center of the greenhouse at canopy height and measured in an aspirated box. The air temperature at the top of the 12 canopies was similar, yielding a mean \pm standard deviation between plots of $25.4 \pm 0.3^\circ\text{C}$. The daytime carbon dioxide was around 410 $\mu\text{mol mol}^{-1}$ and monitored right next to the canopy.

Control of the substrate moisture and fertilization

Control of substrate moisture and fertilization was managed through independent irrigation control at each of the 12 plots, utilizing readings from two soil volumetric water content (VWC) sensors per plot. The plot's solenoid valve was activated for 25 minutes based on the lowest substrate moisture reading from two

TABLE 1 Summary of light sources and levels: supplemental PPFD, cumulative supplemental plus solar treatments (Σ PPFD), average daily light integral (DLI), average PPFD, and light source.

| Supplemental PPFD ^z | Σ PPFD ^y | DLI ^x | Average PPFD ^w | Light source: ^v | | Σ ePAR (400-750 nm) | eDLI (400-750 nm) | PPFD/ePAR |
|--------------------------------------|----------------------------|-----------------------------------|--------------------------------------|----------------------------|-----|----------------------------|-----------------------------------|-----------|
| | | | | Sun | LED | | | |
| $\mu\text{mol m}^{-2} \text{s}^{-1}$ | mol m^{-2} | $\text{mol m}^{-2} \text{d}^{-1}$ | $\mu\text{mol m}^{-2} \text{s}^{-1}$ | | | mol m^{-2} | $\text{mol m}^{-2} \text{d}^{-1}$ | % |
| 151 ± 0 | 376 ± 49 | 17.9 | 277 | 48% | 52% | 408 | 19.5 | 92% |
| 300 ± 2 | 625 ± 8 | 29.8 | 459 | 38% | 62% | 669 | 31.8 | 94% |
| 501 ± 2 | 829 ± 59 | 39.5 | 610 | 22% | 78% | 862 | 41.1 | 96% |
| 703 ± 3 | 1088 ± 55 | 51.8 | 800 | 17% | 83% | 1121 | 53.4 | 97% |

Σ ePAR and eDLI are cumulative photons based on an extended PAR range 400 to 750 nm ([Zhen et al., 2021](#)). Means and standard deviations were calculated from the three greenhouse plots per light level. The photoperiod was maintained at 18 hours.

^zPhotosynthetic photon flux density from 400 to 700 nm) measured with a line quantum sensor (mean \pm standard deviation).

^yMeasured continuously for 21 days with quantum sensors (mean \pm SD). Twenty-one days include one day of LED (~ 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$) + solar radiation and 20 days of LED treatments + solar radiation.

^xDLI = Σ PPFD/21 days.

^wDLI/Photoperiod * time and mol conversion factors.

^vProportions between supplemental (LED) and solar radiation (Sun).

TABLE 2 Solar and LED spectrum measurements and calculations for light quality comparison.

| Plant light parameter | wavelength range (nm) | Units | Greenhouse sunlight (Sun) | LED (151 PPFD) | LED (703 PPFD) | Sun + LED (144 + 151 PPFD) | Sun + LED (144 + 703 PPFD) |
|-----------------------|-----------------------|--------------------------------------|---------------------------|----------------|----------------|----------------------------|----------------------------|
| PPFD | 400-700 | $\mu\text{mol m}^{-2} \text{s}^{-1}$ | 144 | 151 | 703 | 295 | 847 |
| ePAR* | 400-750 | | 170 | 152 | 707 | 321 | 877 |
| UV | 300-400 | | 5.0 | 0.1 | 0.4 | 5.1 | 5.4 |
| Blue | 400-500 | | 34.2 | 27.5 | 121.6 | 61.8 | 155.8 |
| Green | 500-600 | | 53.3 | 0.4 | 1.5 | 53.6 | 54.7 |
| Red | 600-700 | | 56.5 | 123.1 | 580.4 | 179.6 | 636.9 |
| Far Red | 700-800 | | 49.3 | 0.9 | 4.0 | 50.2 | 53.3 |
| Blue/PPFD | | % | 23.8 | 18.2 | 17.3 | 20.9 | 18.4 |
| Green/PPFD | | | 37.0 | 0.2 | 0.2 | 18.2 | 6.5 |
| Red/PPFD | | | 39.3 | 81.5 | 82.5 | 60.9 | 75.2 |
| Red/Blue | | | 1.65 | 4.48 | 4.77 | 2.91 | 4.09 |
| Red/Far Red | | | 1.15 | 132.0 | 143.7 | 3.6 | 11.9 |
| Pfr/Ptotal** | | | 0.73 | 0.88 | 0.88 | 0.83 | 0.86 |

The greenhouse sunlight measurements were adjusted to match the average photosynthetic photon flux density of $144 \mu\text{mol m}^{-2} \text{s}^{-1}$. The calculated solar mean is based on an 18-h photoperiod and an average DLI of $9.3 \text{ mol m}^{-2} \text{d}^{-1}$ from the solar radiation measurements among the 12 plots. The LED and Sun + LED columns show the lowest and highest lighting treatments. The LED columns represent the greenhouse lighting conditions without solar radiation, while the Sun + LED columns represent average lighting conditions in the 18-h lighting period. This table analysis is based on ~20 and 21 days for the LED conditions and the average greenhouse solar radiation, respectively; thereby, they may differ from values in Table 1.

*ePAR represents an extended PAR or PPFD range (Zhen et al., 2021).

**Phytochrome photoequilibrium (Sager et al., 1988).

plants in each treatment area. Irrigation was initiated when the VWC reached or fell below $0.345 \pm 0.014 \text{ m}^3 \text{ m}^{-3}$ (80% of container capacity).

Two irrigation emitters with a combined flow of 5.4 L h^{-1} per pot were employed to ensure uniform moisture and prevent nutrient-salt accumulation. Detailed analysis of nutrient solution composition, pour-thru procedure samples (Table S1), substrate conditions, and moisture calibration can be found in the Supplementary Material section.

Plants for measurements and growing area

the total growing area per plot was made of 20 plants, using a density of $10.8 \text{ plants m}^{-2}$. However, only plants from the center of the plots (6 plants) were used for plant measurements to avoid edge effects.

Vegetative growth measurements

Plant height (measured from the growing tip to substrate level) was assessed every two to four days. Plant biomass and leaf parameters were determined through destructive measurements on day 21. Plant fresh mass was immediately recorded after cutting the plant at ground level. Leaf area, and leaf and stem dry mass were quantified using Li-3100C units (Li-Cor, Lincoln, NE, USA) and drying ovens set at 69°C , respectively. The number of

primary and secondary branches (length $\geq 5 \text{ cm}$) was counted on the main stem and the primary branches, respectively.

Evapotranspiration

Evapotranspiration (ET) and evaporation were quantified by measuring changes in the load cell readings (including the weight of water, substrate, pot, and plant for ET, or the weight of water, substrate, and pot for evaporation) after and immediately before the irrigation events occurred (Supplementary Figure S3A). ET was quantified in all plots, while evaporation was measured using a pot without a plant. Refer to the Supplementary Material section for more details.

WUE (Water Use Efficiency): Shoot water use efficiency was calculated as the ratio between the dry mass of leaves and stems and the liters of evapotranspiration in a 21-day period. Similarly, the ratios between primary, secondary, and total branches and evapotranspiration requirements were calculated. Leaf WUE was calculated as the ratio between photosynthesis (A) and transpiration (E).

Metabolite and gas exchange measurements

These measurements were performed on fully expanded, light-exposed top leaves to compare leaves developed under different

light levels. Crops were subjected to light treatments for 18 days before the readings. Chlorophyll, anthocyanin, and flavonol indexes were measured on the adaxial surfaces using a Dualex Scientific handheld leaf-clip meter (Force-A, Orsay, France). On similar leaves, net CO₂ assimilation (A), stomatal conductance to water vapor (g_{sw}), transpiration (E), internal leaf CO₂ concentration (C_i), and leaf temperature measurements were taken using a Li-6800 with a 2-cm² aperture 6800-01A chamber (Li-Cor, Lincoln, NE, USA). Readings were recorded at the center of the leaflets and after the CO₂ assimilation plateaued for at least two minutes. The chamber maintained 18% blue light, 82% red light, 70% relative humidity (RH), 27°C temperature, and 404 ppm of CO₂. The settings were chosen based on greenhouse conditions and instrument capability.

Statistics

For crop parameters and leaf pigments, linear regression and ANOVA were conducted using the mean of one or more subsamples per plot. Subsamples or observational units (OU) consisted of 6 plants for plant height, 3 plants for biomass, leaf area, and branches, 1-2 load cells for evapotranspiration, and 18 leaves for metabolites. The total sample size was defined as N, and in the same way, the number of true replications in one group or light level was n. Because the plot acted as the experimental unit, N and the number of plots were equivalent to 12 (Lazic et al., 2018).

For leaf gas exchange analysis from 150 to 2000 μmol of light m⁻² s⁻¹ (PPFD), only top leaves grown under DLIs of ~18 and 52 mol m⁻² d⁻¹ were analyzed (N=6, n=3, OU=1 leaf per plot). Split-plot models were used on A, E, g_{sw}, and WUE to evaluate responses based on the effects of DLI, PPFD, and DLI*PPFD. Light intensity (150, 300, 500, 700, 1000, 1300, 1600, and 2000 μmol m⁻² s⁻¹) effects were evaluated at the split-plot level. Consequently, the effects of long light exposure or DLI were assessed at the main plot level. In addition, DLI effects were analyzed using t-tests. T-tests were used to compare specific groups and validate this study's second hypothesis. More specifically, one-tailed t-tests were used to evaluate the effect of DLI on leaf photosynthesis and WUE under elevated PPFDs (≥ 700 μmol m⁻² s⁻¹), while leaves under lower PPFDs were evaluated under two-tailed t-tests. All analyses and graphs were performed using JMP Pro 16.0 and 17.0 from SAS (Cary, NC, USA).

Results

The results were analyzed using the cumulative light per square meter exposure in 21 days (ΣPPFD), given that plant responses to light are influenced by the spatial and temporal aspects of light capture (Rosati and Dejong, 2003). Nevertheless, considering that the standard practice in research and production often involves quantifying cumulative light on a daily basis rather than over the entire growth period, Daily Light Integral (DLI) was also used for the interpretation of results and discussion. A comprehensive

dataset, including specific and general crop responses to light, was provided in Table 3 to facilitate discussion. Additional results, such as leaf temperatures, leaf internal CO₂, and economic feasibility of cutting production, are presented in the Supplementary Material File.

Crop growth, development, and branch production

Plant and crop growth, development, and morphological parameters were evaluated under different greenhouse lighting conditions (Table 1) to characterize the overall plant quality, aiming to produce plants for flower induction and plants for the production of cuttings (stock plants).

Greenhouse lighting from ~18 to 52 mol m⁻² d⁻¹ led to linear increments in fresh mass, dry mass, number of nodes, internode length reduction, stem diameter, leaf area index (LAI), and branches (Table 3, Figures 1–4). In addition, increases in light intensity led to quadratic increments in specific leaf weight (SLW). Notably, under solar and supplemental light intensities between ~700 and 2000 μmol m⁻² s⁻¹ (Supplementary Figure S3B), along with an LAI expansion from 0.04 (initial leaf area) to 4.4 m² m⁻² (Table 3), the vegetative growth of 'Suver Haze' did not exhibit a growth saturation or light efficiency reduction (g mol⁻¹) even when exposed to an average DLI of 52 mol m⁻² d⁻¹. More specifically, the crop's dry mass linearly increased at a rate of 0.26 g mol⁻¹ from 102 to 376 g m⁻² (Figure 2A). In a similar way, the LAI linearly increased from 2.1 to 4.4 m² m⁻² (Figure 2B; Table 3). Furthermore, higher DLIs and LAIs were generally accompanied by increased SLW (specific leaf weight) and top-leaf metabolite concentrations per area (Table 3).

Despite differences in light quantity and quality (Tables 1, 2), the internode length and plant height exhibited marginal or non-significant responses to the light treatments (Table 3; Figure 3). On the other hand, the numbers of primary, secondary, and total branches linearly increased with more light. However, branching rates displayed a significant contrast; primary branches registered a rate of 0.04 branch mol⁻¹ m⁻², while secondary branches exhibited a substantially higher rate of 0.36 branch mol⁻¹ m⁻² (Figure 4) with the increase in light intensity. This difference highlighted the branching dynamics in response to light, with secondary branches surpassing primary branches by nine times.

In conclusion, all the results and their statistical analyses support our hypothesis that increments in the cumulative light dosage would elicit an increase in plant growth and the number of branches. In addition, there was no evidence of light saturation.

Crop water use and efficiency

The water usage (evapotranspiration in 21 days) and water use efficiencies (WUE) to produce shoots and branches were quantified from ~18 to 52 mol m⁻² d⁻¹ (DLI). Light supplementation linearly increased the cumulative crop evapotranspiration from 39.8 to 70.6

TABLE 3 Mean \pm standard deviation for whole canopy and ratios of *Cannabis sativa* 'Suver Haze' at four cumulative solar and LED lighting levels in 21 days (Σ PPFD).

| Σ PPFD (mol m ⁻²) | Shoot fresh mass (g m ⁻²) | Shoot dry mass (g m ⁻²) | Water per dry mass (g g ⁻¹) | Stems (%) | Leaves (%) | |
|--------------------------------------|---------------------------------------|---|---|--|--|--------------------------|
| 376 \pm 49 | 602 \pm 73 | 102 \pm 14 | 4.9 \pm 0.1 | 37 \pm 2 | 63 \pm 2 | |
| 625 \pm 08 | 956 \pm 87 | 161 \pm 19 | 5.0 \pm 0.2 | 38 \pm 2 | 62 \pm 2 | |
| 829 \pm 59 | 1281 \pm 114 | 223 \pm 20 | 4.8 \pm 0.0 | 39 \pm 1 | 61 \pm 1 | |
| 1088 \pm 55 | 1610 \pm 273 | 284 \pm 56 | 4.7 \pm 0.1 | 40 \pm 1 | 60 \pm 1 | |
| | L | L | L | L | L | |
| Σ PPFD (mol m ⁻²) | Main stem height (cm) | N° of nodes on the main stem | Internode length (cm) | Main stem diameter (mm) | LAI (m ² m ⁻²) | SLW (g m ⁻²) |
| 376 \pm 49 | 52.0 \pm 3.8 | 17 \pm 1 | 3.11 \pm 0.06 | 7.4 \pm 0.2 | 2.1 \pm 0.2 | 31.3 \pm 0.7 |
| 625 \pm 08 | 55.3 \pm 0.9 | 18 \pm 0 | 3.08 \pm 0.10 | 8.9 \pm 0.1 | 2.8 \pm 0.3 | 35.1 \pm 1.1 |
| 829 \pm 59 | 57.9 \pm 2.9 | 19 \pm 1 | 3.08 \pm 0.20 | 10.3 \pm 0.5 | 3.6 \pm 0.3 | 37.7 \pm 1.2 |
| 1088 \pm 55 | 55.9 \pm 1.4 | 20 \pm 1 | 2.85 \pm 0.14 | 11.1 \pm 0.9 | 4.4 \pm 0.7 | 38.6 \pm 0.7 |
| | NS | L | L | L | L | Q |
| Σ PPFD (mol m ⁻²) | Total branches m ⁻² | 1° branches m ⁻² | 2° branches m ⁻² | Chlorophyll index | Flavonol index | Anthocyanin index |
| 376 \pm 49 | 218 \pm 70 | 136 \pm 9 | 81 \pm 61 | 32.2 \pm 1.1 | 1.11 \pm 0.03 | 0.122 \pm 0.004 |
| 625 \pm 08 | 287 \pm 29 | 142 \pm 9 | 145 \pm 26 | 33.7 \pm 1.0 | 1.26 \pm 0.02 | 0.128 \pm 0.002 |
| 829 \pm 59 | 390 \pm 34 | 160 \pm 2 | 230 \pm 32 | 36.1 \pm 1.2 | 1.39 \pm 0.03 | 0.138 \pm 0.002 |
| 1088 \pm 55 | 508 \pm 37 | 164 \pm 11 | 344 \pm 34 | 35.9 \pm 1.0 | 1.43 \pm 0.07 | 0.144 \pm 0.006 |
| | L | L | L | L | Q | L |
| Σ PPFD (mol m ⁻²) | Water use (L m ⁻²) | WUE _{Shoot} (g L ⁻¹) | WUE _{Total branches} (Branch L ⁻¹) | WUE _{1° branches} (Branch L ⁻¹) | WUE _{2° branches} (Branch L ⁻¹) | |
| 376 \pm 49 | 39.8 \pm 3.4 | 2.6 \pm 0.3 | 5.5 \pm 1.7 | 3.4 \pm 0.3 | 2.2 \pm 1.2 | |
| 625 \pm 08 | 51.1 \pm 9.5 | 3.2 \pm 0.5 | 5.8 \pm 1.4 | 2.9 \pm 0.7 | 2.9 \pm 0.8 | |
| 829 \pm 59 | 58.7 \pm 3.8 | 3.8 \pm 0.5 | 6.7 \pm 0.9 | 2.7 \pm 0.2 | 3.9 \pm 0.7 | |
| 1088 \pm 55 | 70.6 \pm 10.3 | 4.1 \pm 0.9 | 7.3 \pm 1.4 | 2.4 \pm 0.5 | 5.0 \pm 0.9 | |
| | L | L | NS (p=0.06) | L | L | |

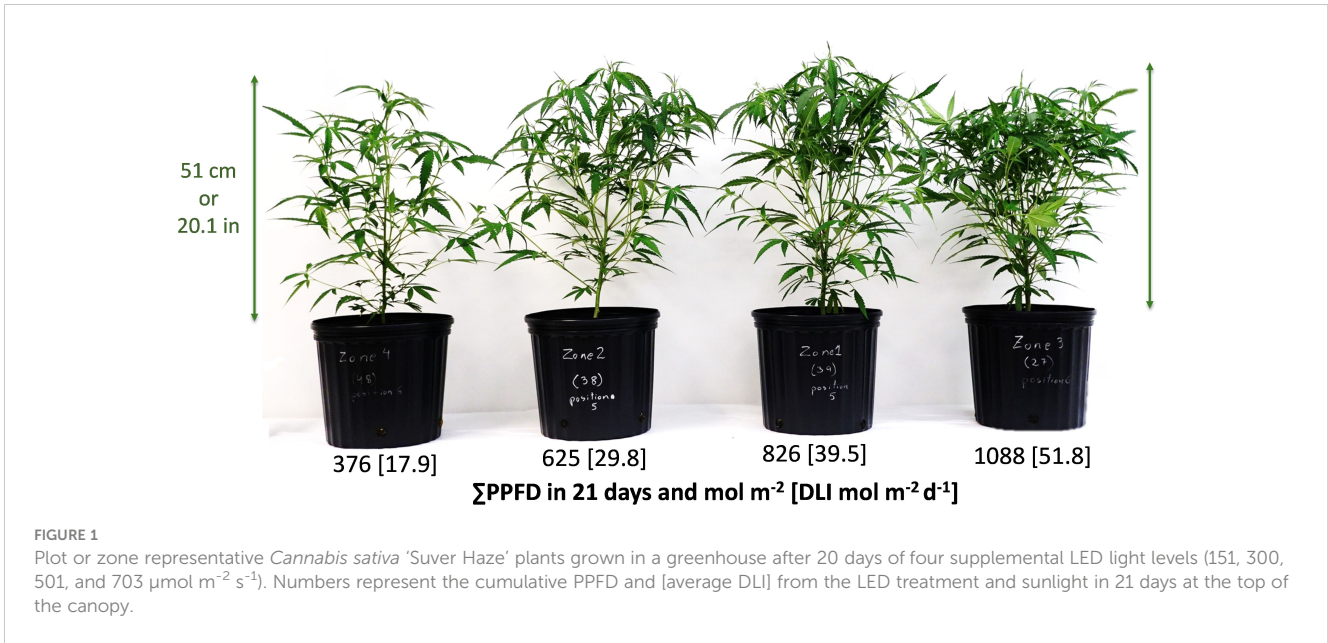
Results in square meter (m²) can be expressed in plant basis by dividing them by density (10.8 plant m⁻²). Shoot = leaf and stem biomass. LAI, leaf area index; SLW, specific leaf weight; 1° and 2°: primary and secondary; WUE, water use efficiency. % are on shoot biomass basis.

L and Q (p<0.05) for linear (L) and quadratic (Q) terms in linear regression analysis; NS (p≥0.05). In all cases, N=12, n=3, and the number of observational units varied, as described in Material and Methods.

liters (L) per square meter (m⁻²) (Figure 5A). Simultaneously, the pot evaporation was measured at 20.9 L m⁻², representing transpiration rates between ~48 to 70% of the evapotranspiration. Despite higher water losses under more light, the shoot WUE increased linearly from 2.7 to 4.2 g L⁻¹ by increasing the supplemental lighting (Figure 5B).

At the branch level, the WUE varied based on the type of branches (Figure 5C) and the branch response to the light (Figure 4). For instance, the WUE of primary branches was reduced with light augmentation (Figure 5C) because of the lower degree of light-level dependency of primary branches (Figure 4).

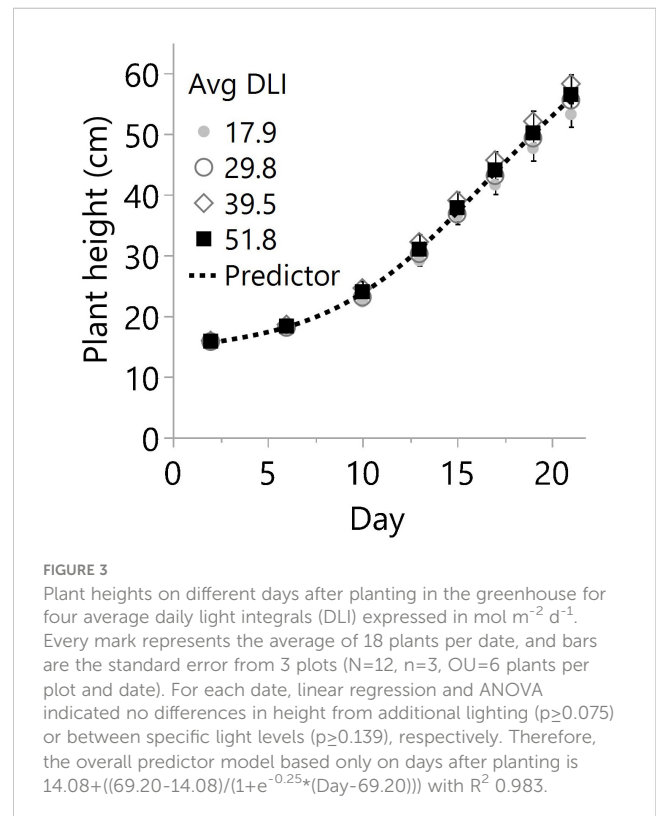
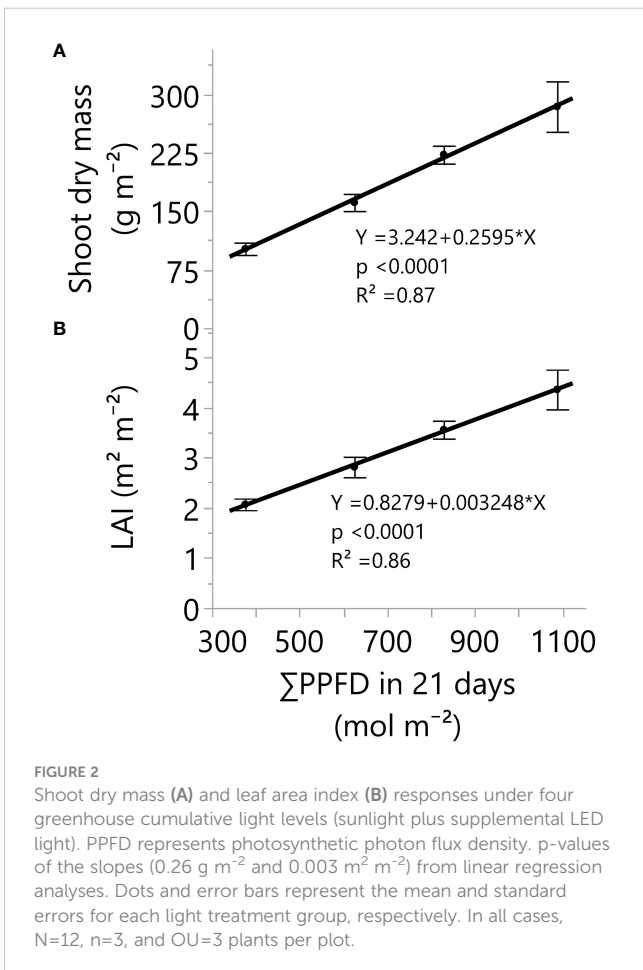
However, the increase of light substantially enhanced the WUE of secondary branch production, elevating it from 2 to 5 branches per liter (Figure 5C). This improvement can be attributed to the more pronounced impact of light on secondary branches compared to its influence on primary branches. Consequently, the combined results of WUE for total branches, primary plus secondary branches, suggest a reduced but still considerable effect by the light augmentation, with WUEs from 5.5 to 7.3 for total branches per liter of water (p=0.06, Figure 5C; Table 3). Nevertheless, the water needs for cutting production can exhibit significant variability, ranging from approximately 14 to 19 liters of water for every 100

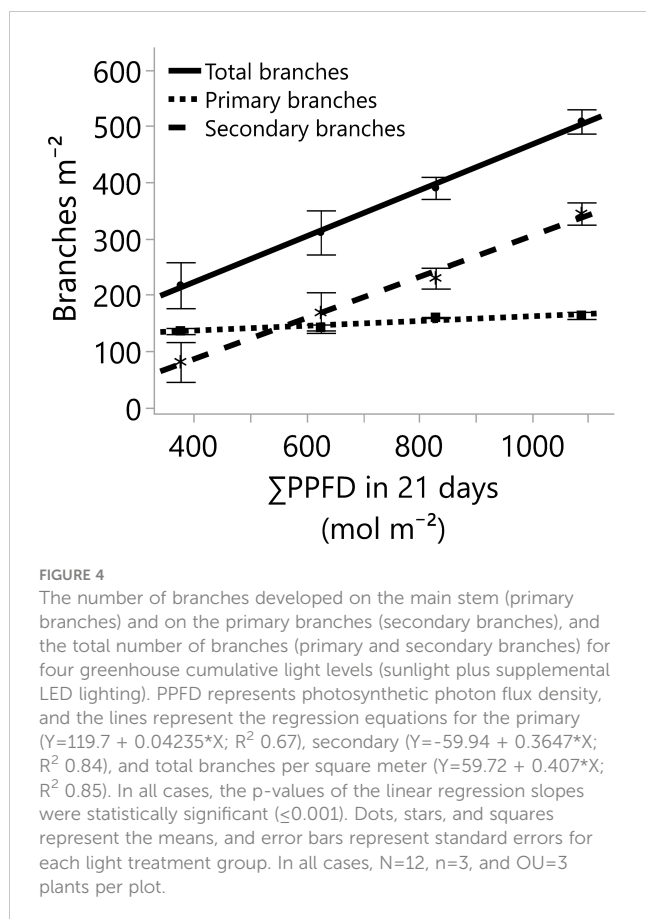


cuttings for highly efficient growers who utilize all available branches to approximately 20 to 50 liters of water for every 100 cuttings for those who selectively employ smaller or larger caliper cuttings (Figure 5D).

Leaf photosynthesis, pigments, stomata, transpiration, and WUE

Top leaves of plants grown under ~ 18 and $52 \text{ mol m}^{-2} \text{ d}^{-1}$ (DLI) were subjected to a range of short-term light intensities from 150 to 2000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (PPFD) to analyze the impact of long-term greenhouse lighting (DLI) on net photosynthesis, transpiration, stomatal conductance, and leaf WUE. Additionally, chlorophyll, flavonol, and anthocyanin indexes (light pigments) were quantified.





The leaf photosynthetic rates (A) of plants grown under 18- and 52-DLI treatments increased even under $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$, but the increments in A with more PPFD were dimmed and only $6.2 \pm 1.9\%$ (mean \pm SD) higher in 2000 than in $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ for both DLI treatments ($p < 0.001$). As a reference, the average proportional increments in A (and PPFD) based on $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ were 14% (150), 30% (300), 51% (500), 66% (700), 79% (1000), 87% (1300), 94% (1600), and 100% (2000). Furthermore, small photosynthetic differences between DLI treatments were observed under low and high PPFDs; for instance, 52-DLI leaves exhibited higher photosynthetic rates when exposed to short-term PPFDs exceeding $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$, but photosynthesis was higher under 18-DLI leaves when PPFDs dropped below $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 6A). Additionally, leaves grown under $52 \text{ mol m}^{-2} \text{d}^{-1}$ presented higher concentrations of photosynthetic (chlorophylls) and non-photosynthetic pigments (flavonols and anthocyanins) compared to $18 \text{ mol m}^{-2} \text{d}^{-1}$ (Table 3).

Unlike photosynthesis, leaf transpiration (E) and stomatal conductance (g_{sw}) increased linearly with PPFD (Figures 6B, C). However, increments in stomatal and transpiration rates were not proportional to the light levels. For example, the transpiration rate per photon of light under $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ was 7.3 times lower than at $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ or 2.9 vs $21.3 \mu\text{mol H}_2\text{O} \mu\text{mol photon}^{-1}$, respectively. Furthermore, despite the raised g_{sw} and E with short-term increments in PPFD, there were no statistical differences by long-term light levels, 18 vs. $52 \text{ mol m}^{-2} \text{d}^{-1}$ ($p \geq 0.29$; Figures 6B, C).

In concordance with the crop WUE, the leaf WUE of upper leaves, or A/E, increased up to a PPFD of $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($p < 0.001$); differences in WUE were not statistically significant from 700 to $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($\alpha=0.05$). Moreover, the main DLI effect (~ 18 vs. $52 \text{ mol m}^{-2} \text{s}^{-1}$) on leaf WUE or the interaction DLI*PPFD was not statistically significant ($\alpha=0.05$). Therefore, there was no evidence to support this study's second hypothesis: Leaves of cannabis plants cultivated under an elevated DLI would display higher WUE due to increased photosynthetic rates than their counterparts exposed to a lower DLI when leaves were evaluated under high PPFDs.

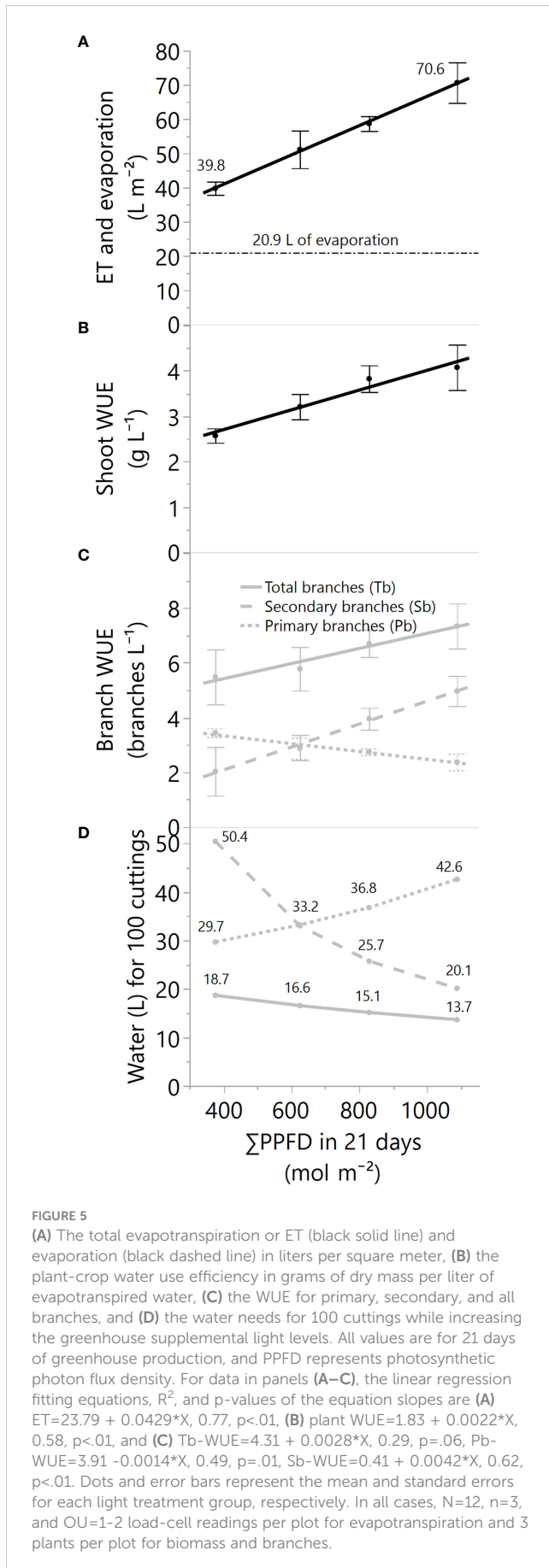
Discussion

Crop growth and development under greenhouse lighting and its effects on water use efficiency

Light effects and DLI recommendations on plant growth and production

The effects of light on plant growth can exhibit substantial variation across plant species, cultivars, developmental stages, and environmental factors, including plant nutrition, water availability, light intensity, and spectra. This variance stems from the diverse photosynthetic capacities inherent to different species, as well as the intricate interplay of multiple environmental factors (Boyer, 1970; Penning de Vries et al., 1989; Rosati and Dejong, 2003; Zhao et al., 2003; Weaver and Van Iersel, 2020). For instance, cultivating crops under identical DLI values but with lower light levels and extended photoperiods can lead to equivalent or even heightened daily growth. This is attributed to the enhanced efficiency of photosynthesis at both the leaf level (Weaver and Van Iersel, 2020) and the crop level (Hui et al., 2001). Furthermore, light wavelengths outside the Photosynthetically Active Radiation (PAR) range, 400 – 700 nm, can significantly influence growth and development. Far-red light (700 to 800 nm), for instance, promotes leaf expansion and photosynthesis in the 700-750 nm range (Zhen et al., 2021). In light of these findings, an extended PAR range (ePAR) from 400 to 750 nm has been proposed to account for photosynthesis enhancements (Zhen et al., 2021). Therefore, due to the complex interplay of these factors, comparing and adopting light levels across different situations is challenging and should ideally be confined to the specific conditions under evaluation. Nevertheless, for general reference, we provide a comparison with other cannabis studies, crops, and indoor systems employing the conventional DLI range (DLI 400-700nm) while offering the extended range values as a reference (Table 1).

Within the realm of cannabis cultivation, this study, as well as prior investigations employing exclusive LED lighting sources (Moher et al., 2021; Rodriguez-Morrison et al., 2021), provides compelling evidence to classify cannabis among the most elevated lighting requiring crops. For comparison, optimal lettuce production is associated with a maximum of $17 \text{ mol m}^{-2} \text{d}^{-1}$ for marketable leaf growth, with higher light levels potentially leading to increased biomass but also an elevated risk of leaf tip burn

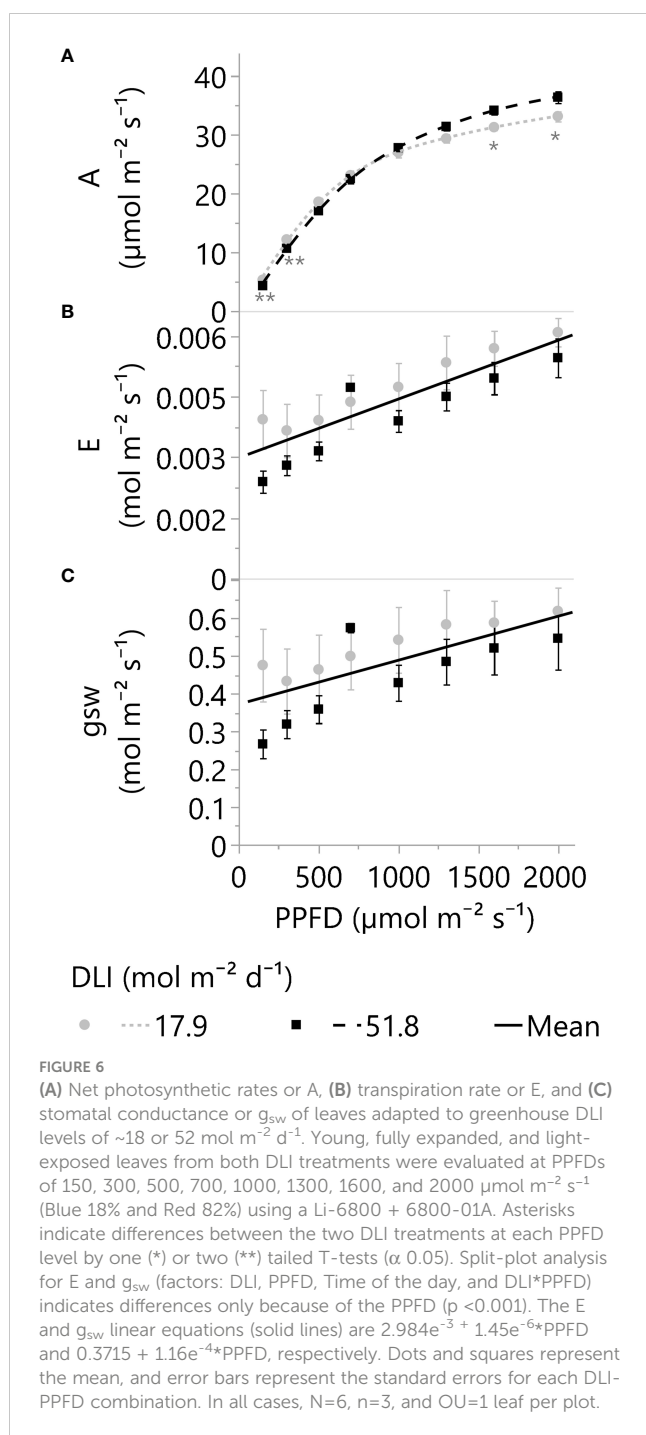


(Both, 2002). High-light greenhouse crops like tomatoes and cucumbers require at least $30 \text{ mol m}^{-2} \text{ d}^{-1}$ for optimal yields and fruit quality (Dorais et al., 2017), although significant research into higher light levels may not have been pursued due to economic feasibility constraints for production (van Iersel and Gianino, 2017). In corn breeding, supplemental greenhouse lighting is recommended to reach up to $750 \mu\text{mol m}^{-2} \text{ s}^{-1}$ or $\geq 38 \text{ mol m}^{-2} \text{ d}^{-1}$, depending on the photoperiod, while corn research in chamber conditions employs as much as $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ or $58 \text{ mol m}^{-2} \text{ d}^{-1}$ (Eddy and Hahn, 2010). Remarkably, indoor farming studies have shown that cannabis crops can utilize $82 \text{ mol m}^{-2} \text{ d}^{-1}$ for shoot growth (Moher et al., 2021) and $78 \text{ mol m}^{-2} \text{ d}^{-1}$ for flower production (Rodriguez-Morrison et al., 2021).

Despite growth increments in the vegetative stage of cannabis under high light, the light-to-biomass conversions in Moher et al. (2021) were lower than in our study. For instance, the biomass yield in the Moher et al. (2021) study was 246 g m^{-2} with $52 \text{ mol m}^{-2} \text{ d}^{-1}$, and it increased to 271 g m^{-2} with $82 \text{ mol m}^{-2} \text{ d}^{-1}$. In contrast, our study yielded an average of 285 g m^{-2} with $52 \text{ mol m}^{-2} \text{ d}^{-1}$ (eDLI: 53; Table 1) and showed no indications of light saturation (Figure 2A); importantly, both studies shared a consistent 21-day timeframe, facilitating a valid DLI-based comparison. It is plausible that the improved greenhouse study result in our research could be attributed to differences in plant genetics (Vanhove et al., 2012; Suchoff et al., 2021), light spectra (Lalge et al., 2017; Zhen et al., 2021), temperature conditions (Chandra et al., 2008, 2011), and/or the effects of water and fertilizer supply. Nevertheless, it is evident that further research into cannabis cultivation and production conditions is necessary to gain a deeper understanding and achieve consistent maximum yields and product quality.

Light effects on LAI

In this investigation, plants were uniformly spaced at intervals of 30.5 cm, resulting in a plant density of 10.8 plants per square meter. In crop production, manipulating plant densities is a strategy to optimize yields and cost-efficiency by capitalizing on resource utilization and enhancing the efficacy of lighting, cooling, and heating per unit area. For instance, elevating plant density leads to an early expanded leaf canopy and improved light interception capacity. Moreover, an optimal leaf area is key to increasing crop photosynthesis and water use efficiency (Hui et al., 2001). However, an excessive plant density does not necessarily translate to increased yields, as seen in cannabis cultivation (Vanhove et al., 2012). The Leaf Area Index (LAI) assesses light interception and quantifies the total leaf area relative to the ground area ($\text{m}^2 \text{ m}^{-2}$). Previous research across multiple plant species has consistently reported that an LAI in the range of 3 to 4 intercepts approximately 95% of the incident light, whereas an LAI of 2 captures roughly 80% (Shibles and Weber, 1965; De Visser et al., 2007; Brodrick et al., 2013; Tabarzad et al., 2016). Given that an LAI of 3 to 4 represents an optimal threshold for light capture, and values exceeding four may not confer any significant advantages in terms of light interception (De Visser et al., 2007; Brodrick et al., 2013; Tabarzad et al., 2016) and growth



(Shibles and Weber, 1965), growers are likely to enhance production efficiency by adjusting plant spacing when the LAI surpasses four. Consequently, for a duration exceeding 21 days and high-light conditions like those presented in this study (leading to an LAI of 4.4), plant density could be reduced or branches trimmed while still maintaining a critical light interception of around 95%. Conversely, for crops subjected to lower light levels (Figures 1, 2B), pruning for harvesting cuttings or conducting formative pruning for flower production may have a negative impact on the LAI and plant growth.

Light effects on plant height and internode elongation

Responses of plant height in conjunction with internode extension are key to understanding whether the plant stretches mainly because of growth or to compete for more light, triggering shade avoidance syndrome or SAS (Kump, 2020).

In this study, the combination of light quantity and quality (Tables 1, 2), did not lead to significant changes in plant height across various light treatments (Figures 1, 3). Still, the light level and spectrum affect SAS responses (Kump, 2020), and perhaps they limited the observed differences in plant height in this study. For example, the lower light condition treatments may have contributed to some stem and internode extensions and vice versa. Furthermore, lower red to far-red ratios have increased stem length in several plant species (Demotes-Mainard et al., 2016). For example, in cucumber and lettuce, stem elongation was only significant when far-red light exceeded 9% at a DLI of 11.5 mol m⁻² d⁻¹, while the effects were marginal under 29 mol m⁻² d⁻¹ (Kusuma and Bugbee, 2023). In the present cannabis study, the daily average far-red ranged from 8% to 3% across DLIs of 18 to 52 mol m⁻² d⁻¹. Therefore, significant light spectrum effects would not be expected.

The overall findings and data suggest that the specific light conditions implemented (as outlined in Tables 1 and 2) had only a minor influence on plant height and internode length. These characteristics would be more substantially influenced by plant genetics and developmental factors than light intensity for cannabis.

Light effects on branching and development

Cannabis branches are fundamental in enhancing cutting production and supporting flowers. To assess the impact of light quantity on branch development, the total number of branches was measured and categorized into two groups: primary branches, which develop directly on the main stem, and secondary branches, which sprout from primary branches. The formation, dormancy, and outgrowth of buds that will give place to new stems, leaves, flowers, and branches are regulated by external and internal factors such as genetics, apical dominance, photoperiod, temperature, nutrition, and the amount and quality of the light, among others (Lortie and Aarssen, 1997; Cooke et al., 2012; Leduc et al., 2014). Among those factors, increasing the quantity of light plays a crucial role in reducing SAS responses like internode extension and axillary bud dormancy, while promoting bud outgrowth and overall growth in various species (Gommers et al., 2013; Leduc et al., 2014). Furthermore, the striking similarities in the main stem heights (Figure 3) and differences in primary and secondary branches (Figure 4) suggest that photochemical resources were strongly prioritized and influenced by the main apical meristem. The impact of this effect can be so pronounced that, in certain species or under specific growth conditions, the strong control of branching by the shoot apex may only cease upon removal (Lortie and Aarssen, 1997). Notably, in our study, cannabis branching proliferation was effectively achieved from ~18 to 52 mol m⁻² d⁻¹ (Figure 4) while maintaining a water and nutrient supply upon plant demand.

Light effects on crop water use and efficiency

The effects of increased supplemental lighting on water use and water use efficiency (WUE) were investigated to understand how water is used at a whole plant and production unit (cutting) in a 21-day production cycle. The primary increases in crop WUE observed in the present study are likely attributed to the pronounced benefits of enhanced photosynthesis at both leaf and crop levels, outweighing the potential drawbacks of increased transpiration under increasing lighting conditions. The increment in WUE at the crop level correlated with higher WUE at the leaf level, which is discussed later in this study. The findings of this study are corroborated by prior research conducted on greenhouse-grown sunflowers, wherein continuous measurements of crop photosynthesis and water use were undertaken under greenhouse solar conditions. In this previous study, it was observed that crop WUE throughout the day aligned closely with solar radiation levels, with peak values coinciding with the highest levels of solar radiation and photosynthesis (Hui et al., 2001).

Despite the correlations observed between branches and WUE (Figure 5C), it is imperative to note that the improvement in water efficiency is likely primarily attributable to impacts on photosynthesis and transpiration rather than the specific type of branches.

Crop water use and efficiency in cutting and flower production

Currently, there exists no standardized protocol for growers and researchers to systematically select the most optimal branch for propagating cuttings. Instead, cutting selection predominantly relies on individual preferences. In order to address the various potential scenarios, Figure 5D elucidates the relationship between branch selection and water usage under different light conditions. Based on these findings, on average, a nursery crop operating under conditions similar to those in this study is anticipated to require between 20 and 33 liters of water for every 100 cuttings, assuming that primary branches are mainly used to produce cuttings from secondary branches. Moreover, it's noteworthy that the water demand per cutting is projected to be higher under lower greenhouse lighting levels ($<18 \text{ mol m}^{-2} \text{ d}^{-1}$).

For flower producers, the shoot WUE results imply that to grow the same shoot biomass in 21 days, growers need 0.37 L g^{-1} under $18 \text{ mol m}^{-2} \text{ d}^{-1}$ of light and only 0.24 L g^{-1} under $52 \text{ mol m}^{-2} \text{ d}^{-1}$, a 35% water reduction in evapotranspiration. Similar effects were found in a greenhouse hydroponic lettuce crop that transpired about 0.5 L g^{-1} under $8 \text{ mol m}^{-2} \text{ d}^{-1}$ and 0.27 L g^{-1} under $22 \text{ mol m}^{-2} \text{ d}^{-1}$, a 46% water reduction in transpiration (Both, 2002).

Influence of greenhouse supplemental lighting on photosynthesis, stomatal conductance, transpiration in leaves, and their interconnection with Water Use Efficiency

Anatomical and physiological leaf characteristics change based on the prevailing light environment (Chabot and Chabot, 1977;

Feng et al., 2019). Leaf photosynthesis (A), stomatal conductance to water vapor (g_{sw}), and transpiration (E) are integral to understanding growth and water usage and efficiency at the leaf level while helping elucidate the broader responses at the crop level.

Photosynthetic responses and significance

This study's results align with similar findings reported for cannabis cultivation under sole-source LED lighting, which exhibited stronger photosynthetic responses and DLI variations (Rodriguez-Morrison et al., 2021). Furthermore, higher leaf maximum-A capacities, as seen in this study and Rodriguez-Morrison et al. (2021), are expected under light augmentation due to increments in leaf weight per area (Penning de Vries et al., 1989). Thicker or denser leaves can produce more RuBisCO (Penning de Vries et al., 1989) and chlorophyll in the same area (Table 3), potentially increasing photosynthesis (Emerson, 1930). On the other hand, higher respiration rates, driven by the elevated biomass and metabolite levels per area (Table 3), may provide an explanation for the lower net photosynthetic rate observed in thicker leaves in cannabis research. In summary, our leaf photosynthetic rates results support the remarkable crop yield increments even at maximum greenhouse radiances of $\sim 2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Supplementary Figure S3B).

Stomatal and transpiration responses and regulation

In this study, the observed rise in E with increased light intensity was attributed to the increased stomatal conductance (g_{sw}). Typically, a higher g_{sw} is linked to either a reduction in leaf internal CO_2 concentration (C_i) or an enhancement in photosynthesis (A) through light supplementation (McAusland et al., 2016; Driesen et al., 2020). However, despite the linear increase in g_{sw} with light intensity, the responses of A and C_i did not parallel the stomatal conductance trend observed in our study (Figures 6C, A; Supplementary S4A).

The linear response in g_{sw} compared to the plateau response of A could be explained by additional mechanisms affected by light other than C_i reduction or photosynthesis enhancement. For instance, blue and red radiation is mentioned as direct influencers on guard-cell water status (Driesen et al., 2020), while shortwave radiation, encompassing most or all solar wavelengths, demonstrated an impact on leaf water status (Lawson et al., 2010; Pieruschka et al., 2010). Pieruschka et al. (2010) proposed a regulatory mechanism where the balance between radiation-driven water vapor production and transpiration rate governs stomatal conductance. Moreover, their observations found no specific wavelength effects, establishing only a linear correlation between stomatal conductance and absorbed radiation energy. Another factor affected by radiation is leaf temperature, a pivotal influence in stomatal conductance. Feller (2007) and Urban et al. (2017) demonstrated significant increments in stomatal aperture across temperatures ranging from $\sim 20^\circ\text{C}$ to over 40°C . Moreover, these increments persisted despite reductions in A and water potential and an increase in C_i (Urban et al., 2017). In the present cannabis study, results showed marginal leaf temperature differences (26.1 ± 0.17 to $26.9 \pm 0.35^\circ\text{C}$) when elevating the PPFD from 150 to $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$

(Supplementary Figure S4B). In conclusion, light can affect photosynthesis and transpiration differently and may act on the stomatal aperture via various mechanisms.

High stomatal conductance and water loss at low radiation levels

Our study found considerably higher E and g_{sw} values per photon of light at lower PFDs than at higher light intensities, as previously shown in the result section. Moreover, leaf transpiration at $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ constituted a noteworthy 57% of the total water loss found under $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 6B). This can be explained by reports across diverse species (Hui et al., 2001; de Dios et al., 2015; Dayer and Gambetta, 2021; Dayer et al., 2021) that suggest rapid and excessive increments in stomatal opening at low light or in the absence of light, which can increase water losses. Furthermore, data from different species, spanning ferns, gymnosperms, and angiosperms, also revealed that at very low light levels ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance constituted between ~16% to 64% of the aperture at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Deans et al., 2019). These results also align with increments above typical nighttime transpiration rates of 5% to 15% (Caird et al., 2007). Moreover, the observed rapid and predawn stomatal aperture increments have been associated with specific wavelengths (Lawson et al., 2010) and circadian regulation (de Dios et al., 2015; Dayer et al., 2021), while stomatal conductance did not correlate with photosynthesis at low light (Deans et al., 2019).

The benefits of enhancing supplemental lighting on leaf transpiration

In addition to increasing photosynthesis, enhancing supplemental lighting strongly reduced the water use per photon of light. These findings align with the broader context of light effects on stomatal conductance observed across various species (Deans et al., 2019), as previously discussed. Additionally, similar trends in crop transpiration have been documented in beans and cotton subjected to maximum PFDs of 500 and $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$. Notably, nocturnal water losses constituted 23% and 12% of daytime requirements under lower and higher PFDs, respectively (de Dios et al., 2015). These results suggest that optimizing lighting conditions can significantly mitigate the impact of both night-time and day-time water losses.

Stomatal slow response implications

Stomatal conductance and transpiration exhibited linear increases by increasing PFD, as detailed earlier. However, the absence of statistical evidence for DLI effects between 18 and 52 $\text{mol m}^{-2} \text{d}^{-1}$ was unexpected. It is plausible that the lack of statistical differences is linked to the substantial variability in transpiration and stomatal conductance due to the interaction of our procedure's sampling speed and stomatal acclimation periods (Lawson and Blatt, 2014; McAusland et al., 2016). Nevertheless, the information and results reviewed emphasize the complex interplay between direct and indirect light effects on guard cells, transpiration, and physiological dynamics, contributing to notable increases in gas exchange within the context of our research. Additionally, it is noteworthy that cannabis leaf temperatures remained similar to or

below an air temperature of 27°C . This observation suggests that transpiration rates were sufficient to maintain optimal metabolic functions without the risk of overheating (Feller, 2007; Nelson and Bugbee, 2015). Our investigation revealed no physiological constraints on stomatal conductance and transpiration, even under the more demanding conditions of $\sim 2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ of actual greenhouse LED lighting and solar radiation (unshown data). This and previous research stress the robustness of optimal cannabis growth under high-light environments.

The impacts of short and long-term lighting exposure on leaf water use efficiency

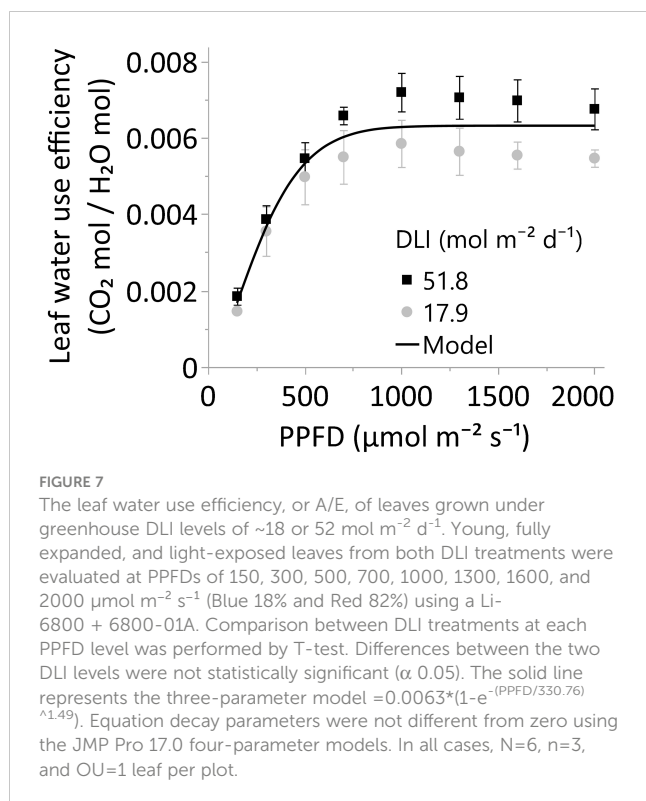
The leaf WUE of upper cannabis leaves remarkably increased as a consequence of the curvilinear and linear responses observed for photosynthesis and transpiration (Figures 6A, B, and 7). Consistent with our findings, Deans et al. (2019) reported a substantial increase in intrinsic WUE (A/g_{sw}) under elevated lighting conditions across species, which was driven by a more pronounced increase in photosynthesis than stomatal conductance. In our study, there was weaker evidence indicating that the leaf WUE of the 52-DLI treatment might surpass that of the 18-DLI WUE under light levels of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ or higher (p-values >0.05 to 0.09); however, at $\alpha 0.05$, the statistical support was not robust enough to substantiate our hypothesis that leaves developed under the 52-DLI treatment exhibit higher WUE than leaves under the 18-DLI treatment.

In conclusion, the observed increase in crop WUE with higher lighting, consistent with other species, lighting conditions (Hui et al., 2001; Both, 2002; de Dios et al., 2015), and supported by increments in leaf WUE across various cannabis cultivars (unpublished data) and fifteen different species (Deans et al., 2019), suggests a potential for elevated WUE and yield in unstressed crops exposed to increased lighting conditions.

Spectral differences between treatments

In the current study, variations in light intensity among treatments were achieved through incremental adjustments in blue and red LED lighting, while similar solar radiation was maintained across treatments (Table 1). Consequently, this led to an augmentation in the proportion of blue and red wavelengths relative to other spectrums within the greenhouse solar spectrum. It is noteworthy that differences in the spectral composition of electrical lighting can significantly impact plant physiology, morphology, and growth in growth chambers (Spalholz et al., 2020) and under conditions of low sunlight, $3\text{--}5 \text{mol m}^{-2} \text{d}^{-1}$ (Hernández and Kubota, 2014; Collado and Hernández, 2022).

While prior research has demonstrated substantial effects of supplemental light spectrum variations under very low sunlight conditions, the impact diminished under higher solar DLIs, $\sim 12\text{--}16 \text{mol m}^{-2} \text{d}^{-1}$, in tomatoes and cucumbers (Hernández and Kubota, 2014; Kaiser et al., 2019). Furthermore, the influence of changing light quantity from 11.5 to $29 \text{mol m}^{-2} \text{d}^{-1}$ was more pronounced on the biomass of seven primary agricultural crops in growth chambers than alterations in light spectrum (blue, green, and red wavelength



ranges: 10.8-27.5%, 1.7-48.0%, and 24.5-86.3%, respectively; a phytochrome photo-equilibrium (PPE) range: 0.83-0.89; Snowden et al., 2016). Similarly, the impact of increasing DLI from 8.7 to 19.7 mol m⁻² d⁻¹ outweighed the effects of modifying the blue and red light ratios in cucumbers (Hernández and Kubota, 2014). In our cannabis research, light exposure ranged from 17.9 to 51.8 mol m⁻² d⁻¹, while spectrum and PPE ranges (Table 2) were similar to those discussed.

In summary, this experiment does not provide evidence to suggest that spectral differences between treatments significantly influenced plant responses under greenhouse supplemental lighting conditions. Instead, the variations in plant responses can be primarily attributed to the differences in total Photosynthetic Photon Flux Density (ΣPPFD). Overall, these findings imply that the effects of light spectrum variations may be relatively smaller, especially in the presence of a complete light spectrum, such as solar radiation.

Conclusions

Enhancing supplemental lighting promoted increased photosynthesis and played a pivotal role in shaping the water use dynamics in cannabis leaves and crops. Most importantly, it could potentially enhance the WUE of most crops while promoting growth. Our findings emphasize the capacity of lighting management to optimize water use efficiency while presenting valuable implications for both research and practical applications in agriculture.

For instance, stakeholders in cannabis nurseries, flower production, and research settings can substantially improve plant growth, yield, and water use efficiency by incorporating supplemental lighting. Specifically, our study indicates a linear

growth response within the range of ~18 to 52 mol m⁻² d⁻¹, suggesting that DLIs exceeding 52 mol m⁻² d⁻¹ could further enhance cuttings and plant growth and surpass existing literature expectations. It is crucial to highlight that these results and expectations are based on meticulous control of water, nutrients, and other growth factors and genetics. In this study, *Cannabis sativa* cv Suver Haze was employed and represents an average-performing cultivar (Suchoff et al., 2021), suggesting that genetic variability may increase or decrease the number of branches and overall plant growth per mole of light.

This study demonstrated notable increases in leaf and crop WUE with elevated light levels while enhancing CO₂ assimilation. Historically, improving WUE without compromising production has been challenging, particularly considering the dependence of photosynthesis on stomata (Lawson and Blatt, 2014; Xing et al., 2022). Furthermore, studies on CO₂ enrichment (Hui et al., 2001) and elevated atmospheric CO₂ (Guerrieri et al., 2019) have also reported improvements in WUE; in alignment with the findings on light supplementation, the enhanced photosynthesis was found to be the main contributing factor. Ultimately, there is evidence showing that the combination of light and CO₂ enrichment can lead to even greater enhancements in water use efficiencies (Hui et al., 2001).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

CC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. SH: Investigation, Methodology, Writing – review & editing. RH: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was funded by the Foundation for Food and Agricultural Research (FFAR) grant number [NexGenHemp000000016]. Gyengsang National University Fund for Professors on Sabbatical Leave, 2020.

Acknowledgments

The authors would like to thank the company CURRENT Horticultural Lighting (Greenville, SC, US) for providing partial funding and LED fixtures. The company was not involved in the study design, collection, analysis, interpretation of data, the writing of

this article, or the decision to submit it for publication. The Authors would like to thank Ryan Patterson from Ryes Greenhouses LLC (Broadway, NC) for providing initial rooted-plug material. The authors would like to thank Bartlett-Golden A., Huber B., Hwang S.J., McLamb S., Shi X., Stanek C., Thompson S.P., and Watson M. for their support during the study. ChatGPT 3.5 was used for language editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Both, A. J. (2002) *Ten years of hydroponic lettuce research*. Available online at: www.researchgate.net/publication/266453402_ten_years_of_hydroponic_lettuce_research (Accessed January 7, 2024).
- Boyer, J. S. (1970). Differing sensitivity of photosynthesis to low leaf water potentials in corn and soybean. *Plant Physiol.* 46, 236–239. doi: 10.1104/pp.46.2.236
- Brodrick, R., Bange, M. P., Milroy, S. P., and Hammer, G. L. (2013). Physiological determinants of high yielding ultra-narrow row cotton: Canopy development and radiation use efficiency. *Field Crops Res.* 148, 86–94. doi: 10.1016/j.fcr.2012.05.008
- Caird, M. A., Richards, J. H., and Donovan, L. A. (2007). Nighttime stomatal conductance and transpiration in C3 and C4 plants. *Plant Physiol.* 143, 4–10. doi: 10.1104/pp.106.092940
- Chabot, B. F., and Chabot, J. F. (1977). Effects of light and temperature on leaf anatomy and photosynthesis in *Fragaria vesca*. *Oecologia* 26, 363–377. doi: 10.1007/BF00345535
- Chandra, S., Lata, H., Khan, I. A., and Elsohly, M. A. (2008). Photosynthetic response of *Cannabis sativa* L. @ to variations in photosynthetic photon flux densities, temperature and CO2 conditions. *Physiol. Mol. Biol. Plants* 14, 299–306. doi: 10.1007/s12298-008-0027-x
- Chandra, S., Lata, H., Khan, I. A., and Elsohly, M. A. (2011). Temperature response of photosynthesis in different drug and fiber varieties of *Cannabis sativa* L. *Physiol. Mol. Biol. Plants* 17, 297–303. doi: 10.1007/s12298-011-0068-4
- Collado, C. E., and Hernández, R. (2022). Effects of light intensity, spectral composition, and paclobutrazol on the morphology, physiology, and growth of petunia, geranium, pansy, and dianthus ornamental transplants. *J. Plant Growth Regul.* 41, 461–478. doi: 10.1007/s00344-021-10306-5
- Conway, J. (2023) *Number of cannabis cultivation licenses in the United States in 2022, by state* (Statista). Available online at: <https://www.statista.com/statistics/1108194/cannabis-cultivation-licenses-by-state-us/> (Accessed January 7, 2024).
- Cooke, J. E. K., Eriksson, M. E., and Junttila, O. (2012). Dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. *Plant Cell Environ.* 35, 1707–1728. doi: 10.1111/j.1365-3040.2012.02552.x
- David Potter, J. P. (2009) (London, UK: King's College). Available online at: <https://archive.org/details/cannabissativaasphytopharmaceutical> (Accessed January 7, 2024).
- Dayer, S., and Gambetta, G. A. (2021). Nighttime transpiration: does it contribute to water stress in grape?: Sourced from the research article “Nighttime transpiration represents a negligible part of water loss and does not increase the risk of water stress in grapevine”. *Plant Cell Environ.* doi: 10.1111/pce.13923
- Dayer, S., Herrera, J. C., Dai, Z., Burrell, R., Lamarque, L. J., Delzon, S., et al. (2021). Nighttime transpiration represents a negligible part of water loss and does not increase the risk of water stress in grapevine. *Plant Cell Environ.* 44, 387–398. doi: 10.1111/pce.13923
- Deans, R. M., Brodribb, T. J., Busch, F. A., and Farquhar, G. D. (2019). Plant water-use strategy mediates stomatal effects on the light induction of photosynthesis. *New Phytol.* 222, 382–395. doi: 10.1111/nph.15572
- DeCarcner, G. A., Kagia, J., Morrissey, K., McCann, M., Tomares, N., Alvarado, I., et al. (2021) *The global cannabis report growth & Trends through 2025* (New Frontier Data). Available online at: <https://newfrontierdata.com/product/the-global-cannabis-report-2021/> (Accessed January 7, 2024).
- de Dios, V. R., Roy, J., Ferrio, J. P., Alday, J. G., Landais, D., Milcu, A., et al. (2015). Processes driving nocturnal transpiration and implications for estimating land evapotranspiration. *Sci. Rep.* 5, 10975. doi: 10.1038/srep10975
- Demotes-Mainard, S., Péron, T., Corot, A., Bertheloot, J., Le Gourrierec, J., Pelleschi-Travier, S., et al. (2016). Plant responses to red and far-red lights, applications in horticulture. *Environ. Exp. Bot.* 121, 4–21. doi: 10.1016/j.envexpbot.2015.05.010
- De Visser, P., van der Heijden, G., and Heuvelink, E. (2007) *3D Modelling of growth and ornamental quality of chrysanthemum at different plant densities*. Available online at: https://www.researchgate.net/publication/40101200_3D_Modelling_of_growth_and_ornamental_quality_of_chrysanthemum_at_different_plant_densities (Accessed January 7, 2024).
- Dieter, C. A., Maupin, M. A., Caldwell, R. R., Harris, M. A., Ivahnenko, T. I., Lovelace, J. K., et al. (2018). Estimated use of water in the United States in 2015. *U.S. Geological Survey* 6–8. doi: 10.3133/cir1441
- Dorais, M., Mitchell, C. A., and Kubota, C. (2017). “Lighting greenhouse fruiting vegetables,” in *Light management in controlled environments*. Eds. R. Lopez and E. S. Runkle (Meister Media Worldwide, Willoughby, OH: Meister Media: Willoughby), 159–169, ISBN: .
- Driesen, E., Van Den Ende, W., De Proft, M., and Saey, W. (2020). Influence of environmental factors light, CO2, temperature, and relative humidity on stomatal opening and development: A review. *Agronomy* 10, 1975. doi: 10.3390/agronomy10121975
- Eddy, R., and Hahn, D. T. (2010). Optimizing greenhouse corn production: what is the best lighting and plant density? *Purdue Methods Corn Growth* 13.
- Emerson, R. (1930). The chlorophyll factor in photosynthesis. *Am. Nat.* 64, 252–260. doi: 10.1086/280314
- Feller, U. (2007). Stomatal opening at elevated temperature: an underestimated regulatory mechanism? *Gen. Appl. Plant Physiol.* 32, 19–31. doi: 10.7892/boris.53995
- Feng, L., Raza, M. A., Li, Z., Chen, Y., Khalid, M. H. B., Du, J., et al. (2019). The influence of light intensity and leaf movement on photosynthesis characteristics and carbon balance of soybean. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.01952
- GAO (2014) *Freshwater: supply concerns continue, and uncertainties complicate planning* (US Government Accountability Office). Available online at: <https://www.gao.gov/assets/gao-14-430.pdf> (Accessed January 7, 2024).
- Gommers, C. M. M., Visser, E. J. W., Onge, K. R. S., Voeselek, L. A. C. J., and Pierik, R. (2013). Shade tolerance: when growing tall is not an option. *Trends Plant Sci.* 18, 65–71. doi: 10.1016/j.tplants.2012.09.008
- Guerrieri, R., Belmecheri, S., Ollinger, S. V., Asbjornsen, H., Jennings, K., Xiao, J., et al. (2019). Disentangling the role of photosynthesis and stomatal conductance on rising forest water-use efficiency. *Proc. Natl. Acad. Sci. U.S.A.* 116, 16909–16914. doi: 10.1073/pnas.1905912116
- Hatfield, J. L., and Dold, C. (2019). Water-use efficiency: advances and challenges in a changing climate. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.00103
- Hernández, R., and Kubota, C. (2014). Growth and morphological response of cucumber seedlings to supplemental red and blue photon flux ratios under varied solar daily light integrals. *Scientia Hortic.* 173, 92–99. doi: 10.1016/j.scientia.2014.04.035
- Hubick, K., and Farquhar, G. (1989). Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. *Plant Cell Environ.* 12, 795–804. doi: 10.1111/j.1365-3040.1989.tb01641.x
- Hui, D., Luo, Y., Cheng, W., Coleman, J. S., Johnson, D. W., and Sims, D. A. (2001). Canopy radiation- and water-use efficiencies as affected by elevated [CO2]: Canopy Radiation- And Water-Use Efficiencies. *Global Change Biol.* 7, 75–91. doi: 10.1046/j.1365-2486.2001.00391.x
- Idris, A., Linatoc, A. C., and Bakar, M. F. B. A. (2019). Effect of light intensity on the photosynthesis and stomatal density of selected plant species of gunung ledang, johor. *Malays. Appl. Biol.* 48, 133–140.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2024.1371702/full#supplementary-material>

- Jackson, P., Basnayake, J., Inman-Bamber, G., Lakshmanan, P., Natarajan, S., and Stokes, C. (2016). Genetic variation in transpiration efficiency and relationships between whole plant and leaf gas exchange measurements in *Saccharum* spp. and related germplasm. *EXBOTJ* 67, 861–871. doi: 10.1093/jxb/erv505
- Kaiser, E., Ouzounis, T., Giday, H., Schipper, R., Heuvelink, E., and Marcelis, L. F. M. (2019). Adding blue to red supplemental light increases biomass and yield of greenhouse-grown tomatoes, but only to an optimum. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.02002
- Kump, B. (2020). The role of far-red light (FR) in photomorphogenesis and its use in greenhouse plant production. *Acta Agric. Slovenica* 116, 93–105. doi: 10.14720/aas.2020.116.1.1652
- Kusuma, P., and Bugbee, B. (2023). On the contrasting morphological response to far-red at high and low photon fluxes. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1185622
- Lalge, A., Cerny, P., Trojan, V., and Vyhnanek, T. (2017). The effects of red, blue and white light on the growth and development of *Cannabis sativa* L. *MendelNet* 24, 646–651.
- Lawson, T., and Blatt, M. R. (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiol.* 164, 1556–1570. doi: 10.1104/pp.114.237107
- Lawson, T., Von Caemmerer, S., and Baroli, I. (2010). “Photosynthesis and stomatal behaviour,” in *Progress in botany*, vol. 72. Eds. U. E. Lüttge, W. Beyschlag, B. Büdel and D. Francis (Springer Berlin Heidelberg, Berlin, Heidelberg), 265–304. doi: 10.1007/978-3-642-13145-5_11
- Lazic, S. E., Clarke-Williams, C. J., and Munafò, M. R. (2018). What exactly is ‘N’ in cell culture and animal experiments? *PLoS Biol.* 16, e2005282. doi: 10.1371/journal.pbio.2005282
- Leduc, N., Roman, H., Barbier, F., Péron, T., Huché-Thélier, L., Lothier, J., et al. (2014). Light signaling in bud outgrowth and branching in plants. *Plants* 3, 223–250. doi: 10.3390/plants3020223
- Lortie, C. J., and Aarssen, L. W. (1997). Apical dominance as an adaptation in *Verbascum thapsus*: effects of water and nutrients on branching. *Int. J. Plant Sci.* 158, 461–464. doi: 10.1086/297456
- McAusland, L., Viallet-Chabrand, S., Davey, P., Baker, N. R., Brendel, O., and Lawson, T. (2016). Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. *New Phytol.* 211, 1209–1220. doi: 10.1111/nph.14000
- Moher, M., Llewellyn, D., Jones, M., and Zheng, Y. (2021). High light intensities can be used to grow healthy and robust cannabis plants during the vegetative stage of indoor production. 1–12. doi: 10.20944/preprints202104.0417.v1
- Nelson, J. A., and Bugbee, B. (2015). Analysis of environmental effects on leaf temperature under sunlight, high pressure sodium and light emitting diodes. *PLoS One* 10, e0138930. doi: 10.1371/journal.pone.0138930
- Penning de Vries, F. W. T., Jansen, D. M., ten Berge, H. F. M., and Bakema, A. (1989) *Simulation of ecophysiological processes of growth in several annual crops* (Pudoc: Wageningen). Available online at: <https://edepot.wur.nl/108856> (Accessed January 7, 2024).
- Pieruschka, R., Huber, G., and Berry, J. A. (2010). Control of transpiration by radiation. *Proc. Natl. Acad. Sci. U.S.A.* 107, 13372–13377. doi: 10.1073/pnas.0913177107
- Pokorny, J. (2019). “Evapotranspiration,” in *Encyclopedia of ecology* (Elsevier), 292–303. doi: 10.1016/B978-0-12-409548-9.11182-0
- Rodriguez-Morrison, V., Llewellyn, D., and Zheng, Y. (2021). Cannabis yield, potency, and leaf photosynthesis respond differently to increasing light levels in an indoor environment. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.646020
- Rosati, A., and Dejong, T. M. (2003). Estimating photosynthetic radiation use efficiency using incident light and photosynthesis of individual leaves. *Annals of Botany* 91, 7, 869–877. doi: 10.1093/aob/mcg094
- Sager, J. C., Smith, W. O., Edwards, J. L., and Cyr, K. L. (1988). Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. *Trans. ASAE* 31, 1882–1889. doi: 10.13031/2013.30952
- Shibles, R. M., and Weber, C. R. (1965). Leaf area, solar radiation interception and dry matter production by soybeans. *Crop Sci.* 5, 575–577. doi: 10.2135/cropsci1965.0011183X000500060027x
- Snowden, M. C., Cope, K. R., and Bugbee, B. (2016). Sensitivity of seven diverse species to blue and green light: interactions with photon flux. *PLoS One* 11, e0163121. doi: 10.1371/journal.pone.0163121
- Spalholz, H., Perkins-Veazie, P., and Hernández, R. (2020). Impact of sun-simulated white light and varied blue:red spectrums on the growth, morphology, development, and phytochemical content of green- and red-leaf lettuce at different growth stages. *Scientia Hort.* 264, 109195. doi: 10.1016/j.scienta.2020.109195
- Suchoff, D., Bloomquist, M., Davis, J., Henriquez Inoa, S., and Learn, K. (2021) *NC state floral hemp variety trial* (North Carolina Extension). Available online at: <https://hemp.ces.ncsu.edu/wp-content/uploads/2021/11/2021-NC-State-Variety-Trial-Report.pdf?fw=0> (Accessed January 7, 2024).
- Tabarza, A., Ghaemi, A. A., and Zand-Parsa, S. (2016). Extinction coefficients and radiation use efficiency of barley under different irrigation regimes and sowing dates. *Agric. Water Manage.* 178, 126–136. doi: 10.1016/j.agwat.2016.09.020
- Urban, J., Ingwers, M. W., McGuire, M. A., and Teskey, R. O. (2017). Increase in leaf temperature opens stomata and decouples net photosynthesis from stomatal conductance in *Pinus taeda* and *Populus deltoides* x *nigra*. *J. Exp. Bot.* 68, 1757–1767. doi: 10.1093/jxb/erx052
- Vanhove, W., Surmont, T., Van Damme, P., and De Ruyver, B. (2012). Yield and turnover of illicit indoor cannabis (*Cannabis* spp.) plantations in Belgium. *Forensic Sci. Int.* 220, 265–270. doi: 10.1016/j.forsciint.2012.03.013
- van Iersel, M. W., and Gianino, D. (2017). An adaptive control approach for light-emitting diode lights can reduce the energy costs of supplemental lighting in greenhouses. *HortScience* 52, 72–77. doi: 10.21273/HORTSCI11385-16
- Vezdos, T., and Martinez, R. (2021) *2021 state of the cannabis cultivation industry report* (Cannabis Business Times). Available online at: <https://www.cannabisbusinesstimes.com/article/special-report-2021-state-of-the-cannabis-cultivation-industry-report/> (Accessed January 7, 2024).
- Weaver, G., and Van Iersel, M. W. (2020). Longer photoperiods with adaptive lighting control can improve growth of greenhouse-grown ‘Little gem’ Lettuce (*Lactuca sativa*). *HortScience* 55, 573–580. doi: 10.21273/HORTSCI14721-19
- Whitehead, D. (1998). Regulation of stomatal conductance and transpiration in forest canopies. *Tree Physiol.* 18, 633–644. doi: 10.1093/treephys/18.8-9.633
- Xing, D., Mao, R., Li, Z., Wu, Y., Qin, X., and Fu, W. (2022). Leaf intracellular water transport rate based on physiological impedance: A possible role of leaf internal retained water in photosynthesis and growth of tomatoes. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.845628
- Zhao, D., Raja Reddy, K., Kakani, V. G., Read, J. J., and Carter, G. A. (2003). Corn (*Zea mays* L.) growth, leaf pigment concentration, photosynthesis and leaf hyperspectral reflectance properties as affected by nitrogen supply. *Plant Soil* 257, 205–218. doi: 10.1023/A:1026233732507
- Zhen, S., and Bugbee, B. (2020). Steady-state stomatal responses of C₃ AND C₄ species to blue light fraction: Interactions with CO₂ concentration. *Plant Cell Environ.* 43, 3020–3032. doi: 10.1111/pce.13888
- Zhen, S., Van Iersel, M., and Bugbee, B. (2021). Why far-red photons should be included in the definition of photosynthetic photons and the measurement of horticultural fixture efficacy. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.693445