REGULATION OF THE LIGHT SPECTRUM TO ELICIT SPECIFIC GROWTH AND QUALITY ATTRIBUTES OF LETTUCE PRODUCED INDOORS

By

Nathan Henry Kelly

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Horticulture – Doctor of Philosophy

2023

ABSTRACT

Indoor-vertical farms enable the production of high-value crops like lettuce (*Lactuca sativa*) year-round in a tightly controlled environment that reduces water and pesticide use. Indoor farms require less land to grow more food compared with field production and can be placed in or near large cities. However, they are entirely reliant on electricity to control temperature and provide electric lighting. Light-emitting diode (LEDs) fixtures are primarily used for their high efficacy and delivery of a specific light spectrum, photon flux density (PFD), and photoperiod. In addition to PFD, the light spectrum can greatly affect biomass accumulation, morphology, and quality traits such as leaf coloration and nutritional content. To further investigate how LEDs can be used to manipulate the light spectrum to regulate plant growth, morphology, and quality, we designed experiments in a temperature-controlled growth room equipped with hydroponic growing racks and light-waveband tunable LED fixtures. During all experiments, lettuce seeds were sown and grown in rockwool cubes under broad-spectrum light until they were transplanted into the hydroponic system after the seedling stage. First, to compare the effects of ultraviolet A (UVA, 315-399 nm) to blue (400-499 nm) light, we grew red-leaf lettuce 'Rouxai' under red (600-699 nm) plus white light with end-of-production supplemental UVA or blue light. UVA and blue light were similarly effective at increasing lettuce leaf coloration and total phenolic and anthocyanin concentrations, while neither affected fresh mass. Next, we investigated the persistency of periodic supplemental UVA or blue light on quality attributes and biomass accumulation by enriching the light spectrum with either waveband during the beginning, middle, or last phase of production as well as the entire production cycle. End-of-production UVA or blue light were as effective at improving lettuce quality as continuous enrichment but the continuous blue light treatment inhibited biomass accumulation.

Next, to more broadly quantify the effects of enriching a white spectrum with various wavebands, we grew lettuce ‘Rouxai’ and ‘Rex’ under two different PFDs supplemented with equal proportions (~30%) of blue, green (500-599 nm), red, far-red (700-799 nm), or white light. Supplemental far-red light increased leaf expansion, while additional red and warm-white light were the most effective at increasing biomass accumulation. Supplemental blue light was the only waveband that increased total anthocyanin concentrations and leaf coloration. Finally, increasing the PFD increased biomass accumulation and total phenolic concentration and responses were generally similar at the low and high PFDs tested. In the last study, we investigated how the efficacy of far-red light depends on other light wavebands, and specifically if the substitution of red light with green light would influence the efficacy of far-red light on increasing plant growth. Lettuce ‘Rouxai’ and ‘Rex’ leaf area continually increased as the far-red light percentage increased, regardless of the green- and red-light percentages. The increase in leaf expansion did not always lead to an increase in fresh mass and was greatest when far-red light represented approximately one-eighth to one-fourth of the total PFD. At higher or lower far-red PFD fractions, fresh mass was similarly lower. Collectively, these studies show that the light spectrum has vast and impactful effects on lettuce growth, morphology, and quality. These studies also highlight the utility of including far-red light and end-of-production blue light in the vertical farming of lettuce. Finally, while changing the light spectrum can elicit certain plant responses, it also influences fixture efficacy and thus, electricity consumption.

ACKNOWLEDGMENTS

I would like to express my gratitude to Dr. Erik Runkle, my academic advisor, for his continued guidance and support throughout my academic career. I would also like to thank my advisory committee members Drs. Ryan Warner, Chieri Kubota, and Leslie Bourquin for assistance and for providing guidance and support to me throughout my experiments and manuscript preparations. I am especially thankful for Mckenna Merkel's assistance in preparing experiments and data collection. I am also grateful to Dr. Ryan Warner for allowing me to use his laboratory for multiple experiments as his laboratory manager, Sue Hammar for assisting me. Furthermore, I am thankful for the help that Dr. Viktorija Vaštakaitė-Kairienė provided me when designing experimental protocols for biochemical analysis and for assisting me during experiments. I am also thankful to our laboratory research technicians Nate Durussel and Annika Kohler and Ph.D. students Eric Stallknecht and Jiyong Shin for their experimental assistance. I am also thankful for the support that all faculty, staff, and students in the Department of Horticulture provided me. I appreciate funding from the Specialty Crops Research Initiative (grant no. 2019-51181-30017) from the USDA National Institute of Food and Agriculture, donations provided by horticultural suppliers, and OSRAM for designing and donating the lighting fixtures used in this research. Last but not least, I would like to thank my friends and family, especially my wife Becca Kelly, parents Byon and Donna Kelly, and siblings Tyler and Emily Kelly for their love and support.

TABLE OF CONTENTS

SECTION I
END-OF-PRODUCTION UVA AND BLUE LIGHT SIMILARLY INCREASE LETTUCE COLORATION AND PHYTOCHEMICAL CONCENTRATIONS

End-of-production UVA and blue light similarly increase lettuce coloration and phytochemical concentrations

This chapter has been published as an open access article and is reprinted here with very minor changes. Kelly N., Runkle E.S. 2023. End-of-production ultraviolet a and blue light similarly increase lettuce coloration and phytochemical concentrations. HortScience 58:525–531. doi: https://doi.org/10.21273/HORTSCI17108-23

Nathan H. Kelly and Erik S. Runkle*

Controlled-Environment Lighting Laboratory, Department of Horticulture, 1066 Bogue Street, Michigan State University, East Lansing, MI 48824

*Corresponding author. Tel.: +1 517 353 0350; fax: +1 517 353 0890. E-mail address: runkleer@msu.edu (E.S. Runkle)

Abstract

Anthocyanins are a group of human-health-promoting phenolic compounds that influence the pigmentation of red-leaf lettuce (*Lactuca sativa*). Ultraviolet (UV) A (UVA; 315 – 399 nm) and blue (B; 400 – 499 nm) light can increase the concentrations of phenolic compounds but also suppress cellular expansion, which can limit harvestable biomass accumulation. It is not known whether UVA or B light is more effective at increasing phenolic compound concentrations when they are each applied at the same photon flux density. Our objective was to evaluate the efficacy of UVA and B light, when added during the end of production (EOP), at promoting phenolic compound synthesis and red-leaf coloration without limiting biomass accumulation. We grew red-leaf lettuce 'Rouxai' in a controlled indoor environment at an air temperature of 22 °C under warm-white and red light-emitting diodes (LEDs). On day 24, 30 or 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from UVA, B, UVA plus B, or red plus green LEDs was added during the last six days of the 30-day production period. UVA and B light, alone or combined, similarly increased leaf redness (by up to 72%), total phenolic concentration (by up to 92%), total anthocyanin concentration (by up to 2.7-fold), relative chlorophyll concentration (by up to 20%), and did not inhibit growth, compared to lettuce grown without EOP supplemental lighting. Considering B light was as effective as UVA light at increasing leaf color and phytonutrient density, and that B LEDs are more electrically effective, economical, and durable, an enriched blue-light spectrum at the EOP is a comparatively sustainable method to increase crop quality without suppressing biomass accumulation.

Introduction

Indoor, vertical farming of leafy green vegetables continues to expand because of its efficient use of land, water, and fertilizer, and no use of pesticides (Kozai and Niu 2016).

Furthermore, the ability to automate most or all cultivation practices and grow near or in large cities can decrease labor and transportation costs compared with field production. Although commercial growers control and optimize environmental factors in indoor farms, such as temperature, carbon dioxide (CO_2) concentration, water vapor-pressure deficit, and light, they are entirely reliant on electricity. Therefore, in the absence of sunlight, electric lighting is one of the most expensive capital and operational expenses, and the least sustainable characteristic, of an indoor farm (Kozai and Niu 2016). Light-emitting diodes (LEDs) are commonplace in indoor farms because of their increasingly high efficacies and longer lifetimes (Kusuma et al., 2020), which have made LEDs more effective than conventional lighting fixtures, such as high-pressure sodium lamps (Radetsky 2018). Additionally, LEDs provide the advantage of precisely controlling the light spectrum for specific plant applications.

Lettuce (*Lactuca sativa*) is a compact leafy green with a short production cycle, which in combination with its high consumer demand, makes it the most-grown species in indoor farms. It is one of the most widely consumed vegetables in the United States (U.S. Department of Agriculture, 2018) because of its versatile culinary use and nutritional value (Kim et al., 2016). Lettuce is also a model crop in horticultural lighting research because of its responsiveness to the light spectrum and flux density. For instance, manipulating the light environment in controlled environments influences lettuce biomass accumulation, plant and leaf morphology, and concentrations of bioactive compounds (Kitazaki et al., 2018; Shin et al., 2014; Son et al., 2017; Vaštakaitė-Kairienė et al., 2021).

Supplementing the light spectrum with short-wavelength light, such as ultraviolet A (UVA; 315 – 399 nm) and blue (B; 400 – 499 nm), can affect plant traits such as extension growth, nutritional quality, and leaf coloration. At least a moderate intensity of B light typically

suppresses extension growth, leading to a smaller leaf area than plants grown with little or no B light (Briggs and Huala 1999; Cosgrove 1981; Son and Oh 2013). For example, 23 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light was enough to suppress fresh weight compared to the 100% R light control, and higher intensities further suppressed growth (Son and Oh 2013). The smaller leaf area decreases the surface area and thus light interception, which can decrease biomass accumulation. For example, lettuce 'Rouxai' grown under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white light supplemented with 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B (peak = 449 nm) had less shoot biomass than plants grown under other supplemental wavelengths, such as an additional 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of green (G; 500 – 599 nm; peak = 526 nm) light (Vaštakaitė-Kairienė et al., 2021) Additionally, as the percentage of B light in a red (R; 600 – 699 nm)+B spectrum increased, lettuce shoot fresh mass was less than that grown under a light spectrum with a higher R:B (Lee et al., 2010; Son and Oh, 2013). To date, few studies have compared UVA and B light on mediating plant growth. In one study, lettuce 'Red Butter' and 'Yanzhi' grown under 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white light supplemented with 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of UVA (peak = 380 nm) light had lower shoot fresh and dry mass than those grown without supplemental light or other wavelengths, such as far-red or far-red+UVA light (He et al., 2021). In another study, supplemental UVA light slightly increased lettuce 'Hongyeom' fresh mass (Lee et al., 2013), indicating that the effects of UVA light on lettuce growth are inconsistent and likely cultivar dependent.

While fresh mass accumulation directly affects yield and profitability, quality attributes such as nutritional density, leaf coloration, and taste are traits also important to growers, as well as consumers, and may affect their willingness to buy a product. UVA and B light can potentially increase nutritional quality by increasing the concentration of various secondary metabolites and vitamins (Alrifai et al., 2019; Hasan et al., 2017; Thoma et al., 2020). Phenolic compounds are

one of the most abundant secondary metabolites in plants and help protect against abiotic and biotic stresses, are involved in pigment accumulation, and influence taste (Balasundram et al., 2006; Naikoo et al., 2019; Soares et al., 2013). Phenolic compounds are antioxidants that have numerous potential health benefits to humans, such as anti-allergenic, anti-inflammatory, cardioprotective, and vasodilatory properties (Balasundram et al., 2006). These bioactive phenolic compounds are not synthesized in mammalian tissues, which makes their acquisition in the diet from plant sources such as fruits and vegetables essential (Lin et al., 2016). Lettuce phenolic concentrations can increase under small doses of UVA light. For example, in lettuce 'Hongyeom', total phenolic concentration (TPC) increased by 30% when 11 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of UVA (peak = 352 nm) light was added to 185 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white light (Lee et al., 2013). Conversely, an increase in the photon flux density (PFD) of UVA (peak = 373 nm) from 5 to 21 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in a white-light background did not affect TPC (Li and Kubota 2009). B light has a more consistent effect on lettuce TPC. For instance, lettuce 'Sunmang' and 'Grand Rapids TBR' TPC increased by up to 200% when the percentage of B light in an R+B spectrum increased from 0 to 59% (Son and Oh 2013). Additionally, lettuce 'Rouxai' TPC increased by about 25% relative to the control when 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light was added to the light spectrum (Vaštakaitė-Kairienė et al., 2021). Therefore, while UVA and B light both have the potential to increase TPC in lettuce, and B light may be more effective, more research is needed since no studies have compared their efficacy at the same PFD and duration.

Anthocyanins are a subset of plant phenolic compounds that play a significant role in influencing red-leaf pigmentation, especially in red- and purple-leaf plants such as red-leaf lettuce. In general, a light spectrum that increases the TPC in lettuce also increases total anthocyanin concentration (TAC). For example, TAC in lettuce 'Red Cross' increased by 11%

when the PFD of UVA increased (Li and Kubota 2009). In the same study, lettuce TAC increased by 30% as the B light percentage increased from 23 to 55% in a white-light background. Finally, lettuce 'Hongha' TAC increased by up to 6.9-fold as the percentage of B light in an R+B spectrum increased to 43% (Lee et al., 2010).

End-of-production (EOP) lighting refers to adding additional light to a light spectrum for a short period (e.g., several days) prior to the harvest or modifying the PFD. EOP white-red light of different PFDs (0 – 470 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was added to the last six or seven days of production and increased lettuce nutritional quality and improved postharvest performance indicators such as appearance, texture, and odor (Min et al., 2021). EOP lighting can be a potentially useful technique to mitigate possible disadvantages of using a high PFD of UVA or B light throughout production, such as less fresh mass accumulation, while enhancing the nutritional quality and red-leaf pigmentation. EOP lighting can lower electrical costs by only delivering an enriched spectrum for a limited portion of the production cycle compared to the entire time. Therefore, we grew lettuce 'Rouxai' under various EOP treatments to 1) determine how EOP lighting with UVA and B light influences biomass accumulation, TPC, TAC, and leaf coloration; and 2) to compare the effects of UVA and B light when applied at the same PFD. We hypothesized that 1) both UVA and B light would slightly inhibit plant growth; and 2) UVA and B light would be equally effective at increasing TPC, TAC, and leaf coloration when delivered at the same PFD.

Materials and Methods

Plant material and propagation conditions

The red-leaf lettuce cultivar 'Rouxai' (Johnny's Selected Seeds, Winslow, ME, USA) was selected for this study because of its commercial relevance, sensitivity to the light spectrum, and relevant previous experiments. On May 21, 2019 (Rep. 1) and June 23, 2019 (Rep. 2), we

presoaked 200-cell (2.5 cm × 2.5 cm) rockwool plugs (AO 25/40 Starter Plugs; Grodan, Milton, ON, Canada) in deionized water with a pH of 4.5 and sowed 200 seeds of lettuce 'Rouxai' that were presoaked in deionized water with a pH of 4.5. The pH was adjusted using 10% sulfuric acid (H_2SO_4; ACS, ≥ 99.5–98.0%). H_2SO_4 and all other chemicals used during this experiment were purchased from Sigma–Aldrich (Merck KGaA, Darmstadt, Germany). From seed sow to day 7, clear plastic humidity domes covered the trays. We grew the lettuce seedlings in a temperature-controlled growth room (the Controlled Environment Lighting Laboratory at Michigan State University) at 23 °C throughout each replication. We germinated the seeds under a total PFD (TPFD; 315 – 800 nm) of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ delivered from warm-white (WW; peak = 639 nm, correlated color temperature = 2700 K) LEDs (PHYTOFY RL; OSRAM, Beverley, MA) controlled by customized software (Spartan Control Software; OSRAM) for 24 $h \cdot d^{-1}$. Beginning on day 3, seedlings were grown under a TPFD of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from R (peak = 664 nm) plus 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from WW LEDs for 20 $h \cdot d^{-1}$ (daily light integral = 12.96 $mol \cdot m^{-2} \cdot d^{-1}$) until EOP treatments began. We hand-watered the seedlings until transplant on day 10 with deionized water supplemented with a water-soluble fertilizer (12N–4P_2O_5–16K_2O RO Hydro FeED; JR Peters, Inc., Allentown, PA) and magnesium sulfate (Epsom salt; Pennington Seed, Inc., Madison, GA) to achieve the following nutrient solution (in $mg \cdot L^{-1}$): 125 N, 42 P, 167 K, 73 Ca, 49 Mg, 39 S, 1.7 Fe, 0.52 Mn, 0.56 Zn, 0.13 B, 0.47 Cu, and 0.13 Mo. The pH was 5.6 and the electrical conductivity (EC) was 1.6 $mS \cdot cm^{-1}$, as measured by a pH/EC meter (HI9814; Hanna Instruments, Woonsocket, RI).

Growth conditions and lighting treatments

We utilized two vertical hydroponic growing racks with three canopies each to create six different EOP lighting treatments. On day 10, seedlings were transplanted into floating 36-cell

rafts (Beaver Plastics, Ltd., Acheson, AB, Canada) with 2.5-cm-wide holes that were spaced 20 × 15 cm apart. The nutrient solution used in the hydroponic growing racks was the same mixture provided to the seedlings, but the concentrations were increased by 20% (e.g., 150 mg $N \cdot L^{-1}$). The pH and EC of the hydroponic tanks were measured (as previously described) daily and had an average of 5.8 and 1.7 $mS \cdot cm^{-1}$, respectively. The pH was adjusted to 5.5–5.8 using potassium bicarbonate and H_2SO_4. The air temperature setpoint was 23 °C, although the actual air temperature was 23.5±0.8 °C for each replication. Infrared sensors were used to monitor plant canopy temperature, which averaged 24.1±0.8 °C (Rep. 1) and 24.7±0.8 °C (Rep. 2). Relative humidity and CO_2 concentrations were not controlled but were measured at 51±8% (Rep. 1 and 2) and 381±18 ppm (Rep. 1) and 393±19 ppm (Rep. 2), respectively. Additional information about the experimental conditions, equipment, and sensors can be found in Kelly et al. (2020).

On day 24, we added EOP supplemental lighting treatments to the original R+WW LED spectrum for the last six days of production using the same lighting fixtures previously described. EOP lighting treatments (Figure I-1; Table I-1) consisted of a control (no additional light) or supplemental lighting from UVA (peak = 386 nm), B (peak = 449 nm), or G (peak = 532 nm) plus R LEDs. The EOP G_{20}+R_{40} treatment was provided to evaluate EOP light with a higher TPFD but without additional UVA or B light. Treatments delivered a TPFD of 180 to 240 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 20 $h \cdot d^{-1}$. The TPFD and light spectrum of all lighting treatments were measured using a portable spectroradiometer (PS200; Apogee Instruments, Inc., Logan, UT.). Measurements were taken from nine representative spots at plant canopy level and averaged before the experiment began.

Biochemical analysis

Within each lighting treatment, we harvested three biological samples (leaf tissue from separate plants) for TPC analysis and TAC analysis. From each biological sample, we performed two or three technical replicates, depending on the assay. On day 30 after seed sow, we collected leaf tissue directly exposed to the lighting treatments from three randomly selected plants for TPC and TAC analysis, which we then froze in liquid nitrogen and stored in a −80 °C freezer until analysis. We determined lettuce 'Rouxai' TPC spectrophotometrically based on the protocols reported by Ainsworth and Gillespie (2007), with slight modifications. We mixed 0.5 g of frozen plant tissue from each biological sample with 5 mL of 80% methanol (≥ 99.9%) in a ceramic mortar. We then transferred the mixture to a 15 mL polypropylene conical centrifuge tube (Falcon, Fisher Scientific; Hampton, NH) and incubated it for 24 h in a 4 °C refrigerator. Afterward, we centrifuged (Heraeus Megafuge, Thermo Fisher Scientific) the samples for 5 min at a relative centrifugal force of 4000× *g* before filtering the supernatant through a 70 mm qualitative filter paper (Whatman Grade No. 1; Maidstone, United Kingdom) into a 2 mL Eppendorf microcentrifuge tube (Dot Scientific; Burton, MI) that was stored in a −20 °C freezer. Next, we created three technical replicates by adding 100 µL of the filtrate to three different 1.5 mL plastic cuvettes (DOT Scientific; Burton, MI). We diluted the filtrate with 200 µL of 10% (vol/vol) Folin & Ciocalteu's phenol (F–C) reagent and 800 µL of sodium carbonate (Na_2CO_3; ≥ 99.0%) and mixed cuvettes thoroughly before covering and leaving them to sit for 20 min at room temperature. We measured the absorbance of each biological and technical replicate at 765 nm using a spectrophotometer (BioSpec–mini; Shimadzu, Japan). We calculated the TPC in lettuce 'Rouxai' on a fresh weight ($mg \cdot g^{-1}$ FW) basis using a gallic acid (GA; anhydrous) standard curve ($R^2 > 0.95$).

We determined the TAC of lettuce 'Rouxai' using a modified pH differential method (AOAC Official Method 2005.2) (Lee et al., 2005). We mixed 0.3 g of frozen plant tissue with 5 mL of 1% hydrochloric acid (HCL; ACS, ≥ 37.0%) in a ceramic mortar. Similar to the previous TPC protocol, we transferred the mixture to a 15-mL centrifuge tube and incubated it for 24 h in a 4 °C refrigerator. We centrifuged the samples for 5 min at a relative centrifugal force of 4000× *g*. We then filtered the supernatant through a 70 mm qualitative filter paper into a 2 mL Eppendorf tube and stored the supernatant in a −20 °C freezer. Next, we created two separate technical replicates, added 400 µL of the filtrate to two cuvettes, and mixed it with 2 mL of 0.025 M potassium chloride (KCL; ACS, ≥ 99.0%). Additionally, we added 400 µL of the filtrate to two other cuvettes and mixed it with 2 mL of 0.4 M sodium acetate (CH_3COONa; ACS, ≥ 99.0%). We covered all cuvettes, and after sitting for 20 min at room temperature, measured the absorbance of each cuvette at 530 nm and 700 nm using the same spectrophotometer. The dilution factor was 6 and we calculated the TAC in each lettuce 'Rouxai' plant on a fresh weight (mg/g^{-1} FW) basis.

Morphological data collection and analysis

On day 30, we collected morphological data from ten randomly selected plants that were not used for biochemical analysis from each treatment. We cut lettuce shoots at the substrate surface and weighed each one using an analytical balance (AG245; Mettler Toledo, Columbus, OH). We dried the same shoots for 5 d at 60 °C in a drying oven (Blue M, Blue Island, IL) then weighed each with the same balance. We measured leaf length (cm) and width (cm) of the fifth fully expanded leaf and counted leaf number (> 2 cm in length). Additionally, we took overhead pictures of three randomly selected plants for reference and coloration analysis. We used the pictures to measure the *L*a*b** color space of each photo using an R code developed to

determine the lightness (black: $L^* = 0$; white: $L^* = 100$), redness (green: $a^* = -128$; red: $a^* = 127$), and blueness (blue: $b^* = -128$; yellow: $b^* = 127$) of each pixel in an imported TIFF picture. Finally, we measured the relative chlorophyll concentrations of each plant using a SPAD meter (SPAD-502; Konica Minolta Sensing, Inc., Osaka, Japan) by selecting ten random plants from each treatment and measuring and averaging three spots on one fully expanded leaf directly exposed to light.

We arranged the experiment as a randomized complete block design with two replications in time (May 21, 2019–June 20, 2019; June 23, 2019–July 23, 2019) and performed statistical analysis using R statistical analysis software (R Core Team 2014) (version 3.5.1; R Foundation for Statistical Computing, Vienna, Austria). We conducted analysis of variance (ANOVA) and Tukey's honestly significant difference test ($\alpha = 0.5$) using the R packages 'dplyr' (Wickham et al., 2022) and 'agricolae' (Mendiburu 2021).

Results

Total phenolic and anthocyanin concentration

The TPC of lettuce 'Rouxai' increased when we added UVA or B light to 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of WW+R light for the last six days of production (Figure I-2). However, there were no significant differences in the effectiveness of UVA or B light at increasing TPC. For instance, 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B (B_{30}) or UVA (UVA_{30}) light at the end of production increased TPC by 92% or 79%, respectively, compared to the control. Adding 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light to these treatments (B_{60}, UVA_{30}+B_{30}) did not further increase the TPC in 'Rouxai'. Increasing the TPFD without adding UVA or B light (G_{20}+R_{40}) provided an intermediate response and TPC was statistically similar to all of the other treatments.

Similar to TPC, TAC of lettuce 'Rouxai' increased when we added UVA or B light at the end of production (Figure I-2). UVA_{30}, B_{30}, UVA_{30}+B_{30}, or B_{60} for the last six days of production increased TAC by 224%, 258%, 303%, and 273%, respectively, compared with the control. There were no significant differences in the effectiveness between the UVA_{30} and B_{30} treatments or the UVA_{30}+B_{30} and B_{60} treatments. Likewise, the higher TPFD treatments containing UVA or B light did not increase TAC more than the lower TPFD treatments. Finally, G_{20}+R_{40} did not increase TAC and was similar to the control and the UVA_{30} treatment.

Leaf pigmentation and relative chlorophyll concentration

UVA_{30}+B_{30} or B_{60} added at the end of production increased 'Rouxai' leaf redness (more positive a* value) by 72% and 66%, respectively, compared to the control (Figure I-3). Increasing the TPFD without UVA or B light (G_{20}+R_{40}) did not increase leaf redness. Leaf redness under a lower TPFD of UVA or B light was similar to all other treatments. We also measured L* (darkness – lightness) and b* (blue – yellow) but there were no significant differences between any of the EOP treatments and the control (Figure I-3).

EOP lighting treatments containing UVA or B light increased the SPAD index (relative chlorophyll concentration) of lettuce 'Rouxai' (Figure I-3). For example, when we added UVA_{30} or B_{30} to the base WW+R spectrum, the SPAD index increased by 15% and 20%, respectively. The TPFD of UVA or B light did not differentially affect the SPAD index. The G_{20}+R_{40} EOP lighting treatment did not affect the SPAD index.

Plant morphology and shoot mass

Plant morphology was generally similar under all EOP lighting treatments. No EOP treatment containing UVA or B light influenced leaf length, leaf width, or leaf number, except for B_{60}, which increased leaf number by 13% compared to the control. The G_{20}+R_{40} treatment

increased leaf width by 7% compared to the B_{60} treatment (Table I-2). In addition, the EOP lighting treatments did not affect shoot fresh mass (Figure I-4; Table I-2), but some treatments slightly increased shoot dry mass (Table I-2). Specifically, all EOP treatments that increased the TPFD by 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (B_{60}, UVA_{30}+B_{30}, G_{20}+R_{40}) increased shoot dry mass compared to the control treatment, irrespective of the light spectrum. For instance, the addition of UVA_{30}+B_{30}, B_{60}, or G_{20}+R_{40} increased shoot dry mass by 35%, 32%, and 27%, respectively, but there was no statistical difference among those treatments.

Discussion

UVA and blue light both increased secondary metabolite production and leaf pigmentation

Red-leaf lettuce has high concentrations of phenolic compounds, including anthocyanins, which influence its nutritional quality and taste. Environmental factors, including short-wavelength light, can differentially regulate the concentration of these metabolites, but there are inconsistent trends on what wavelengths and PFDs are most effective. Li and Kubota (2009) reported that partial substitution of white light with approximately 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B (peak = 476 nm) or 18 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of UVA (peak = 373 nm) light increased lettuce 'Red Cross' TAC by 31% and 11%, respectively, but neither affected TPC. In the current study, adding UVA_{30} or B_{30} to a WW+R light spectrum for the last six days of production increased the TPC and TAC of lettuce 'Rouxai' (Figure I-2). Interestingly, there were no differences in the effectiveness of UVA and B light, which contrasts with some other studies. For instance, when 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light (peak = 449 nm) was added to WW light for 18 days, TPC and TAC of baby leaf lettuce 'Rouxai' increased by 25% and 95%, respectively, but UVA (peak = 385 nm) at 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ did not affect either TPC or TAC (Vaštakaitė-Kairienė et al., 2021). Discrepancies between these studies could be attributed to a variety of factors such as cultivar selection, plant

maturity, UVA or B light application duration, PFD of UVA or B light applied, or the spectral quality and PFD of the background spectrum.

UVA and B light differentially regulate specific groups of phenolic compounds that lead to a cumulative increase in total content (Verdaguer et al., 2017). The largest group of phenolic compounds is flavonoids, from which anthocyanins are derived. Their strong absorption of UV and B light is often associated with an increased expression of genes that regulate flavonoid biosynthesis, such as those from the R2R3-MYB, WD40, and bHLH transcription factor families (Falcone Ferreyra et al., 2012; Naikoo et al., 2019; Zoratti et al., 2014). Cryptochromes, specifically cryptochrome 1 (cry1) and cryptochrome 2 (cry2), are the primary UV/B sensing photoreceptors and control many UV- and B-light responses (Briggs and Huala 1999). The increase in TPC and TAC in lettuce 'Rouxai' can be attributed to cry1's role in mediating flavonoid and anthocyanin biosynthesis by regulating the transcription of *CHALCONE SYNTHASE* (*CHS*), which encodes the first, committed enzyme in the flavonoid biosynthesis pathway (Jenkins et al., 2001; Wade et al., 2001; Weisshaar and Jenkinst 1998). Additionally, cry2 is involved in anthocyanin regulation, but only under low-intensity UV or B light, since cry2 begins to degrade under a higher PFD of these wavebands (Ahmad et al., 1998; Christie and Briggs 2001; Lin et al., 1998). This could explain why TPC or TAC did not increase in the present study when the PFD of UVA and/or B light at the end of production increased from 30 to 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$. We speculate that 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of short-wavelength light was sufficiently high to cause cry2 degradation and flavonoid synthesis to slow. It is also plausible that the cry1-mediated response was saturated with 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of UVA or B light.

In lettuce, red-leaf pigmentation is closely associated with anthocyanin accumulation in leaf tissue (Park et al., 2008), which suggests leaf coloration can be used as a predictor of

anthocyanin content (Yang et al., 2016). Gazula and colleagues (2007) reported that anthocyanin concentrations in nine lettuce cultivars were closely associated with both instrument assessment of color and panelist rating of red coloration. Although there is a strong association between anthocyanin concentrations and red-leaf coloration in lettuce leaves, few studies have measured the effects of the light spectrum on both the anthocyanin concentration and coloration values of lettuce. Owen and Lopez (2015) quantified leaf coloration of multiple lettuce varieties grown in a greenhouse with or without various supplemental EOP lighting treatments. Leaf redness, and presumably anthocyanin concentration, increased after 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of R, B, or R+B EOP lighting was applied for at least three days. In our study, the highest PFD tested (60 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of UVA+B or B increased leaf redness compared to the control treatment (Figure I-3), which correlated with an increase in TAC.

UVA and blue light at the EOP did not suppress biomass accumulation

A moderate to high PFD of B light typically suppresses plant growth and leaf expansion (Cosgrove 1981; Ohashi-Kaneko et al., 2007; Shin et al., 2014; Son and Oh 2015) but the effects of UVA on plant growth are less clear and vary among species (Verdaguer et al., 2017). Some studies indicate that UVA can promote plant growth and leaf expansion (Chen et al., 2019; Hooks et al., 2021) while others reported inhibitory effects, similar to B light (Krizek et al., 1998; Tsormpatsidis et al., 2008). In the present study, EOP lighting treatments did not increase shoot fresh mass (Figure I-4; Table I-2), but treatments with a TPFD of 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$, regardless of the spectrum, increased shoot dry mass by up to 35% compared to the control treatment. Since this biomass response was not specific to a light spectrum, the increase in shoot dry mass can be attributed to an increase in the daily light integral during the six days of EOP lighting (Kelly et al., 2020). The light spectrum before the EOP lighting treatments began was

the same, so leaf area and therefore light interception can be assumed to be equal. Lettuce grown under EOP treatments with a TPFD of 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had a greater shoot dry mass, but there were no morphological changes, except for B_{60}, which slightly inhibited leaf width compared to the $G_{20}+R_{40}$ treatment and had more leaves than the control treatment (Table I-2).

UVA and blue LEDs: technical considerations

While light from UVA and B LEDs can have similar effects on plant growth, morphology, and quality attributes at the end of lettuce production, there are differences in the LED types that need to be considered such as photon efficacy ($\mu mol \cdot J^{-1}$), photon flux, and worker safety. From a horticultural perspective, the efficacy of an LED is the photon flux ($\mu mol \cdot s^{-1}$) per watt ($J \cdot s^{-1}$) of input power and thus represents an important performance metric (Kusuma et al., 2020). As of 2022, B LEDs have a photon efficacy of 1.6 to 3.5 $\mu mol \cdot J^{-1}$ while UVA LEDs have a photon efficacy of up to 0.9 $\mu mol \cdot J^{-1}$, but these values depend on peak wavelength, current density, and junction temperature (Kusuma et al., 2020; Kusuma et al., 2022). Since UVA LEDs produce fewer photons per unit of input power, more energy is required to deliver the same photon flux as B LEDs and thus, are less sustainable.

Another consideration is the effect of UVA and B light on worker safety and photopic vision. UVA photons are less energetic and thus less damaging to humans than UV B (280 – 315 nm) and UVC (100 – 280 nm) photons, but acute exposure can cause visual irritation, and long-term exposure can cause eye and skin damage (Burke and Wei, 2009; Ivanov et al., 2018). B light is not as physiologically harmful to humans, but can still cause visual irritation or photochemical damage with excessive exposure (Ouyang et al., 2020). Another concern of B light is the impact on the color rendering index (CRI) and correlated color temperature (CCT; K) of the work environment. The CRI is a scale of 0 – 100 that describes how well a light source

reveals the true colors of objects. At a CRI of 0, all colors look the same and at a CRI of 100, all true colors of objects are apparent. CCT is the color temperature of a white-light source. The higher the CCT, the cooler (i.e., more blue and less red) the white light appears. Increasing the percentage of B in a light spectrum increases the CCT and generally lowers the CRI of a light source. Therefore, light with a low CRI (e.g., <80) can create a less desirable work environment for employees, cause visual eye strain, and make it more challenging to identify insects, diseases, or nutritional disorders. Since UVA light is less visible than B light, it has a negligible effect on the CRI and CCT of a light source.

End-of-production lighting as a production tool

Other studies have investigated the effects of EOP LED lighting on leafy greens production and have found it to be an effective method to increase plant growth and quality. For instance, lettuce 'Cherokee' grown in a greenhouse had increased leaf redness when 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of EOP R, B or R+B light was added for at least the last three days of production (up to 14 days) (Owen and Lopez, 2015). Furthermore, 171 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of supplemental lighting that included low-wavelength B (peak = 403 nm) or R+B light in a greenhouse increased shoot fresh and dry mass, leaf area, TPC, TAC, and carotenoid concentration of lettuce 'Red Mist', but the magnitude depended on the daily light integral, duration of lighting (2 or 4 days), and whether it was applied at night or during the day (Hooks et al., 2021). In another study, anthocyanin content, but not phenolic content, of lettuce 'Codex' and 'Rouxai' increased 2-fold when high-intensity EOP light with a high percentage of B light (69% B + 31% R) was applied for the last four days of production (Gómez and Jiménez, 2020). In the same study, EOP light with UVA light (5% UVA + 33% B + 62% R) did not increase anthocyanin or phenolic content. Finally, shoot fresh and dry mass, leaf area, leaf number, and TAC increased when 10

μmol·m^{-2}·s^{-1} of UVA (peak = 365 nm) light was applied to indoor-grown lettuce 'Klee' for 5 to 15 days before harvest (Chen et al., 2019). Similar to our results, EOP lighting with UVA increased TAC and shoot dry mass, although we applied a higher PFD and longer peak wavelength of UVA light. Therefore, EOP short-wavelength lighting can have little or no negative impact on leaf expansion or biomass accumulation, which can occur if delivered during the entire production period, yet increase the nutritional quality and leaf coloration.

Conclusion

EOP lighting with short-wavelength light, such as UVA or B, is a production technique that can enhance lettuce nutritional attributes, leaf coloration, and potentially biomass accumulation. Compared to continuous application of UVA or B light, EOP lighting with 30 μmol·m^{-2}·s^{-1} of UVA or B light had less of an effect on growth and leaf expansion inhibition, but increased phenolic and anthocyanin concentrations as well as leaf coloration in at least some cultivars of lettuce. Additionally, when UVA and B light were applied at the same PFD as EOP lighting, they were equally effective at increasing TPC, TAC, and leaf coloration. Moreover, EOP light with longer wavelengths (i.e., G_{20}+R_{40} treatment) did not increase TPC, TAC, or leaf redness. More research is needed to determine the most effective peak wavelength and dose (PFD and duration) of light to achieve desired plant outcomes while also considering sustainability, including the technical performance of LEDs. Furthermore, additional research is needed to determine how the background spectrum and PFD interact with EOP lighting treatments.

Acknowledgements

This work was supported by the Specialty Crops Research Initiative (grant no. 2019-51181-30017) and Hatch project 192266 from the USDA National Institute of Food and

Agriculture. We thank Ryan Warner for use of his laboratory and Mckenna Merkel and Viktorija Vaštakaitė-Kairienė for experimental assistance. We also thank Leslie Bourquin, Chieri Kubota, and Ryan Warner for their critical review of this manuscript.

LITERATURE CITED

Ahmad, M., Jarillo, J.A., Cashmore, A.R. 1998. Chimeric proteins between cry1 and cry2 *Arabidopsis* blue light photoreceptors indicate overlapping functions and varying protein stability. Plant Cell. 10, 197–207. doi:10.1105/TPC.10.2.197.

Ainsworth, E.A., Gillespie, K.M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc 24, 875–877. doi:10.1038/nprot.2007.102.

Alrifai, O., Hao, X., Marcone, M.F., Tsao, R. 2019. Current review of the modulatory effects of LED lights on photosynthesis of secondary metabolites and future perspectives of microgreen vegetables. J Agric Food Chem. 67, 6075–6090. doi:10.1021/acs.jafc.9b00819.

Balasundram, N., Sundram, K., Samman, S. 2006. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem. 99, 191–203. doi:10.1016/J.FOODCHEM.2005.07.042.

Briggs, W.R., Huala, E. 1999. Blue-light photoreceptors in higher plants. Annu Rev Cell Dev Biol. 15, 33–62. doi:10.1146/annurev.cellbio.15.1.33.

Burke, K., Wei, H. 2009. Synergistic damage by UVA radiation and pollutants. Toxicol Ind Health. 25, 219–224. doi:10.1177/0748233709106067.

Chen, Y., Li, T., Yang, Q., Zhang, Y., Zou, J., Bian, Z., Wen, X. 2019. UVA radiation is beneficial for yield and quality of indoor cultivated lettuce. Front Plant Sci. 10, 1563. doi:10.3389/FPLS.2019.01563/BIBTEX.

Christie, J.M., Briggs, W.R.. 2001. Blue light sensing in higher plants. J Biol Chem. 276, 11457–11460. doi:10.1074/JBC.R100004200.

Cosgrove, D.J. 1981. Rapid suppression of growth by blue light. Plant Physiol. 67, 584–590. doi:10.1104/pp.67.3.584.

Falcone Ferreyra, M.L., Rius, S.P., Casati, P. 2012. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Front Plant Sci. 3,222. doi:10.3389/fpls.2012.00222.

Gómez, C., Jiménez, J. 2020. Effect of end-of-production high-energy radiation on nutritional quality of indoor-grown red-leaf lettuce. HortScience. 55, 1055–1060. doi:10.21273/HORTSCI15030-20.

Gazula, A., Kleinhenz, M.D., Scheerens, J.C., Ling P.P. 2007. Anthocyanin levels in nine lettuce (*Lactuca sativa*) cultivars: influence of planting date and relations among analytic,

instrumented, and visual assessments of color. HortScience 42, 232–238. doi:10.21273/HORTSCI.42.2.232.

Hasan, M., Bashir, T., Ghosh, R., Lee, S.K., Bae, H. 2017. An overview of LEDs' effects on the production of bioactive compounds and crop quality. Molecules. 22, 1420. https://doi.org/10.3390/molecules22091420

He, R., Zhang, Y., Song, S., Su, W., Hao, Y., Liu, H. 2021. UV-A and FR irradiation improves growth and nutritional properties of lettuce grown in an artificial light plant factory. Food Chem. 345, 128727. doi:10.1016/j.foodchem.2020.128727.

Hooks, T., Masabni, J., Sun, L., Niu, G. 2021. Effect of pre-harvest supplemental UV-A/blue and red/blue LED lighting on lettuce growth and nutritional quality. Horticulturae. 7, 80. doi:10.3390/horticulturae7040080.

Ivanov, I.V., Mappes, T., Schaupp, P., Lappe, C., Wahl S. 2018. Ultraviolet radiation oxidative stress affects eye health. J Biophotonics. 11, e201700377. doi:10.1002/JBIO.201700377.

Jenkins, G.I., Long, J.C., Wade, H.K., Shenton, M.R., Bibikova, T.N. 2001. UV and blue light signalling: pathways regulating chalcone synthase gene expression in *Arabidopsis*. New Phytol. 151, 121–131. doi:10.1046/J.1469-8137.2001.00151.X.

Kelly, N., Choe, D., Meng, Q., Runkle, E.S. 2020. Promotion of lettuce growth under an increasing daily light integral depends on the combination of the photosynthetic photon flux density and photoperiod. Sci Hortic. 272, 109565. doi:10.1016/j.scienta.2020.109565.

Kim, M.J., Moon, Y., Tou, J.C., Mou, B., Waterland, N.L. 2016. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). J Food Compos Anal. 49, 19–34. doi:10.1016/J.JFCA.2016.03.004.

Kitazaki, K., Fukushima, A., Nakabayashi, R., Okazaki, Y., Kobayashi, M., Mori, T., Nishizawa, T., Reyes-Chin-Wo, S., Michelmore, R.W., Saito, K., Shoji, K., Kusano, M. 2018. Metabolic reprogramming in leaf lettuce grown under different light quality and intensity conditions using narrow-band LEDs. Sci Rep. 8, 1–12. doi:10.1038/s41598-018-25686-0.

Kozai, T., Niu, G. 2016. Plant factory as a resource-efficient closed plant production system, p 69–90. In: Plant factory: an indoor vertical farming system for efficient quality food production. doi:10.1016/B978-0-12-801775-3.00004-4.

Krizek, D.T., Britz, S.J., Mirecki, R.M. 1998. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cv. New Red Fire lettuce. Physiol Plant. 103, 1–7. doi:10.1034/j.1399-3054.1998.1030101.x.

Kusuma, P., Pattison, P.M., Bugbee, B. 2020. From physics to fixtures to food: current and potential LED efficacy. Hortic Res. 7, 56. doi:10.1038/s41438-020-0283-7.

Kusuma, P., Pattison, P.M., Bugbee, B. 2022. Photon efficacy in horticulture: turning LED packages into LED luminaires, p 115–128. In: Plant factory basics, applications, and advances. doi:10.1016/B978-0-323-85152-7.00006-9.

Lee, J., Durst, R.W., Wrolstad, R.E. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J AOAC Int. 88, 1269–1278. doi:10.1093/JAOAC/88.5.1269.

Lee, J.G., Oh, S.S., Cha, S.H., Jang, Y.A., Kim, S.Y., Um, Y.C., Cheong, S.R. 2010. Effects of red/blue light ratio and short-term light quality conversion on growth and anthocyanin contents of baby leaf lettuce. J Bio-Environ Control. 19, 351–359.

Lee, M-J., Son, J.E., Oh, M-M. 2013. Growth and phenolic compounds of *Lactuca sativa* L. grown in a closed-type plant production system with UV-A, -B, or -C lamp. J Sci Food Agric. 94, 197–204. doi:10.1002/jsfa.6227.

Li, Q., Kubota, C. 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ Exp Bot. 67, 59–64. doi:10.1016/j.envexpbot.2009.06.011.

Lin, C., Yang, H., Guo, H., Mockler, T., Chen, J., Cashmore, A.R. 1998. Enhancement of blue-light sensitivity of *Arabidopsis* seedlings by a blue light receptor cryptochrome 2. Proc Natl Acad Sci USA. 95, 2686–2690. doi:10.1073/PNAS.95.5.2686.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., Chen, S. 2016. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules. 21, 1374. doi:10.3390/molecules21101374.

Mendiburu, F. 2021. Agricolae: statistical procedures for agricultural research. https://CRAN.R-project.org/package=agricolae.

Min, Q., Marcelis, L.F.M., Nicole, C.C.S., Woltering, E.J. 2021. High light intensity applied shortly before harvest improves lettuce nutritional quality and extends the shelf life. Front Plant Sci. 12, 615355. doi:10.3389/fpls.2021.615355.

Naikoo, M.I., Dar, M.I., Raghib, F., Jaleel, H., Ahmad, B., Raina, A., Khan, F.A., Naushin, F. 2019. Role and regulation of plants phenolics in abiotic stress tolerance: an overview, p 157–168. In: Plant signaling molecules. doi:10.1016/B978-0-12-816451-8.00009-5.

Ohashi-Kaneko, K., Tarase, M., Noya, K.O.N., Fujiwara, K., Kurata, K. 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. Environ Control Biol. 45, 189–198. doi:10.2525/ecb.45.189.

Ouyang, X., Yang, J., Hong, Z., Wu, Y., Xie ,Y., Wang, G. 2020. Mechanisms of blue light-induced eye hazard and protective measures: a review. Biomed Pharmacother. 130, 110577. doi:10.1016/J.BIOPHA.2020.110577.

Owen, W.G., Lopez, R.G. 2015. End-of-production supplemental lighting with red and blue light-emitting diodes (LEDs) influences red pigmentation of four lettuce varieties. HortScience, 50, 676–684. doi:/10.21273/HORTSCI.50.5.676.

Park, J.S., Kim, J.B., Cho, K.J., Cheon, C.I., Sung, M.K., Choung, M.G., Sung, M.K., Choung, M.G., Roh, K.H. 2008. *Arabidopsis* R2R3-MYB transcription factor AtMYB60 functions as a transcriptional repressor of anthocyanin biosynthesis in lettuce (*Lactuca sativa*). Plant Cell Rep. 27, 985–994. doi:10.1007/s00299-008-0521-1.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria https://www.r-project.org/.

Radetsky, L.C. 2018. LED and HID horticultural luminaire testing report. Lighting Research Center, Rensselaer Polytechnic Institute: Troy, NY. 2018. NY. https://www.lrc.rpi.edu/programs/energy/pdf/HorticulturalLightingReport-Final.pdf.

Shin, Y-S., Lee, M-J., Lee, E-S., Ahn, J-H., Kim, M-K., Lee, J-E., Do, H-W., Cheung, J-D., Park, J-U., Um, Y-G., Park, S-D., Chae, J-H. 2014. Effect of light emitting diodes treatment on growth and quality of lettuce (*Lactuca sativa* L. 'Oak Leaf'). J Life Sci. 24, 148–153. doi:10.5352/JLS.2014.24.2.148.

Soares, S., Kohl, S., Thalmann, S., Mateus, N., Meyerhof, W., De Freitas, V. 2013. Different phenolic compounds activate distinct human bitter taste receptors. J Agric Food Chem. 61, 1525–1533. doi:10.1021/jf304198k.

Son, K-H., Lee, J-H., Oh, Y., Kim, D., Oh, M-M., In, B.C. 2017. Growth and bioactive compound synthesis in cultivated lettuce subject to light-quality changes. HortScience. 52, 584–591. doi:10.21273/HORTSCI11592-16.

Son, K-H., Oh, M-M. 2013. Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. HortScience. 48, 988–995. doi:10.21273/HORTSCI.48.8.98.

Son, K-H., Oh, M-M.. 2015. Growth, photosynthetic and antioxidant parameters of two lettuce cultivars as affected by red, green, and blue light-emitting diodes. Hortic Environ Biotechnol. 56,639–653. doi:10.1007/S13580-015-1064-3.

Thoma, F, Somborn-Schulz, A., Schlehuber, D., Keuter, V., Deerberg, G. 2020. Effects of light on secondary metabolites in selected leafy greens: a review. Front Plant Sci. 11, 497. doi:10.3389/fpls.2020.00497.

Tsormpatsidis, E., Henbest, R.G.C., Davis, F.J., Battey, N.H., Hadley, P., Wagstaffe, A. 2008. UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce "Revolution" grown under polyethylene films. Environ Exp Bot. 63, 232–239. doi:10.1016/j.envexpbot.2007.12.002.

U.S. Department of Agriculture. 2018. Agricultural Marketing Resource Center. 11 November 2019. <https://www.agmrc.org/commodities-products/vegetables/lettuce>.

Vaštakaitė-Kairienė, V., Kelly, N., Runkle, E.S. 2021. Regulation of the photon spectrum on growth and nutritional attributes of baby-leaf lettuce at harvest and during postharvest storage. Plants. 10, 549. doi:10.3390/PLANTS10030549.

Verdaguer, D., Jansen, M.A.K., Llorens, L., Morales, L.O., Neugart, S. 2017. UV-A radiation effects on higher plants: exploring the known unknown. Plant Sci. 255, 72–81. doi:10.1016/j.plantsci.2016.11.014.

Wade, H.K., Bibikova, T.N., Valentine, W.J., Jenkins, G.I. 2001. Interactions within a network of phytochrome, cryptochrome and UV-B phototransduction pathways regulate chalcone synthase gene expression in *Arabidopsis* leaf tissue. Plant J. 25,675–685. doi:10.1046/J.1365-313X.2001.01001.X.

Weisshaar, B., Jenkinst, G.I. 1998. Phenylpropanoid biosynthesis and its regulation. Curr Opin Plant Biol. 1, 251–257. doi:10.1016/S1369-5266(98)80113-1.

Wickham, H., François, R., Henry, L., Müller, K. 2022. dplyr: A Grammar of Data Manipulation. https://CRAN.R-project.org/package=dplyr.

Yang, X., Zhang, J., Guo, D., Xiong, X., Chang, L., Niu, Q., Huang D. 2016. Measuring and evaluating anthocyanin in lettuce leaf based on color information. IFAC-PapersOnLine 49, 96–99. doi:10.1016/J.IFACOL.2016.10.018.

Zoratti, L., Karppinen, K., Escobar, A.L., Häggman, H., Jaakola, L. 2014. Light-controlled flavonoid biosynthesis in fruits. Front Plant Sci. 5, 534. doi:10.3389/FPLS.2014.00534/BIBTEX.

APPENDIX

Table I-1. End-of-production supplemental lighting treatments were provided to lettuce plants beginning on day 24 after seeding. Except for the control, each treatment consisted of additional ultraviolet A (UVA; 315 – 399 nm) and/or blue (B; 400 – 499 nm) light or green (G; 500 – 599 nm) plus red (R; 600 – 699 nm) light, which increased the total photon flux density (TPFD; 315 – 800 nm) to 210 or 240 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and the extended daily light integral (eDLI, 315 – 800 nm) to 15.1 to 17.3 $mol \cdot m^{-2} \cdot d^{-1}$.

Treatment	Supplemental lighting ($\mu mol \cdot m^{-2} \cdot s^{-1}$)				TPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	eDLI ($mol \cdot m^{-2} \cdot d^{-1}$)
	UVA	Blue	Green	Red		
Control	0	0	0	0	180	13.0
UVA_{30}	30	0	0	0	210	15.1
B_{30}	0	30	0	0	210	15.1
$UVA_{30}+B_{30}$	30	30	0	0	240	17.3
B_{60}	0	60	0	0	240	17.3
$G_{20}+R_{40}$	0	0	20	40	240	17.3

Table I-2. Shoot dry mass (g), leaf length (cm), leaf width (cm), and leaf number of lettuce 'Rouxai' grown without (control) or with supplemental end-of-production lighting of ultraviolet A (UVA, 315 – 399 nm), blue (B; 400 – 499 nm), green (G; 500 – 599 nm), and/or red (R; 600 – 699 nm) light for the last six days of production. Subscript values following each waveband represents its photon flux density in $\mu mol \cdot m^{-2} \cdot s^{-1}$. Data are the mean of two replications with 10 samples in each replication. Means with different letters are significantly different according to Tukey's honestly significant difference test ($\alpha = 0.05$).

Treatment	Dry mass (g)	Leaf length (cm)	Leaf width (cm)	Leaf number
Control	1.59 b	12.8 a	20.1 ab	17.1 b
UVA_{30}	1.98 ab	13.0 a	19.7 ab	18.2 ab
B_{30}	1.88 ab	12.6 a	19.7 ab	18.4 ab
$UVA_{30}+B_{30}$	2.15 a	12.7 a	19.3 ab	18.3 ab
B_{60}	2.10 a	12.4 a	18.9 b	19.4 a
$G_{20}+R_{40}$	2.02 a	12.8 a	20.3 a	18.0 ab

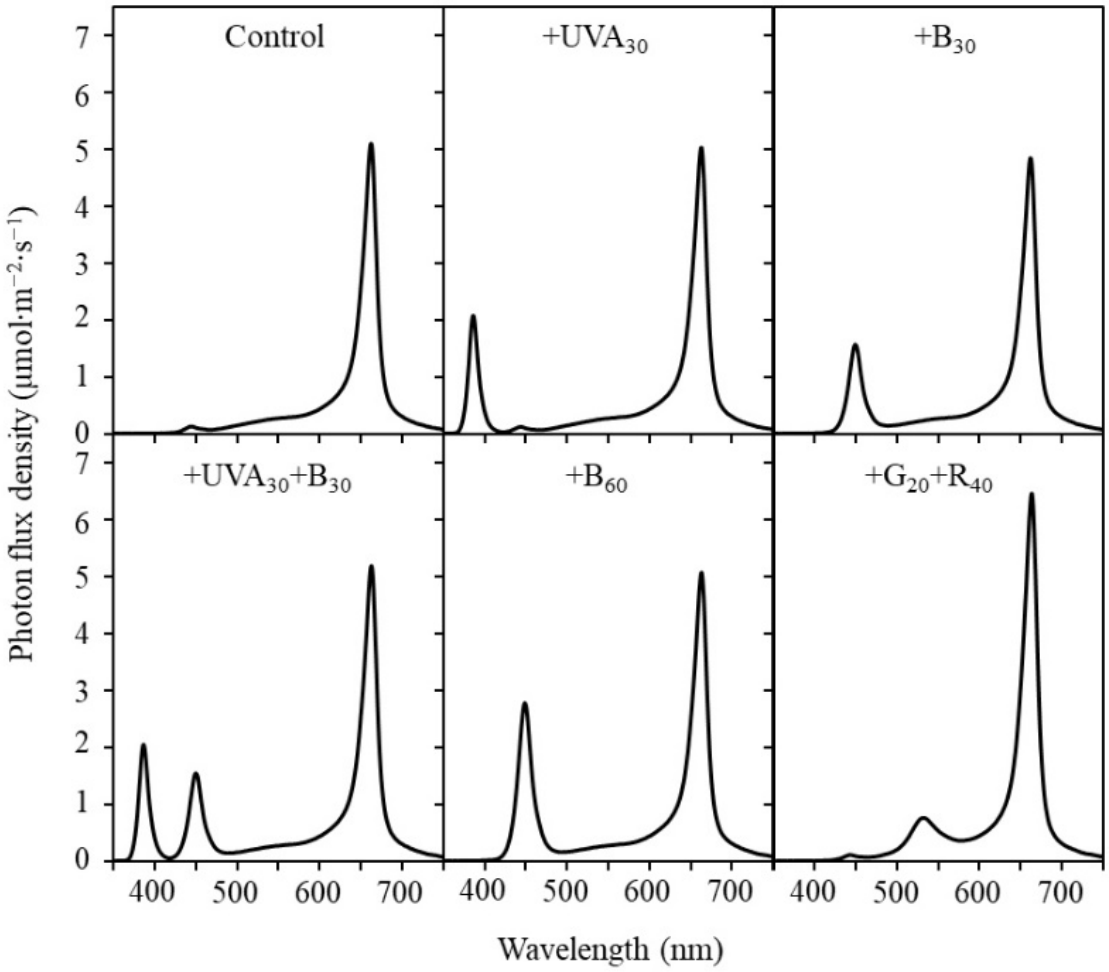

Figure I-1. The spectral distribution of the base warm-white and red LED lighting spectrum plus end-of-production lighting treatments that were added for the last six days of production. End-of-production lighting treatments consisted of additional ultraviolet A (UVA; 315 – 399 nm) and/or blue (B; 400 – 499 nm) light or green (G; 500 – 599 nm) plus red (R; 600 – 699 nm) light.

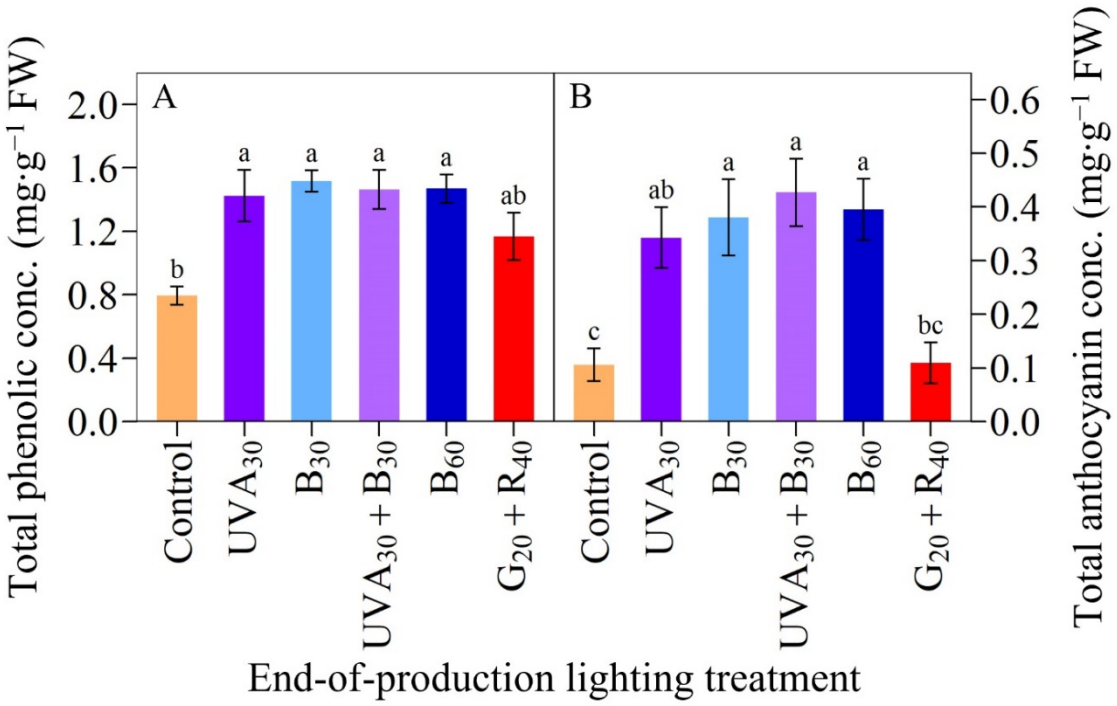

Figure I-2. (A) Mean total phenolic concentration and (B) total anthocyanin concentration on a fresh weight (FW) basis of lettuce 'Rouxai' grown without (control) or with supplemental end-of-production lighting of ultraviolet A (UVA, 315 – 399 nm), blue (B; 400 – 499 nm), green (G; 500 – 599 nm), and/or red (R; 600 – 699 nm) light for the last six days of production. Subscript values following each waveband represents its photon flux density in $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each bar represents the mean of two replications with three biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars indicate the standard error of each treatment.

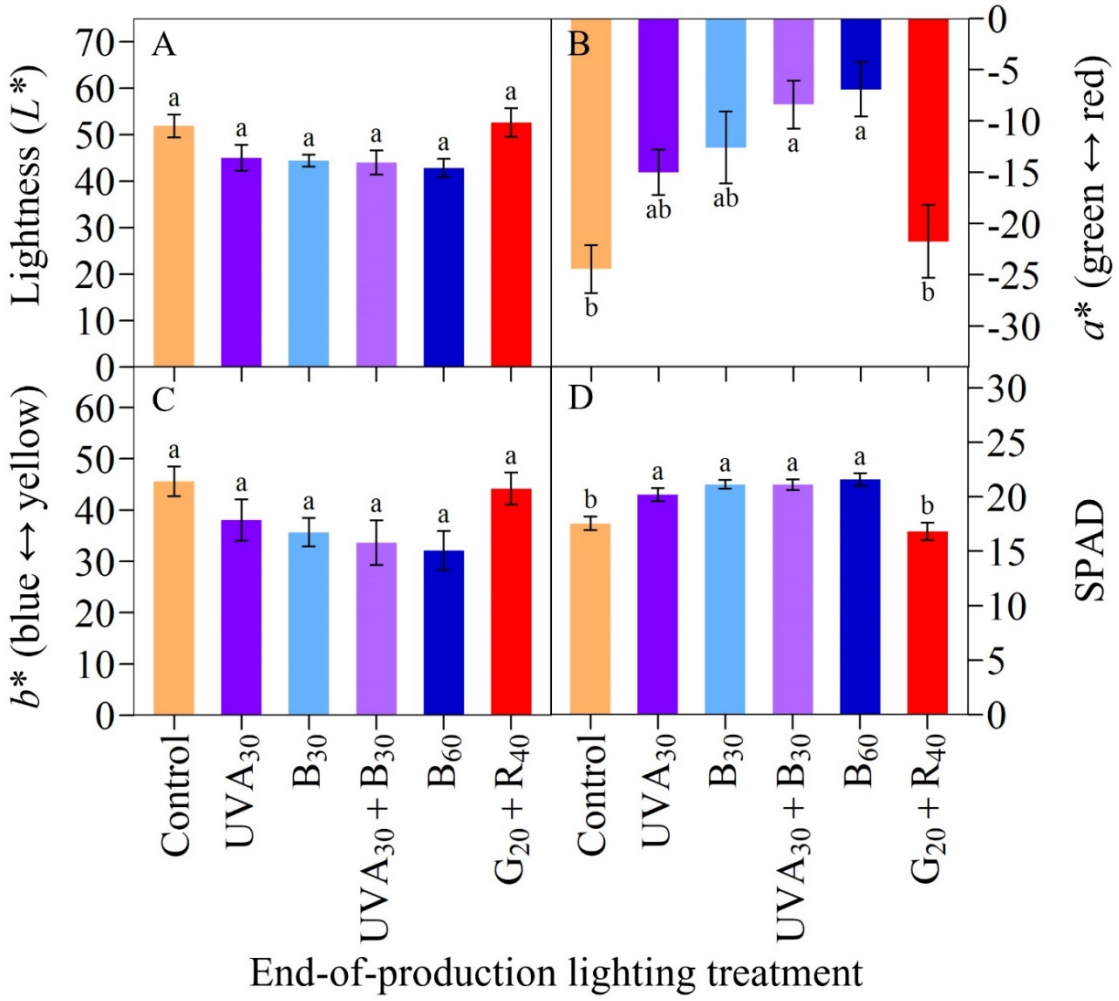

Figure I-3. (A, B, C) Mean leaf pigmentation indicated by L*a*b* values and (D) relative chlorophyll concentration (SPAD) of lettuce 'Rouxai' grown without (control) or with supplemental end-of-production lighting of ultraviolet A (UVA, 315 – 399 nm), blue (B; 400 – 499 nm), green (G; 500 – 599 nm), and/or red (R; 600 – 699 nm) light for the last six days of production. Subscript values following each waveband represents its photon flux density in $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each bar represents the mean of two replications with three biological samples per treatment and replication, except for SPAD where there were 10 samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars indicate the standard error of each treatment.

Treatment	Control	UVA_{30}	B_{30}	$UVA_{30}+B_{30}$	B_{60}	$G_{20}+R_{40}$
Fresh mass (g)	70.0	73.7	73.8	72.9	74.7	73.8

Figure I-4. Shoot fresh mass (g) of lettuce 'Rouxai' grown without (control) or with supplemental end-of-production lighting of ultraviolet A (UVA, 315 – 399 nm), blue (B; 400 – 499 nm), green (G; 500 – 599 nm), and/or red (R; 600 – 699 nm) light for the last six days of production. Subscript values following each waveband represents its photon flux density in $\mu mol \cdot m^{-2} \cdot s^{-1}$. Data are the mean of two replications with 10 samples in each replication. There were no significant differences according to Tukey's honestly significant difference test (α = 0.05). Pictures are representative plants from each treatment.

SECTION II
UVA AND BLUE LIGHT TRANSIENTLY REGULATE TOTAL PHENOLIC AND ANTHOCYANIN CONCENTRATIONS IN INDOOR-GROWN RED-LEAF LETTUCE

UVA and blue light transiently regulate total phenolic and anthocyanin concentrations in indoor-grown red-leaf lettuce

Nathan H. Kelly and Erik S. Runkle*

Controlled-Environment Lighting Laboratory, Department of Horticulture, 1066 Bogue Street, Michigan State University, East Lansing, MI 48824

*Corresponding author. Tel.: +1 517 353 0350; fax: +1 517 353 0890. E-mail address: runkleer@msu.edu (E.S. Runkle)

Abstract

In controlled environments, supplementing a light spectrum with ultraviolet A (UVA; 315-399 mn) or blue (B; 400-499 nm) light increases the concentrations of phenolic compounds that can increase quality attributes, such as leaf pigmentation and nutritional quality of lettuce (*Lactuca sativa*). However, UVA and B light inhibit leaf expansion and biomass accumulation when applied continuously, whereas applying it only at the end of the production cycle can increase lettuce quality with little to no effect on crop yield. Our objective was to quantify the persistency of periodic supplemental UVA or B light during indoor production on quality attributes and biomass accumulation. We hypothesized supplemental UVA or B light would be more effective later, rather than earlier, during production at increasing lettuce quality attributes. We grew red-leaf lettuce 'Rouxai' hydroponically at 23 °C air temperature under of 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of red (peak = 664 nm) plus 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of warm-white light provided by light-emitting diodes. The lighting treatments consisted of adding 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of UVA (peak= 386 nm) or B (peak = 449 nm) light during the seedling phase (P1; day 4-12), growth phase (P2; day 12-20), finishing phase (P3; day 20-28), or the entire time (ET; day 4-28). Supplemental UVA or B light applied at any individual phase did not inhibit biomass accumulation whereas enriched B light during the entire production period inhibited fresh mass. Additionally, supplemental UVA or B light during P3 or ET similarly increased total phenolic and anthocyanin concentrations. Finally, applying UVA or B light during P1 or P2 had no residual effect on mature plants at harvest. We conclude that the end of the production cycle is the optimal time to apply supplemental UVA or B light, that earlier application elicits transient responses, and that continuous application inhibits fresh mass accumulation.

Introduction

Indoor (vertical) farming is growing in popularity because of its efficient use of resources (e.g., water, fertilizer, and land), limited or no use of pesticides, and consistent, year-round production (Kozai and Niu, 2016). Lettuce (*Lactuca* sativa) is a versatile culinary crop that has a compact growth habit and short production cycle, which makes it ideal for indoor farming. Inside indoor farms, light-emitting diodes (LEDs) allow for the precise control of the light spectrum, photon flux density (PFD), and photoperiod, which allow growers to manipulate the growth and biochemical properties of lettuce and other specialty crops. For example, a light spectrum with a high percentage of red (R; 600-699 nm) and/or far-red (FR; 700-799 nm) light promotes leaf expansion and growth (Legendre and van Iersel, 2021; Park and Runkle, 2017; Son and Oh, 2013), while ultraviolet A (UVA; 315-400 nm) and blue (B; 400-499 nm) light promote the synthesis of phenolic compounds including anthocyanins (Li and Kubota, 2009; Son and Oh, 2013). Typically, these wavebands of light are delivered to plants during the entire crop production cycle and thus the effects of each are persistent from transplant until harvest.

However, growers can dynamically change the light spectrum during specific stages of the production cycle to differentially regulate growth and biochemical processes. For instance, FR light could be applied to lettuce seedlings to promote early leaf expansion, allowing for greater light interception and subsequent photosynthesis later in production (Klassen et al., 2003; Legendre and van Iersel, 2021; Park and Runkle, 2017). Alternatively, UVA or B light can be delivered at the end of production (EOP) to increase total anthocyanin concentration (TAC) and subsequent leaf pigmentation of lettuce (Chen et al., 2019; Kelly and Runkle, 2023; Owen and Lopez, 2015). In addition, the delivery of short-waveband light at the EOP has a limited or no effect on suppressing extension growth or biomass accumulation (Chen et al., 2019). Finally,

compared to a static lighting spectrum during the entire production cycle, limiting the use of some wavebands to a production phase can decrease electricity consumption (Pinho et al., 2013; Schwend et al., 2016). The challenge of dynamic or phasic lighting strategies is that growers need a comprehensive understanding of when and how to change their lighting spectrum. Additionally, multi-waveband, tunable LED packages must be available at a reasonable price (Viršile et al., 2017) or they must have multiple growing areas with different light spectra during the production process.

While the harvestable yield of lettuce is of paramount importance when designing the indoor lighting environment, nutritional composition and density, taste, and leaf coloration affect marketability and also likely influence consumers' willingness to buy the product again. Short-wave radiation in the form of UVA and B light can potentially increase the concentrations of nutritious secondary metabolites that also influence taste and leaf pigmentation. For instance, plant phenolic compounds influence perceived taste, while a specific group of phenolic compounds, anthocyanins, are red/purple pigments that affect the color of red-leaf lettuce (de Pascual-Teresa and Sanchez-Ballesta, 2008; Soares et al., 2013). Cryptochrome 1 is the dominant photoreceptor regulating phenolic compound biosynthesis, and specifically, a functional cryptochrome promotes phenolic compound synthesis in response to UVA and B light (Brelsford et al., 2019). While the same photoreceptor regulates phenolic compound synthesis in response to UVA and B light, there is conflicting information on whether these wavebands are equally effective at increasing total phenolic concentration (TPC) in lettuce. This is at least partly because studies compared different PFDs of UVA and B light. For instance, 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of UVA (peak = 385 nm) added to white light did not affect lettuce 'Rouxai' TPC, but 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light increased TPC by 25% (Vaštakaitė-Kairienė et al., 2021). Additionally,

Li and Kubota (2009) reported that increasing the percentage of UVA or B light in a white-light spectrum from 1 to 6% or 23 to 55%, respectively, did not affect TPC, but did increase TAC by up to 31% in lettuce 'Red Cross'. Finally, UVA light (peak = 365 nm) applied to lettuce 'Klee' increased both TPC and TAC (Chen et al., 2019). The inconsistent effects of UVA light on plant phenolics suggest that the effects depend on the lettuce cultivar, peak waveband, and/or dose (PFD and duration) of UVA application.

A potential detriment of delivering more than a modest percentage of UVA or B light in a lighting spectrum is their suppression of extension growth, which can decrease the leaf area available for light capture, whole-plant photosynthesis, and biomass accumulation (Cosgrove, 1981). Thus, plants grown under a moderate intensity of B light typically have less biomass than those grown under identical conditions but with less or no B light (Son and Oh, 2013). The effects of UVA on extension growth are less clear than B light. Some studies indicate that there is an inhibitory effect on growth, similar to B light (Krizek et al., 1998; Tsormpatsidis et al., 2008), while others report no effect (Vaštakaitė-Kairienė et al., 2021) or even a promotion of leaf expansion and plant growth (Chen et al., 2019; Lee et al., 2013).

While studies have reported favorable results with EOP lighting on indoor-grown leafy greens (Chen et al., 2019; Hooks et al., 2021; Kelly and Runkle, 2023), we are not aware of studies that have directly compared EOP lighting to continuous lighting with the same spectrum or for different phases of production (e.g., seedling stage or middle of production) to determine if there are any persistent effects on harvested lettuce. Therefore, the objectives of this study were to determine 1) when UVA or B light could be applied during production to increase lettuce quality and nutritional attributes and 2) if EOP UVA or B light is as effective at increasing lettuce phytonutrients and leaf pigmentation as applying UVA or B light during the entire

production cycle. We postulated that increased UVA or B light applied 1) during the final phase of production would be the most effective dynamic strategy to increase phytonutrients and leaf pigmentation at harvest, and 2) during the entire production cycle would increase concentrations of phytonutrients and leaf pigmentation but at the expense of fresh mass (FM).

Materials and Methods

Chemicals

We used sulfuric acid (H_2SO_4; ACS, ≥ 99.5–98.0%), methanol (≥ 99.9%), Folin & Ciocalteu's phenol reagent (F–C reagent), sodium carbonate (Na_2CO_3; ≥ 99.0%), gallic acid (GA; anhydrous), hydrochloric acid (HCL; ACS, ≥ 37.0%), potassium chloride (KCL; ACS, ≥ 99.0%), and sodium acetate (CH_3COONa; ACS, ≥ 99.0%) from Sigma–Aldrich (Merck KGaA, Darmstadt, Germany) for both replications of the experiment.

Plant material and propagation

We sowed 500 seeds of the red-leaf lettuce cultivar 'Rouxai' (Johnny's Selected Seeds, Winslow, ME, USA) in a refrigerated growth room (the Controlled Environment Lighting Laboratory) at Michigan State University. 'Rouxai' was chosen because of its commercial relevance, sensitivity to the light spectrum, and use in previous experiments. Seeds were sown on July 8, 2020 (Rep. 1) and August 13, 2020 (Rep.2) in 200-cell (2.5 cm × 2.5 cm) rockwool plugs (AO 25/40 Starter Plugs; Grodan, Milton, ON, Canada) that were presoaked in deionized water adjusted to a pH of 4.5 using 10% diluted H_2SO_4. From day 0 (seed sow) to day 4, we covered the seedling trays with clear plastic domes to increase humidity. We grew them at a constant 23 °C under a 24-h photoperiod at a total PFD (TPFD; 315-800 nm) of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by warm-white (WW; peak = 639 nm, correlated color temperature = 2700 K) LEDs (PHYTOFY RL; OSRAM, Beverley, MA) controlled by customized software (Spartan Control

Software; OSRAM). On day 4, we removed the humidity domes and separated the seedlings into ten separate groups. We grew the seedlings under 75 μmol·m^{-2}·s^{-1} from R LEDs (peak = 664 nm) plus 75 μmol·m^{-2}·s^{-1} from WW LEDs and their respective phase 1 (P1) treatments (Figure II-1; Table II-1; Table II-2) at a 20-h photoperiod until day 12. We hand-watered the seedlings until transplant on day 12 with deionized water supplemented with a water-soluble fertilizer (12N–4P_2O_5–16K_2O RO Hydro FeED; JR Peters, Inc., Allentown, PA) and magnesium sulfate (Epsom salt; Pennington Seed, Inc., Madison, GA) with the following nutrients (in mg·L^{-1}): 125 N, 42 P, 167 K, 73 Ca, 49 Mg, 39 S, 1.7 Fe, 0.52 Mn, 0.56 Zn, 0.13 B, 0.47 Cu, and 0.13 Mo. The pH and the electrical conductivity (EC) were set to 5.6 and 1.6 mS·cm^{-1}, respectively, as measured by a pH/EC meter (HI9814; Hanna Instruments, Woonsocket, RI).

Growth conditions and dynamic lighting treatments

Inside the growth room, we used four vertical hydroponic growing racks with three canopies each to create ten different lighting treatments during each phase. On day 12, we transplanted seedlings from each P1 treatment into floating 36-cell rafts (Beaver Plastics, Ltd., Acheson, AB, Canada) with 2.5-cm-wide holes that were spaced 20 × 15 cm apart. We used the same nutrient solution as described above but increased the nutrient concentrations by 20% (150 mg N·L^{-1}). We measured and adjusted the pH and EC daily (as previously described) using potassium bicarbonate and H_2SO_4 to maintain an average of 5.7 and 1.9 mS·cm^{-1}, respectively. Additionally, we set an air temperature of 23 °C during the day and night; however, the actual air temperature averaged 22.8 ± 1.6 °C in both replications. Finally, sensors were used to monitor plant canopy temperature (25.0 ± 1.3 °C in both replications), relative humidity (Rep. 1 = 64.2 ± 4.7%, Rep. 2 = 60.7 ± 6.2%), and CO_2 concentration (Rep. 1 = 377 ± 7 μmol·mol^{-1}, Rep. 2 = 381

± 10 µmol·mol^{-1}). Specific information about experimental conditions, equipment, and sensors can be found in Kelly et al. (2020).

Lighting treatments were delivered during three distinct phases of plant growth: P1, phase 2 (P2; day 12 to 20), and phase 3 (P3; day 20 to 28). During each phase, we grew the plants under a base spectrum delivered by R LEDs and WW LEDs, each at 75 µmol·m^{-2}·s^{-1}, and supplemental lighting treatments during none, one of the three phases, or all three phases. Supplemental lighting treatments (Figure II-1; Table II-1; Table II-2), regardless of when they were delivered, consisted of 30 µmol·m^{-2}·s^{-1} of UVA (peak = 386 nm), 30 µmol·m^{-2}·s^{-1} of B (peak = 449 nm), or 20 µmol·m^{-2}·s^{-1} of R and 10 µmol·m^{-2}·s^{-1} of green (G; peak = 532 nm) light, which increased the TPFD of the light spectrum to 180 µmol·m^{-2}·s^{-1} for at least one phase of the experiment (Table II-1). We created one treatment with no supplemental lighting (control), one with R+G light applied during all three phases to act as another control at a higher TPFD, one with UVA and one with B applied during all three phases, and six treatments with either UVA or B light applied during one of the three phases (Table II-2). We measured the TPFD and light spectrum using a portable spectroradiometer (PS200; Apogee Instruments, Inc., Logan, UT.) from nine representative spots at the plant canopy and averaged them.

Data collection and analysis

On days 12, 20, and 28, we collected leaf tissue from three randomly selected plants from each treatment for TPC and TAC analysis. For TPC analysis, we collected 0.5 g of light-exposed leaf tissue from each biological sample, immediately froze it in liquid nitrogen, and processed and analyzed samples spectrophotometrically according to the protocol used in Kelly and Runkle (2023), which was based on the Ainsworth and Gillespie (2007) protocol, with slight modifications. Similarly, for TAC analysis, we collected 0.3 g of light-exposed leaf tissue from

each biological sample, froze it in liquid nitrogen, and processed and analyzed the samples using a modified version of the Lee et al. (2005) pH differential method (AOAC Official Method 2005.2) described in Kelly and Runkle (2023). Additionally, on days 12, 20, and 28, we used the same three plants used for biochemical analysis to measure relative chlorophyll concentration using a SPAD meter (SPAD-502; Konica Minolta Sensing, Inc., Osaka, Japan) by measuring and averaging three spots on one fully expanded leaf exposed to direct light. Finally, before biochemical analysis, we took overhead pictures of each plant for leaf coloration analysis using an R code developed to determine the lightness (black: $L^* = 0$; white: $L^* = 100$), redness (green: $a^* = -128$; red: $a^* = 127$), and blueness (blue: $b^* = -128$; yellow: $b^* = 127$) of each pixel of an imported TIFF picture. The L^*, a^*, and b^* values of each pixel were generated and averaged to quantify the average coloration of an entire plant from overhead.

On day 28, we collected morphological data from ten randomly selected plants from each lighting treatment that were not used for biochemical analysis. We cut the lettuce shoots from the rockwool substrates and weighed each using an analytical balance (AG245; Mettler Toledo, Columbus, OH). We also measured plant diameter (cm), length and width of the fifth fully expanded leaf, and counted leaf number (> 2 cm in length). We then packed the shoots into paper bags and dried them for 7 days at 60 °C in a drying oven (Blue M, Blue Island, IL) then weighed each with the same balance.

We arranged the experiment as a randomized complete block design with two replications in time (July 8, 2020–August 5, 2020; August 13, 2020–September 10, 2020) and performed statistical analysis using R statistical analysis software (R Core Team, 2014; version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria). We conducted an analysis of

variance (ANOVA) and Tukey's honestly significant difference test ($\alpha = 0.5$) using the R packages 'dplyr' (Wickham et al., 2022) and 'agricolae' (Mendiburu, 2021).

Results

Shoot fresh and dry mass

We measured lettuce shoot FM at the end of P3 (final harvest), which was day 28 (Figure II-2). Generally, dynamic supplemental lighting treatments applied during P1, P2, or P3 did not affect FM at harvest, except $P1B_{30}$, which increased FM by 23% compared to the control. Delivery of ET UVA light did not affect FM, but ET R_{20}+G_{10} increased it by 21% compared to the control, while ET B_{30} decreased FM by 24%. Similarly, $P1B_{30}$ and ET R_{20}+G_{10} increased shoot dry mass by 27% and 21%, respectively, compared to the control treatment. In addition, both EOP treatments ($P3UVA_{30}$ and $P3B_{30}$) increased shoot dry mass by 18% and 20% (Table II-3).

Total phenolic and anthocyanin concentration

At harvest (day 28), 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of UVA or B light delivered during P3 or ET increased TPC of lettuce 'Rouxai' (Figure II-3). For example, UVA ($P3UVA_{30}$) or B ($P3B_{30}$) light delivered during P3 increased TPC by 74% and 109%, respectively, compared to the control treatment with no supplemental light, but TPC was similar between the $P3UVA_{30}$ and $P3B_{30}$ treatments. Likewise, ET UVA_{30} or B_{30} added to the broad-band spectrum similarly increased lettuce TPC by 93% and 135%, respectively, compared to the control. Additionally, the duration of supplemental UVA or B light application before harvest did not affect lettuce 'Rouxai' TPC. Both $P3UVA_{30}$ and ET UVA_{30} led to similar TPC at harvest, as did $P3B_{30}$ and ET B_{30} light. The only exception was that ET B_{30} increased lettuce TPC by 35% compared to $P3UVA_{30}$. Providing supplemental UVA or B light during P1 or UVA light during P2 did not

affect TPC, although B light applied during P2 slightly increased TPC compared to the control treatment. Finally, increasing the TPFD during the entire production period with additional R and G light (ET R_{20}+G_{10}) did not affect TPC.

Comparable to TPC, 30 μmol·m^{-2}·s^{-1} of EOP or ET UVA or B light increased TAC of lettuce 'Rouxai' (Figure II-3). P3UVA_{30}, P3B_{30}, ET UVA_{30}, and ET B_{30} similarly increased TAC by 321%, 423%, 356%, and 414%, respectively, compared to the control treatment. UVA and B light were similarly effective when applied as EOP lighting or continuously. Additionally, EOP and ET supplemental lighting led to similar TAC. Supplemental UVA or B light applied during P1 or P2 did not influence TAC of lettuce 'Rouxai' when harvested at the end of P3. Finally, increasing the TPFD by providing additional ET R_{20}+G_{10} did not affect TAC.

We also harvested lettuce 'Rouxai' and measured TPC and TAC at the end of P1 (day 12) and P2 (day 20). At the end of P1, TPC was similar among all treatments. When we harvested plants at the end of P2, there were some significant differences between treatments. Compared to the control treatment, P2B_{30}, ET UVA_{30}, and ET B_{30} similarly increased TPC, by 35% to 42%. Lettuce grown under all other supplemental lighting treatments was statistically similar to the control treatment.

Unlike TPC, TAC of lettuce 'Rouxai' harvested at the end of P1 was influenced by supplemental lighting treatments. P1UVA_{30} and P1B_{30} greatly increased TAC, by 6500% and 14300%, respectively, whereas ET R_{20}+G_{10} did not affect TAC compared to the control treatment. At the end of P1, B light was more effective at increasing TAC of lettuce 'Rouxai' than UVA light. Furthermore, at the end of P2, supplemental light applied during P2, or continuous light, led to the greatest change in TAC. P2UVA_{30}, P2B_{30}, and ET UVA_{30} similarly increased TAC, by 187% to 255%. ET B_{30}, on the other hand, increased TAC by 347%

(compared to the control), which was similar to $P2B_{30}$ but greater than both UVA treatments. Supplemental UVA or B light applied during P1 or containing R plus G light did not affect lettuce 'Rouxai' TAC.

Leaf coloration and relative chlorophyll concentration

At final harvest, $P3UVA_{30}$, $P3B_{30}$, ET UVA_{30}, and ET B_{30} increased leaf redness (more positive a^* value) compared to the control treatment by 22%, 55%, 39%, and 71%, respectively (Figure II-4). Both EOP and ET UVA supplemental light produced similar leaf redness, as did both B supplemental lighting treatments. Although all four treatments consisting of either EOP or ET UVA or B light increased leaf redness compared to the control treatment, both B treatments were more effective than their analogous UVA treatments. For example, lettuce grown under the EOP B treatment ($P3B_{30}$) had 42% redder leaves than plants under the $P3UVA_{30}$ treatment. Additionally, ET B light produced 52% redder leaves than ET UVA light. Lettuce grown with supplemental UVA or B light during P1 or P2, or ET R+G light, had a similar leaf redness as the control treatment. Lettuce 'Rouxai' blueness increased when supplemental EOP or ET light was applied. $P3B_{30}$, ET UVA_{30}, and ET B_{30} decreased b^* (increased leaf blueness) by 33%, 21%, and 41%, respectively, compared to the control. Additionally, lettuce grown under ET B light had bluer leaves than all treatments except lettuce grown under EOP B light, whose leaves were similarly blue. Finally, the lightness and darkness (L^* values) of the lettuce leaves were similar among treatments (Table II-4).

At the end of P3, we measured relative chlorophyll concentrations using a SPAD meter. Similar to TAC and leaf redness, relative chlorophyll concentration was the greatest in lettuce grown under EOP or ET B light (Figure II-4). $P3B_{30}$ and ET B_{30} increased the SPAD index by 31% and 26%, respectively, compared to the control, and were similar to plants under the

P3UVA$_{30}$ and ET UVA$_{30}$ treatments. Interestingly, EOP UVA light increased the SPAD index of lettuce by 23% compared to the control treatment, but lettuce grown under ET UVA light had similar SPAD values to that of the control treatment.

We measured TPC, TAC, and leaf coloration earlier in production at the end of P1 and P2 to determine whether the effects of supplemental lighting were transient or persistent. When plants were harvested at the end of P1, leaf coloration followed similar trends as plants harvested at the end of P3 (Table II-4). Lettuce grown under the P1UVA$_{30}$ and P1B$_{30}$ treatments had statistically similar leaf redness to each other, but only P1B$_{30}$ increased leaf redness compared to the control treatment (46% increase). Although lettuce grown under P1B$_{30}$ and P1UVA$_{30}$ had similar leaf blueness, P1B$_{30}$ produced 29% bluer leaves than the control treatment, while P1UVA$_{30}$ did not. Lettuce grown under P1UVA$_{30}$ or P1B$_{30}$ was darker than the control treatment, by 12% and 20%, respectively, although B light led to slightly darker leaves than UVA light. Compared to the control treatment, lettuce grown under P1UVA$_{30}$ or P1B$_{30}$ had a 24% to 26% greater relative chlorophyll concentration. At the end of P2, P2UVA$_{30}$, P2B$_{30}$, ET UVA$_{30}$, and ET B$_{30}$ increased leaf redness by 72%, 90%, 73%, and 95%, respectively, compared to the control treatment, but leaf redness was 83% greater under ET B$_{30}$ than P2UVA$_{30}$. These same supplemental lighting treatments led to bluer (and darker) leaves by 43% (28%), 56% (35%), 46% (33%), and 58% (38%), respectively, compared to the control treatment. Finally, P2B$_{30}$ was the only treatment that increased the SPAD index of lettuce compared to that of the control (by 19%), but it was statistically similar to that under the P2UVA$_{30}$, ET UVA$_{30}$, and ET B$_{30}$ treatments.

Plant morphology

There were few plant morphological differences between treatments except that lettuce grown under $P1B_{30}$ was 12% wider and had 17% more leaves than the control treatment (Table II-3). Additionally, lettuce grown under ET B_{30} had 14% shorter leaves than the control treatment. Other supplemental lighting treatments did not affect plant diameter or leaf length, width, or number. Figure II-5 shows representative plants from each light treatment and the slight differences in plant shape and size.

Discussion

UVA and blue light are similarly effective at increasing secondary metabolite production

UV and B light-absorbing phenolic compounds, such as flavonoids and anthocyanins, accumulate in plants grown under UVA and B light as a stress response to limit damage caused by environmental factors like short-wave radiation (Naikoo et al., 2019). Under high-energy radiation, plants accumulate reactive-oxygen species, which act as a signal for the activation of phenylalanine ammonia-lyase (PAL) (Rabelo et al., 2020; Surjadinata et al., 2017). PAL catalyzes the first committed step of the phenylpropanoid biosynthesis pathway, from which phenolic compounds such as flavonoids are derived (Bate et al., 1994; Vogt, 2010). *CHALCONE SYNTHASE (CHS)*, the gene that encodes the first committed enzyme in the flavonoid biosynthesis pathway, is expressed under UVA or B light in plants with a functional cry1 (Jenkins et al., 2001; Wade et al., 2001) and is associated with increased concentrations of flavonoids (Park et al., 2007). Plants with a non-functional cryptochrome 1 (*cry1*) showed reduced B-light induction of *CHS* and no induction of *CHS* under UVA light, indicating that UVA or B light induces the expression of *CHS* to advance the flavonoid biosynthesis pathway (Fuglevand et al., 1996; Jackson and Jenkins, 1995; Jenkins et al., 2001). Additionally,

cryptochrome 2 (cry2) induces anthocyanin biosynthesis under UV or B light, but it begins to degrade under a higher PFD (Ahmad et al., 1998; Lin et al., 1998). The similar increase in lettuce 'Rouxai' TPC and TAC grown under UVA or B light can be attributed to cry1's, and possibly cry2's, role in mediating flavonoid biosynthesis.

There are some inconsistencies between studies on whether UVA or B light is the most effective light at increasing concentrations of specific phenolic compounds and total content. For example, Vaštakaitė-Kairienė et al. (2021) added 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light (peak = 449 nm) to WW light for 18 days and found TPC and TAC of baby leaf lettuce 'Rouxai' increased by 25% and 95%, respectively. However, when they added 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of UVA (peak = 385 nm) light to WW light, there was no effect on TPC or TAC. Additionally, supplemental UVA or B light may not always increase both TPC and TAC, especially if B light is already present in the light spectrum. Increasing the B (peak = 476 nm) light percentage from 23% to 55% in a white spectrum or supplementing an additional 18 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of UVA (peak = 373 nm) light increased TAC but not TPC of lettuce 'Red Cross' (Li and Kubota, 2009). The discrepancies between the effectiveness of UVA and B light is presumably due to differences in the PFDs of each waveband used and the background light spectrum.. When UVA (peak = 386 nm) or B (peak = 449 nm) light was added to WW+R light for the last six days of production at the same PFD (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$), TPC and TAC of lettuce 'Rouxai' similarly increased (Kelly and Runkle, 2023). Similarly, in the current study, adding 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of UVA (peak = 386 nm) or B (peak = 449 nm) light to WW+R light similarly increased TPC and TAC of lettuce 'Rouxai' when applied for the last eight days of production or the entire production cycle. Therefore, when UVA and B light are applied at the same PFD, they are likely to be similarly effective at

advancing key steps in the phenylpropanoid and flavonoid biosynthesis pathways, leading to phenolic compound synthesis and accumulation.

Furthermore, no studies have directly compared EOP to ET UVA or B light, but data presented here indicate both lighting strategies are similarly effective at increasing phytochemical content. Young leaves that accumulated phenolic compounds under the P1 or P2 lighting treatments were eventually covered and shaded by new leaves that emerged by the end of the production cycle. During this time, the red-purple coloration of shaded leaves began to decrease, and phenolic compounds, particularly anthocyanins, began to degrade under less light (Oren-Shamir, 2009; Zhao et al., 2021). Additionally, as young leaves continued to grow, the anthocyanin compounds became more diluted and concentrations decreased likely due to a decrease in anthocyanin biosynthesis, an increase in chlorophyll concentrations, and an increase in leaf expansion (Oren-Shamir, 2009). Therefore, since phenolic compounds in the lower leaves began to degrade as new leaves covered them, only new growth that occurred during the final eight days of production, when both the P3 and ET treatments were active, had elevated TPC at harvest.

Phase 1 and 2 supplemental light did not affect plants at harvest

We delivered supplemental lighting treatments at the beginning (P1) or middle (P2) of the production cycle to test if there was any residual effect of UVA or B light on plants at harvest. Plants harvested immediately after supplemental UVA or B light during P1 or P2 had greater TPC and TAC than plants grown without supplemental light (Table II-3). However, these increases diminished following cessation of the supplemental UVA or B light, indicating that there is no residual effect of supplemental UVA or B light on lettuce at harvest. Similarly, lettuce seedlings treated with B light had greater anthocyanin concentrations when harvested on day 17,

but not when B light was deficient for the remaining production period (Johkan et al., 2010). Finally, mature lettuce plants are more capable of accumulating anthocyanins than young plants (Sng et al., 2021), which further negates the benefit of enriching the light spectrum with UVA or B light during the early stages of production.

ET blue light suppressed biomass accumulation

A moderate or high PFD of B light suppresses extension growth and biomass accumulation of many plant species (Cosgrove, 1981; Ohashi-Kaneko et al., 2007). Our results with B light are consistent with other studies that have shown ET B light can suppress biomass accumulation compared to other wavebands within the photosynthetic active radiation (400-700 nm) range (Shin et al., 2014; Son and Oh, 2015). For example, continuously applied B light inhibited leaf extension growth metrics such as plant canopy size, leaf area, and leaf width and length, which inhibited biomass accumulation (Meng et al., 2020; Son and Oh, 2013). During the young plant stages (days 4-20), B light continuously suppressed extension growth, leading to a smaller plant canopy, which inhibited leaf surface area available for light capture and photosynthesis. Plants grown without supplemental B light during the young plant stages developed more fully expanded leaves, which increased light capture and subsequent biomass accumulation. Plants grown with supplemental B light during P3 did not experience early growth suppression, leading to a larger plant canopy than plants grown under supplemental B light continuously.

Conclusion

If equipment is available to dynamically light plants, such as spectrally tunable LED fixtures, enriching the light spectrum with UVA or B light only towards the EOP cycle can increase secondary metabolite concentrations without negatively impacting biomass

accumulation. Continuous enrichment of the light spectrum with UVA or B light provides similar advantages to EOP lighting, except that supplemental B light inhibits leaf expansion and biomass accumulation. Moreover, providing UVA or B light during P1 or P2 had no lasting effect on TPC, TAC, or leaf coloration of lettuce harvested at the end of production. More research is needed to determine specific PFDs and wavebands of UVA or B light that are most efficient at enhancing desired quality attributes.

Acknowledgements

This work was supported by the Specialty Crops Research Initiative (grant no. 2019-51181-30017) and Hatch project 192266 from the USDA National Institute of Food and Agriculture. We thank Ryan Warner for use of his laboratory and Mckenna Merkel and Viktorija Vaštakaitė-Kairienė for experimental assistance. We also thank Leslie Bourquin, Chieri Kubota, and Ryan Warner for their critical review of this manuscript.

LITERATURE CITED

Ahmad, M., Jarillo, J.A., Smirnova, O., Cashmore, A.R., 1998. Cryptochrome blue-light photoreceptors of *Arabidopsis* implicated in phototropism. Nature 392, 720–723. https://doi.org/10.1038/33701

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc. 24, 875–877. https://doi.org/10.1038/nprot.2007.102

Bate, N.J., Orr, J., Ni, W., Meromi, A., Nadler-Hassar, T., Doerner, P.W., Dixon, R.A., Lamb, C.J., Elkind, Y., 1994. Quantitative relationship between phenylalanine ammonia-lyase levels and phenylpropanoid accumulation in transgenic tobacco identifies a rate-determining step in natural product synthesis. Proc. Natl. Acad. Sci. 91, 7608–7612. https://doi.org/10.1073/pnas.91.16.7608

Brelsford, C.C., Morales, L.O., Nezval, J., Kotilainen, T.K., Hartikainen, S.M., Aphalo, P.J., Robson, T.M., 2019. Do UV-A radiation and blue light during growth prime leaves to cope with acute high light in photoreceptor mutants of *Arabidopsis thaliana*? Physiol. Plant. 165, 537–554. https://doi.org/10.1111/PPL.12749

Chen, Y., Li, T., Yang, Q., Zhang, Y., Zou, J., Bian, Z., Wen, X., 2019. UVA radiation is beneficial for yield and quality of indoor cultivated lettuce. Front. Plant Sci. 10, 1563. https://doi.org/10.3389/FPLS.2019.01563/BIBTEX

Cosgrove, D.J., 1981. Rapid Suppression of growth by blue light, Plant Physiol. 63, 584–590. https://doi.org/10.1104%2Fpp.67.3.584

de Pascual-Teresa, S., Sanchez-Ballesta, M.T., 2008. Anthocyanins: from plant to health. Phytochem. Rev. 7, 281–299. https://doi.org/10.1007/s11101-007-9074-0

Fuglevand, G., Jackson, J.A., Jenkins, G.I., 1996. UV-B, UV-A, and blue light signal transduction pathways interact synergistically to regulate chalcone synthase gene expression in *Arabidopsis*. Plant Cell 8, 2347–2357. https://doi.org/10.1105/TPC.8.12.2347

Hooks, T., Masabni, J., Sun, L., Niu, G., 2021. Effect of pre-harvest supplemental UV-A/blue and red/blue LED lighting on lettuce growth and nutritional quality. Horticulturae 7, 80. https://doi.org/10.3390/horticulturae7040080

Jackson, J.A., Jenkins, G.I., 1995. Extension-growth responses and expression of flavonoid biosynthesis genes in the *Arabidopsis* hy4 mutant. Planta 197, 233–239. https://doi.org/10.1007/BF00202642

Jenkins, G.I., Long, J.C., Wade, H.K., Shenton, M.R., Bibikova, T.N., 2001. UV and blue light signalling: pathways regulating chalcone synthase gene expression in *Arabidopsis*. New Phytol. 151, 121–131. https://doi.org/10.1046/J.1469-8137.2001.00151.X

Johkan, M., Shoji, K., Goto, F., Hashida, S. nosuke, Yoshihara, T., 2010. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. HortScience 45, 1809–1814. https://doi.org/10.21273/hortsci.45.12.1809

Kelly, N., Choe, D., Meng, Q., Runkle, E.S., 2020. Promotion of lettuce growth under an increasing daily light integral depends on the combination of the photosynthetic photon flux density and photoperiod. Sci. Hortic. 272, 109565. https://doi.org/10.1016/j.scienta.2020.109565

Kelly, N., Runkle, E.S., 2023. End-of-production ultraviolet A and blue light similarly increase lettuce coloration and phytochemical concentrations. HortScience 58, 525–531. https://doi.org/10.21273/HORTSCI17108-23

Klassen, S.P., Ritchie, G., Frantz, J.M., Pinnock, D., Bugbee, B., 2003. Real-time imaging of ground cover: relationships with radiation capture, canopy photosynthesis, and daily growth rate, in: Digital Imaging and Spectral Techniques: Applications to Precision Agriculture. John Wiley & Sons, Ltd, pp. 1–14. https://doi.org/10.2134/ASASPECPUB66.C1

Kozai, T., Niu, G., 2016. Plant factory as a resource-efficient closed plant production system, in: Plant Factory: An Indoor Vertical Farming System for Efficient Quality Food Production. pp. 69–90. https://doi.org/10.1016/B978-0-12-801775-3.00004-4

Krizek, D.T., Britz, S.J., Mirecki, R.M., 1998. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cv. New Red Fire lettuce. Physiol. Plant. 103, 1–7. https://doi.org/10.1034/j.1399-3054.1998.1030101.x

Lee, J., Durst, R.W., Wrolstad, R.E., Barnes, K.W., Eisele, ; T, Giusti, ; M M, Haché, ; J, Hofsommer, ; H, Koswig, ; S, Krueger, D.A., Kupina, ; S, Martin, ; S K, Martinsen, ; B K, Miller, T.C., Paquette, ; F, Ryabkova, ; A, Skrede, ; G, Trenn, ; U, Wightman, J.D., 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J. AOAC Int. 88, 1269–1278. https://doi.org/10.1093/JAOAC/88.5.1269

Lee, M.-J., Son, J.E., Oh, M.-M., 2013. Growth and phenolic compounds of *Lactuca sativa* L. grown in a closed-type plant production system with UV-A, -B, or -C lamp. J. Sci. Food Agric. 94, 197–204. https://doi.org/10.1002/jsfa.6227

Legendre, R., van Iersel, M.W., 2021. Supplemental far-red light stimulates lettuce growth: disentangling morphological and physiological effects. Plants 2021 Vol 10 Page 166 10, 166. https://doi.org/10.3390/PLANTS10010166

Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67, 59–64. https://doi.org/10.1016/j.envexpbot.2009.06.011

Lin, C., Yang, H., Guo, H., Mockler, T., Chen, J., Cashmore, A.R., 1998. Enhancement of blue-light sensitivity of *Arabidopsis* seedlings by a blue light receptor cryptochrome 2. Proc. Natl. Acad. Sci. U. S. A. 95, 2686–2690. https://doi.org/10.1073/PNAS.95.5.2686

Mendiburu F. 2021. Agricolae: statistical procedures for agricultural research. https://CRAN.R-project.org/package=agricolae.

Meng, Q., Boldt, J., Runkle, Erik.S., 2020. Blue radiation interacts with green radiation to influence growth and predominantly controls quality attributes of lettuce. J. Am. Soc. Hortic. Sci. 145, 75–87.

Naikoo, M.I., Dar, M.I., Raghib, F., Jaleel, H., Ahmad, B., Raina, A., Khan, F.A., Naushin, F., 2019. Role and regulation of plants phenolics in abiotic stress tolerance: an overview. Plant Signal. Mol. Role Regul. Stress. Environ. 157–168. https://doi.org/10.1016/B978-0-12-816451-8.00009-5

Ohashi-Kaneko, K., Tarase, M., Kon, N., Fujiware, K., Kurata, K., 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. Environ. Control Biol. 45, 189–198. https://doi.org/10.2525/ecb.45.189

Oren-Shamir, M., 2009. Does anthocyanin degradation play a significant role in determining pigment concentration in plants? Plant Sci. 177, 310–316. https://doi.org/10.1016/j.plantsci.2009.06.015

Owen, W.G., Lopez, R.G., 2015. End-of-production supplemental lighting with red and blue light-emitting diodes (LEDs) influences red pigmentation of four lettuce varieties, HortScience 50, 676–684. https://doi.org/10.21273/HORTSCI.50.5.676

Park, J.-S., Choung, M.-G., Kim, J.-B.J.-B., Hahn, B.-S., Kim, J.-B.J.-B., Bae, S.-C., Roh, K.-H., Kim, Y.-H., Cheon, C.-I., Sung, M.-K., Cho, K.-J., 2007. Genes up-regulated during red coloration in UV-B irradiated lettuce leaves. Plant Cell Rep. 26, 507–516. https://doi.org/10.1007/s00299-006-0255-x

Park, Y., Runkle, E.S., 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Exp. Bot. 136, 41–49. https://doi.org/10.1016/J.ENVEXPBOT.2016.12.013

Pinho, P., Hytönen, T., Rantanen, M., Elomaa, P., Halonen, L., 2013. Dynamic control of supplemental lighting intensity in a greenhouse environment: Light. Res. Technol. 45, 295–304. https://doi.org/10.1177/1477153512444064

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rabelo, M.C., Bang, W.Y., Nair, V., Alves, R.E., Jacobo-Velázquez, D.A., Sreedharan, S., de Miranda, M.R.A., Cisneros-Zevallos, L., 2020. UVC light modulates vitamin C and phenolic biosynthesis in acerola fruit: role of increased mitochondria activity and ROS production. Sci. Rep. 10, 21972. https://doi.org/10.1038/s41598-020-78948-1

Schwend, T., Beck, M., Prucker, D., Peisl, S., Mempel, H., 2016. Test of a PAR sensor-based, dynamic regulation of LED lighting in greenhouse cultivation of Helianthus annuus. Eur. J. Hortic. Sci. 81, 152–156. https://doi.org/10.17660/EJHS.2016/81.3.3

Shin, Y.-S., Lee, M.-J., Lee, E.-S., Ahn, J.-H., Kim, M.-K., Lee, J.-E., Do, H.-W., Cheung, J.-D., Park, J.-U., Um, Y.-G., Park, S.-D., Chae, J.-H., 2014. Effect of light emitting diodes treatment on growth and quality of lettuce (*Lactuca sativ*a L. 'Oak Leaf'). J. Life Sci. 24, 148–153. https://doi.org/10.5352/JLS.2014.24.2.148

Sng, B.J.R., Mun, B., Mohanty, B., Kim, M., Phua, Z.W., Yang, H., Lee, D.-Y., Jang, I.-C., 2021. Combination of red and blue light induces anthocyanin and other secondary metabolite biosynthesis pathways in an age-dependent manner in Batavia lettuce. Plant Sci. 310, 110977. https://doi.org/10.1016/j.plantsci.2021.110977

Soares, S., Kohl, S., Thalmann, S., Mateus, N., Meyerhof, W., De Freitas, V., 2013. Different phenolic compounds activate distinct human bitter taste receptors. J. Agric. Food Chem. 61, 1525–1533. https://doi.org/10.1021/jf304198k

Son, K.H., Oh, M.M., 2015. Growth, photosynthetic and antioxidant parameters of two lettuce cultivars as affected by red, green, and blue light-emitting diodes. Hortic. Environ. Biotechnol. 2015 565 56, 639–653. https://doi.org/10.1007/S13580-015-1064-3

Son, K.-H., Oh, M.-M., 2013. Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. HortScience 48, 988–995.

Surjadinata, B.B., Jacobo-Velázquez, D.A., Cisneros-Zevallos, L., 2017. UVA, UVB and UVC light enhances the biosynthesis of phenolic antioxidants in fresh-cut carrot through a synergistic effect with wounding. Molecules 22, 668. https://doi.org/10.3390/molecules22040668

Tsormpatsidis, E., Henbest, R.G.C., Davis, F.J., Battey, N.H., Hadley, P., Wagstaffe, A., 2008. UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. Environ. Exp. Bot. 63, 232–239. https://doi.org/10.1016/J.ENVEXPBOT.2007.12.002

Vaštakaitė-Kairienė, V., Kelly, N., Runkle, E.S., 2021. Regulation of the photon spectrum on growth and nutritional attributes of baby-leaf lettuce at harvest and during postharvest storage. Plants 2021 Vol 10 Page 549 10, 549–549. https://doi.org/10.3390/PLANTS10030549

Viršile, A., Olle, M., Duchovskis, P., 2017. LED lighting in horticulture, in: Light Emitting Diodes for Agriculture: Smart Lighting. Springer Singapore, pp. 113–147. https://doi.org/10.1007/978-981-10-5807-3_7/TABLES/3

Vogt, T., 2010. Phenylpropanoid biosynthesis. Mol. Plant 3, 2–20. https://doi.org/10.1093/mp/ssp106

Wade, H.K., Bibikova, T.N., Valentine, W.J., Jenkins, G.I., 2001. Interactions within a network of phytochrome, cryptochrome and UV-B phototransduction pathways regulate chalcone synthase gene expression in *Arabidopsis* leaf tissue. Plant J. 25, 675–685. https://doi.org/10.1046/J.1365-313X.2001.01001.X

Wickham, H., François, R., Henry, L., Müller, K., RStudio, 2022. dplyr: a grammar of data manipulation.

Zhao, Y.-W., Wang, C.-K., Huang, X.-Y., Hu, D.-G., 2021. Anthocyanin stability and degradation in plants. Plant Signal. Behav. 16, 1987767. https://doi.org/10.1080/15592324.2021.1987767

APPENDIX

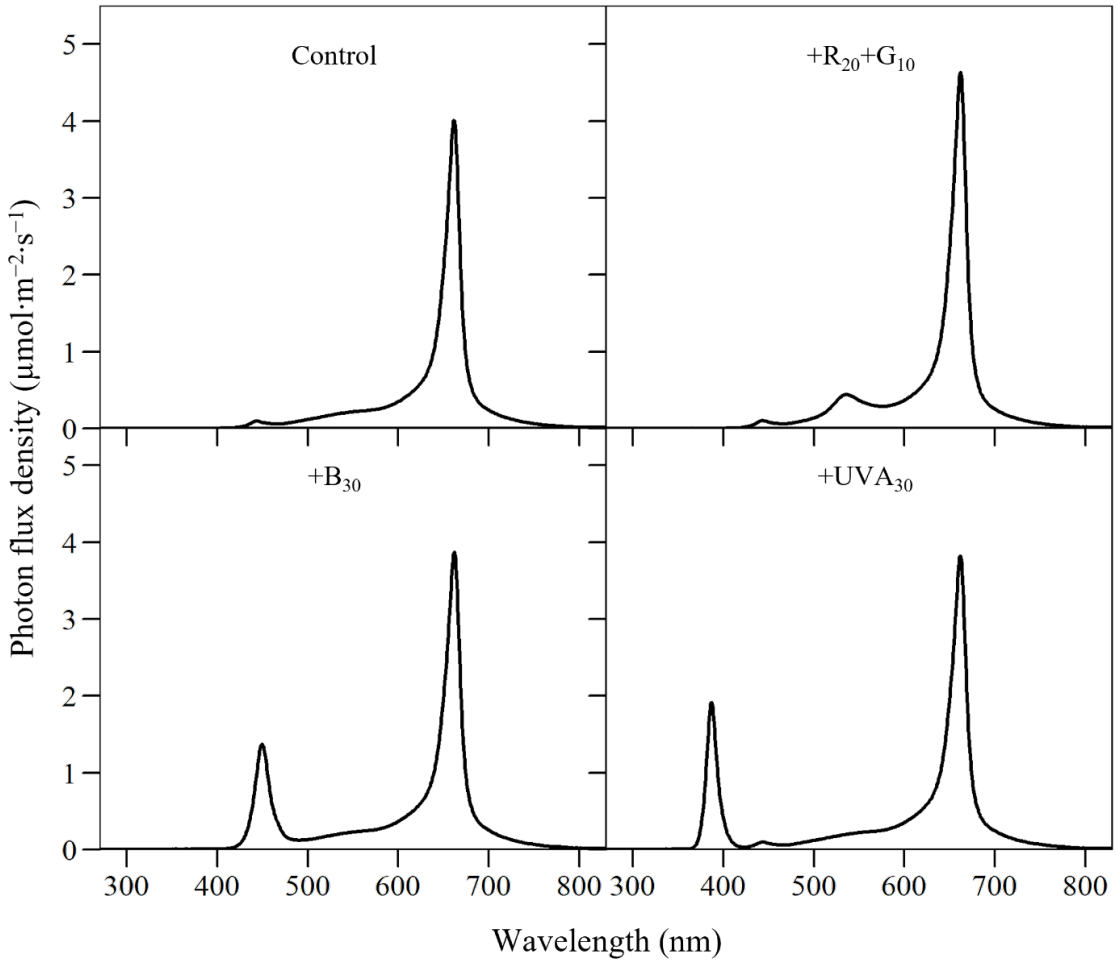

Figure II-1. The distribution of the control light spectrum delivered by warm-white and red light-emitting diodes (LEDs) and supplemental lighting treatments delivered during one or all three phases of the experiment. Supplemental lighting treatments were delivered by ultraviolet A (UVA; peak = 386 nm), blue (B; peak = 449 nm), or red (R; peak = 664 nm) and green (G; peak = 532 nm) LEDs.

Table II-1. Measured ultraviolet A (UVA), blue (B), green (G), red (R), and far-red (FR) photon flux density, as well as the photosynthetic photon flux density (PPFD) and total photon flux density (TPFD) in $\mu mol \cdot m^{-2} \cdot s^{-1}$ of the control or supplemental lighting treatments delivered during none, one, or all three phases of the experiment.

Light waveband	Supplemental lighting treatments			
	None (control)	B_{30}	UVA_{30}	$R_{20}+G_{10}$
UVA (315-399 nm)	0.2	0.3	28.6	0.2
B (400-499 nm)	5.6	34.5	8.6	6.0
G (500-599 nm)	21.3	22.5	21.6	31.5
R (600-699 nm)	116.6	115.2	113.2	132.8
FR (700-799 nm)	8.5	9.0	8.8	8.9
PPFD (400-700 nm)	143.5	172.2	143.4	170.3
TPFD (315-800 nm)	152.2	181.4	180.7	179.4

Table II-2. We applied supplemental lighting treatments to lettuce plants during phase 1 (P1), phase 2 (P2), phase 3 (P3), or all three phases (ET) of the experiment except for the control treatment, which had no supplemental light applied to it. Each treatment consisted of additional light from ultraviolet A (UVA; 315-399 nm), blue (B; 400-499 nm), or red (R; 600-6 99 nm) plus green (G; 500-599 nm) light-emitting diodes, which increased the total photon flux density (TPFD; 315-800 nm) from 150 to 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for at least one phase of the experiment. The cumulative light integral (CLI; 400-700 nm) and cumulative extended light integral (CeLI; 315-800 nm) represents the cumulative amount of light output by the lighting fixtures during the 24 days (day 4-28) where lighting treatments were applied.

Supplemental lighting	Phase 1 (day 4-12)	Phase 2 (day 12-20)	Phase 3 (day 20-28)	CLI ($mol \cdot m^{-2}$)	CeLI ($mol \cdot m^{-2}$)
None (control)				248.0	263.0
$ETR_{20}+G_{10}$				294.3	310.0
$P1UVA_{30}$				247.9	279.4
$P2UVA_{30}$				247.9	279.4
$P3UVA_{30}$				247.9	279.4
$ETUVA_{30}$				247.8	312.2
$P1B_{30}$				264.5	279.8
$P2B_{30}$				264.5	279.8
$P3B_{30}$				264.5	279.8
ETB_{30}				297.6	313.5

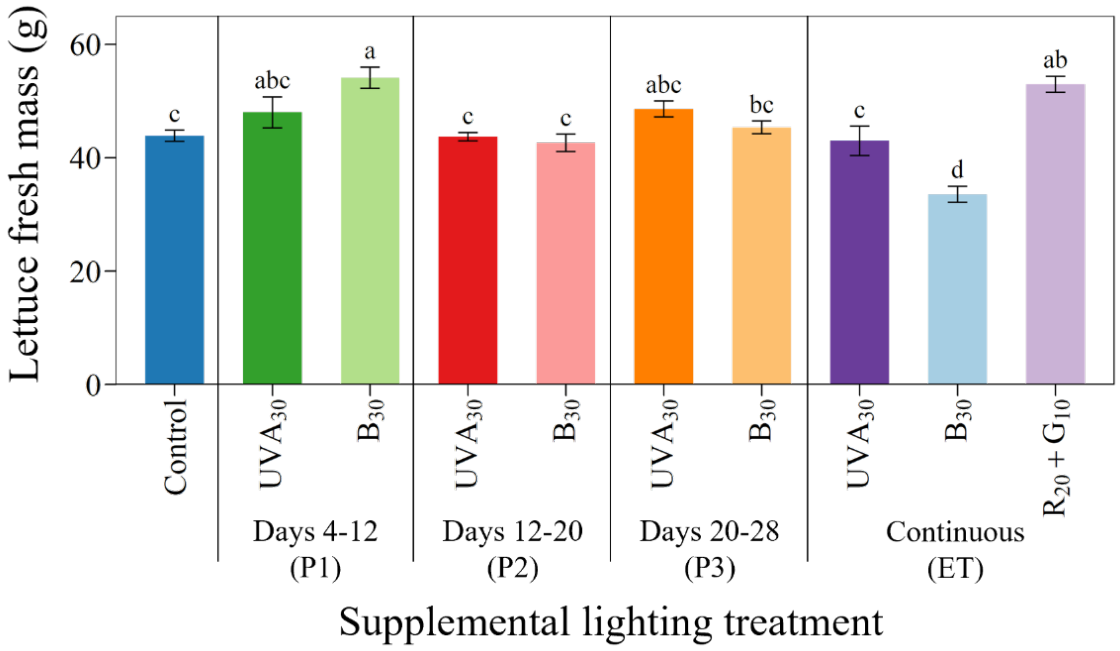

Figure II-2. Mean shoot fresh mass (g) of lettuce 'Rouxai' grown without (control) or with supplemental ultraviolet A (UVA, 315-399 nm), blue (B; 400-499 nm), green (G; 500-599 nm), and/or red (R; 600-699 nm) light during one of three eight-day phases (P1, P2, P3), or continuously (ET). Subscript values indicate the photon flux density of each waveband, in $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each bar represents the mean of two replications with ten biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars indicate the standard error of each treatment.

Table II-3. Mean shoot dry mass (g), plant diameter (cm), leaf length (cm) and width (cm) of the fifth leaf, leaf number, and leaf pigmentation indicated by *L*a*b** coloration index values of lettuce 'Rouxai' harvested on day 28. See Figure II-2 caption for treatment information. Each value represents the mean of two replications with three or ten biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$).

Supplemental lighting	Dry mass (g)	Plant diameter (cm)	Leaf length (cm)	Leaf width (cm)	Leaf number	*L**
Control	1.71 cd	21.9 bcd	13.4 abc	16.8 abc	13.5 b	46.6 a
$ETR_{20}+G_{10}$	2.06 ab	22.4 bc	13.3 abc	18.1 ab	14.5 ab	45.8 a
$P1UVA_{30}$	1.91 abc	23.3 abc	13.8 a	17.9 ab	14.5 ab	44.7 a
$P2UVA_{30}$	1.70 cd	23.7 ab	13.9 a	18.1 ab	13.3 b	43.5 a
$P3UVA_{30}$	2.01 ab	22.7 bc	13.4 abc	18.2 ab	13.9 b	45.2 a
$ETUVA_{30}$	1.83 bcd	22.0 bc	12.5 cd	16.5 bc	14.1 b	43.9 a
$P1B_{30}$	2.16 a	24.6 a	13.9 a	18.3 a	15.7 a	43.9 a
$P2B_{30}$	1.80 bcd	23.5 ab	13.7 ab	18.3 a	14.0 b	45.1 a
$P3B_{30}$	2.05 ab	21.4 cd	12.7 bc	17.3 ab	14.0 b	40.4 a
ETB_{30}	1.64 d	20.0 d	11.5 d	15.2 c	13.3 b	38.9 a

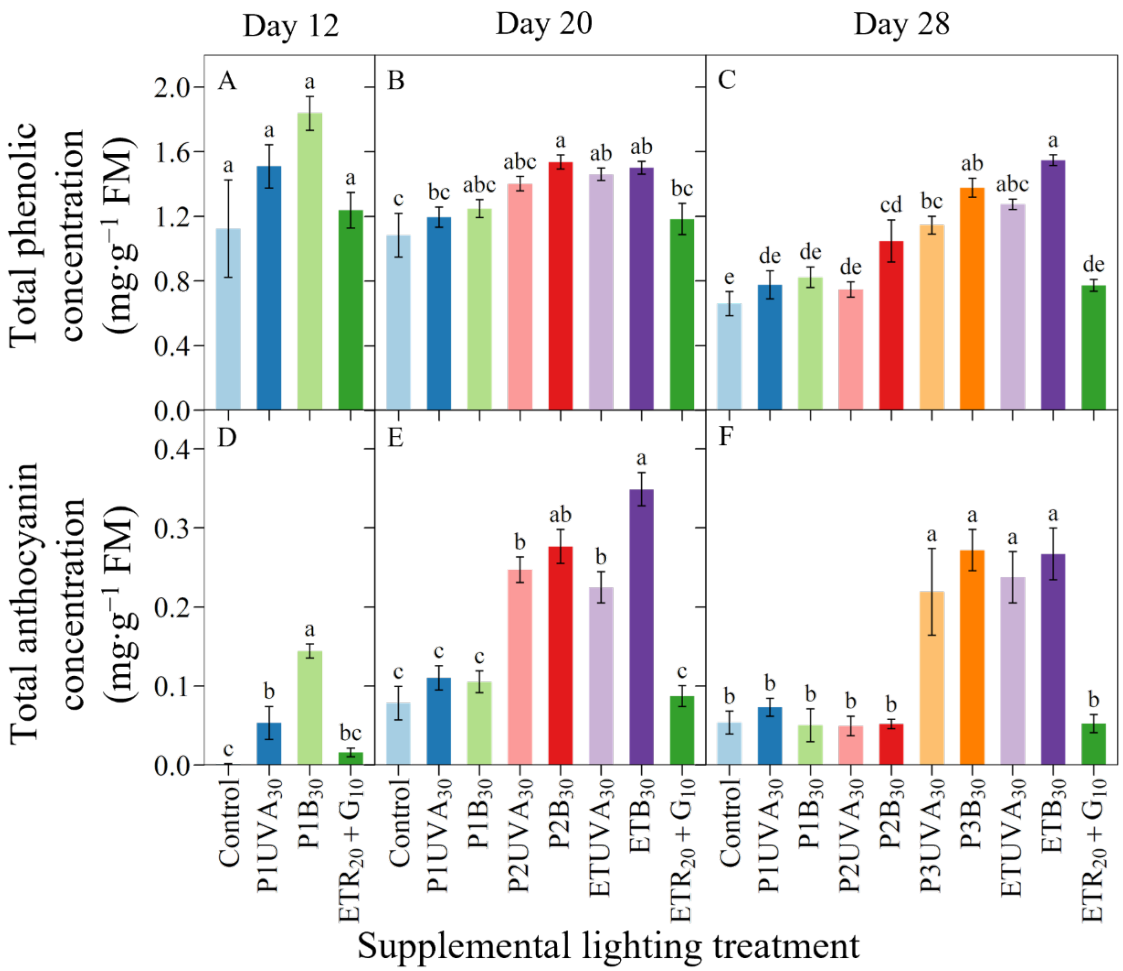

Figure II-3. (A-C) Mean total phenolic concentration and (D-F) mean total anthocyanin concentration on a fresh mass (FM) basis of lettuce 'Rouxai' harvested at the end of each phase (day 12, 20, or 28). See Figure II-2 caption for treatment information. Each bar represents the mean of two replications with three biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars indicate the standard error of each treatment.

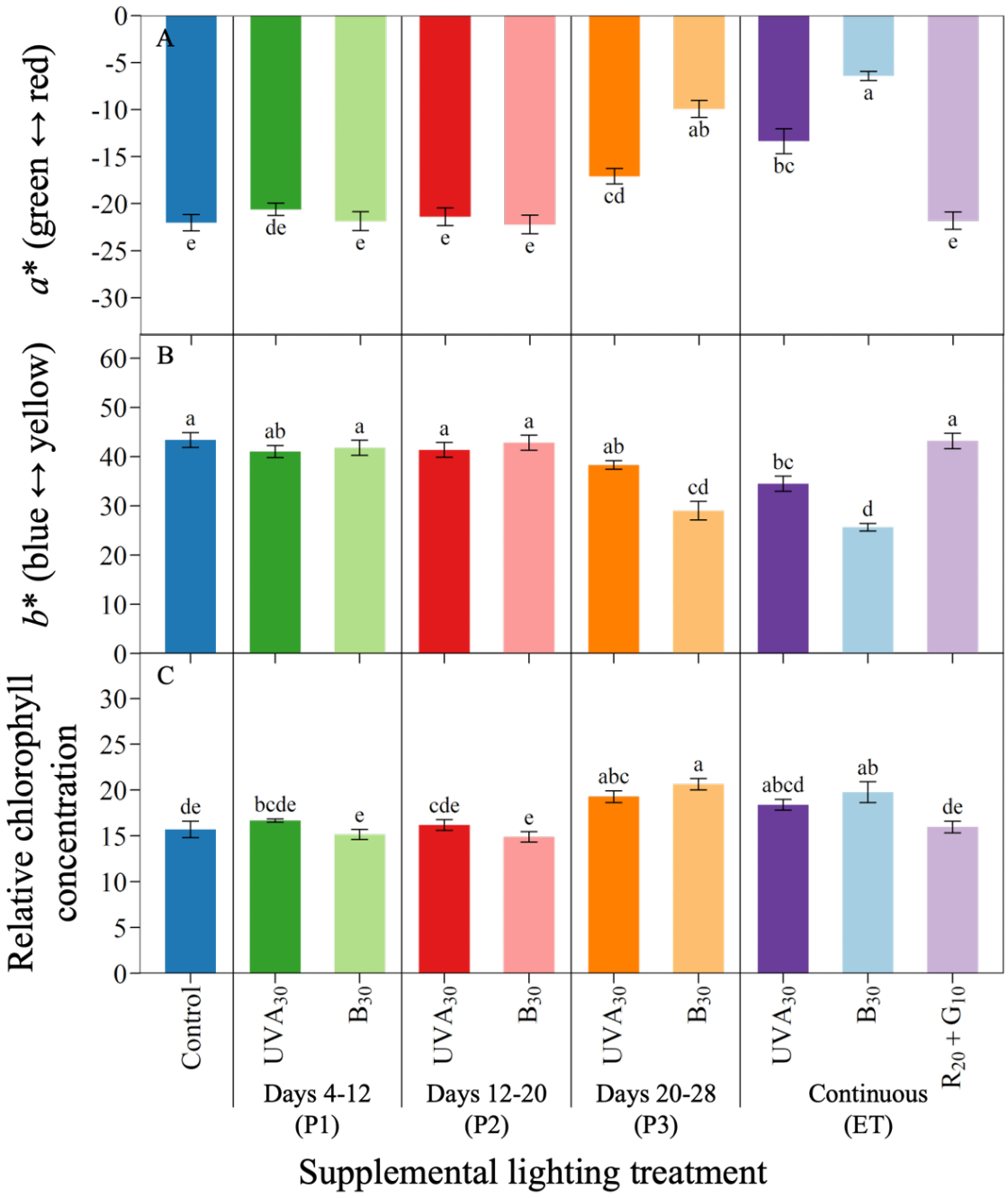

Figure II-4. (A, B) Mean leaf pigmentation indicated by $L^*a^*b^*$ coloration index values (L^* not shown graphically) and (C) mean relative chlorophyll concentration of lettuce 'Rouxai' harvested at the end of phase 3. See Figure II-2 caption for treatment information. Each bar represents the mean of two replications with three biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars indicate the standard error of each treatment.

Table II-4. Mean leaf pigmentation indicated by *L*a*b** coloration index values and relative chlorophyll concentration (SPAD) of lettuce 'Rouxai' harvested at the end of phase 1 (P1) or phase 2 (P2). See Figure II-2 caption for treatment information. Each value represents the mean of two replications with three biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$).

Phase	Treatment	*L**	*a**	*b**	SPAD
P1	None (control)	59.4 a	-27.5 b	50.0 a	13.9 b
	$ETR_{20}+G_{10}$	58.0 a	-28.4 b	50.8 a	15.8 ab
	$P1UVA_{30}$	52.2 b	-22.6 ab	42.2 ab	17.2 a
	$P1B_{30}$	47.2 c	-14.9 a	35.7 b	17.5 a
P2	None (control)	41.8 a	-17.3 cd	39.2 ab	17.2 bcd
	$ETR_{20}+G_{10}$	41.2 a	-16.6 c	37.4 ab	17.3 bcd
	$P1UVA_{30}$	37.3 ab	-14.0 c	33.7 b	17.1 cd
	$P2UVA_{30}$	30.1 bcd	-4.9 b	22.4 c	19.2 abc
	$ETUVA_{30}$	27.8 bcd	-4.6 ab	21.1 c	19.8 abc
	$P1B_{30}$	41.6 a	-16.4 c	37.6 ab	17.3 bcd
	$P2B_{30}$	27.3 cd	-1.7 ab	17.4 c	20.5 a
	ETB_{30}	25.8 d	-0.8 a	16.6 c	20.3 ab

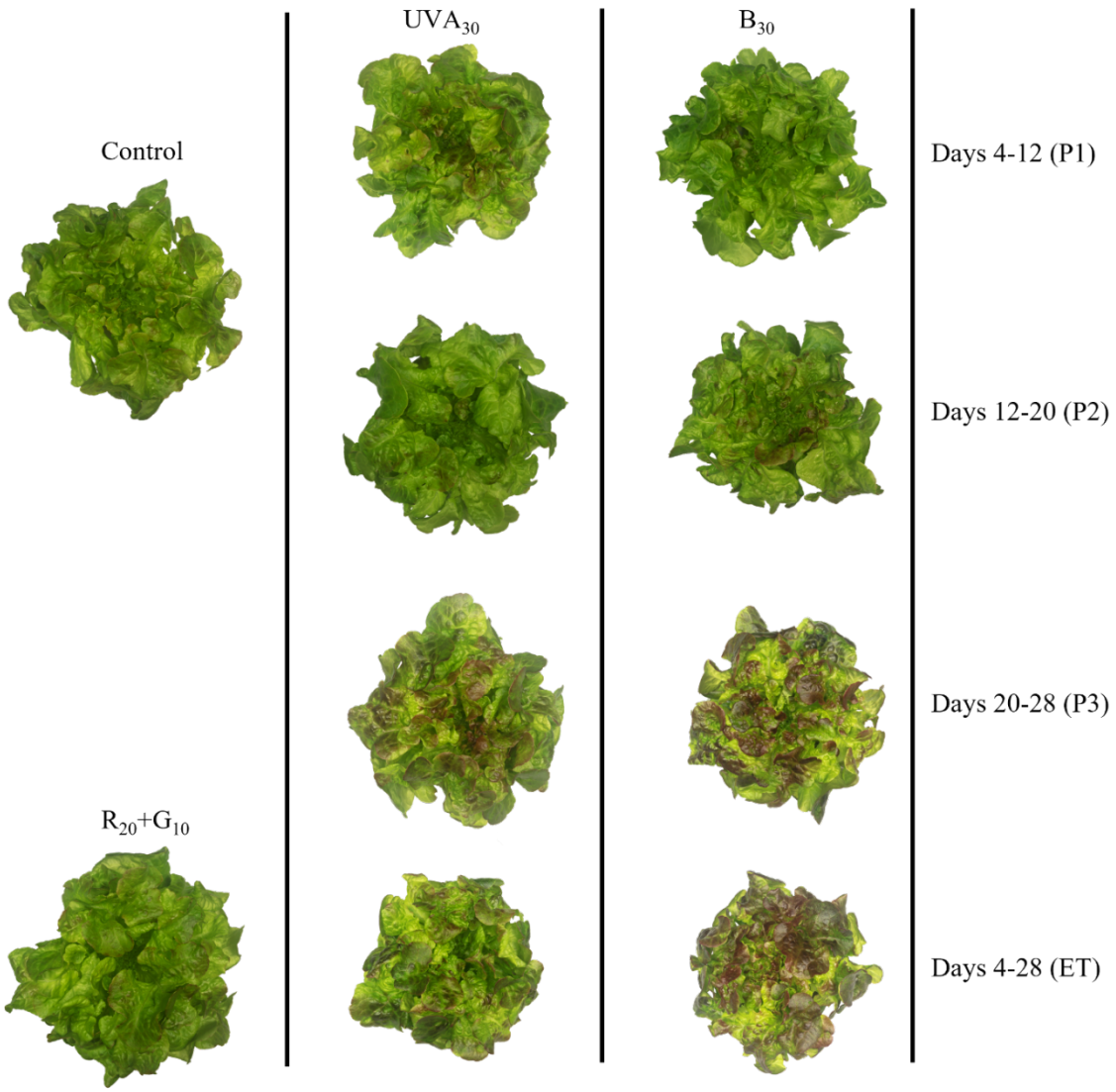

Figure II-5. Representative plants from each lighting treatment. Plants were grown under the base spectrum (control) or supplemental red (R; 600-699 nm) and green (G; 500-599 nm) light (R20+G10) from day 4 to 28. The remaining eight treatments consisted of supplemental ultraviolet A (UVA30; 315-399 nm) or blue (B30; 400-499 nm) light applied during one of three phases, or continuously.

SECTION III
SUPPLEMENTAL NARROW-BAND LIGHT INCREASES LETTUCE GROWTH AND PHYTOCHEMICAL CONCENTRATIONS

Supplemental narrow-band light increases lettuce growth and phytochemical concentrations

Nathan H. Kelly and Erik S. Runkle*

Controlled-Environment Lighting Laboratory, Department of Horticulture, 1066 Bogue Street, Michigan State University, East Lansing, MI 48824

*Corresponding author. Tel.: +1 517 353 0350; fax: +1 517 353 0890. E-mail address: runkleer@msu.edu (E.S. Runkle)

Abstract

Plant cultivation in indoor farms is reliant on electric lighting to drive plant photosynthesis and control plant morphology. While electric lighting accounts for one of the greatest operational costs in indoor farming, light-emitting diodes are a highly efficient light source that enables growers to precisely control the photon flux density, spectrum, and photoperiod. The photon flux density and spectrum have profound effects on plant photosynthesis, growth, morphology, and quality traits like secondary metabolite biosynthesis. We supplemented a broad-band spectrum (i.e., white light) blue (B; 400-499 nm), green (G; 500-599 nm), red (R; 600-699 nm), far-red (FR; 700-750 nm), and additional white light at two total PFDs to determine their effects on lettuce (*Lactuca sativa*) growth, morphology, and secondary metabolite synthesis. We hypothesized that supplemental B light would increase total phenolic and anthocyanin concentrations but decrease biomass; supplemental FR light would have the opposite effect; and the spectral effects of supplemental light would be diminished at a higher PFD. We grew lettuce 'Rouxai' and 'Rex' hydroponically at an air temperature of 23 °C and under 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ or 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of warm-white light plus 40 or 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B, G, R, FR, or warm-white light provided by light-emitting diodes. Increasing the PFD, regardless of the waveband, increased biomass accumulation and the total phenolic concentration of lettuce 'Rouxai'. At each PFD, supplemental R, FR, and warm-white light increased biomass accumulation the most, while B light increased total phenolic and anthocyanin concentrations. Supplemental light effects were generally similar at both photon flux densities, indicating that the high photon flux density of white light did not attenuate supplemental light effects. We conclude that supplemental light is effective at increasing biomass accumulation, secondary

metabolite concentration, and leaf coloration, and is as effective at higher photon flux densities where the percentage of supplemental light remained constant.

Introduction

In indoor farms, electric lighting can be used to precisely control the light environment to optimize plant growth. The ability to control the light spectrum and photon flux density (PFD) allows growers to control the yield, morphology, leaf coloration, and nutritional quality of leafy-green vegetables, microgreens, and other crops suitable for indoor farming. Increasing the PFD of photosynthetically active radiation (PAR; 400-700 nm) typically increases plant growth and leaf coloration, but also increases capital and operational costs. In particular, increasing the percentage of red (R; 600-699 nm) light in the light spectrum can increase biomass accumulation compared to blue (B; 400-499 nm) light, but at the consequence of lower nutritional quality (Son and Oh, 2013). Furthermore, light that induces shade-avoidance responses, particularly green (G; 500-599 nm) and especially far-red (FR; 700-750 nm) light, can increase leaf expansion and sequent plant growth (Meng et al., 2019).

As a general rule, a 1% increase in PAR leads to a 0.75% to 1% increase in growth and yield of horticultural crops (Marcelis et al., 2006). However, continually increasing the photosynthetic PFD (PPFD; 400-700 nm; $\mu mol \cdot m^{-2} \cdot s^{-1}$) does not continuously increase growth at the same rate. Electron transport rate (ETR) and the quantum yield of photosystem II photochemistry (ΦPSII) are both indicators of overall plant photosynthesis (Fu et al., 2012; Maxwell and Johnson, 2000). Lettuce (*Lactuca sativa*) ETR and ΦPSII increased when the PFD increased from 100 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at an air temperature of 20/16 °C (day/night) and CO_2 concentration of 400 $\mu mol \cdot mol^{-1}$, but then began to increase at a decreasing rate as the PFD increased to 400, 600, or 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, indicating that the relative efficacies of photons

where greater at a low to moderate PFD than a higher PFD (Fu et al., 2012). However, in the same study, lettuce fresh mass (FM) increased as the PFD increased up to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, despite having lower instantaneous photosynthetic rates. Increasing the PFD can also increase lettuce leaf coloration (Kelly et al., 2020) and secondary metabolite concentrations including phenolic compounds (Pérez-López et al., 2018). Therefore, increasing the PFD can increase FM and secondary metabolite accumulation but is also more expensive.

Light-emitting diode (LED) fixtures are widely used in commercial indoor farms to control the PFD and light spectrum by utilizing diodes that emit narrow wavebands, broad wavebands (i.e., white light), or both (Kusuma et al., 2020, 2022; van Iersel, 2017). Although white LEDs are less electrically effective than R or B LEDs, they are often included in lighting devices because of their low cost, broad spectrum, and high color-rendering index (Kusuma et al., 2020, 2022). A base spectrum of white light can be enriched with one or more specific wavebands (supplemental light; SL) to achieve specific growth responses or enhance specific quality attributes. For example, adding 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light to a warm-white (WW) light spectrum during the entire production period increased total phenolic concentration (TPC) and total anthocyanin concentration (TAC) of baby-leaf lettuce ‘Rouxai’ at harvest, but did not affect FM (Vaštakaitė-Kairienė et al., 2021). Similarly, adding 30 or 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light to a WW+R light spectrum for the last six days of production increased the TPC, TAC and leaf redness of lettuce ‘Rouxai’ whereas additional G+R at the same PFD did not (Kelly and Runkle, 2023). FR light is not included in the PAR waveband but can increase FM by increasing photosynthesis, leaf expansion, and light capture (Legendre and van Iersel, 2021; Zhen et al., 2019; Zhen and van Iersel, 2017). However, an increased percentage of FR light in the light spectrum decreased secondary metabolite concentration and leaf coloration (Li and Kubota,

2009; Meng et al., 2019). Finally, different LED types (including those of the same waveband but with different peak wavebands and manufacturing processes) have different efficacies, so the selection of a light spectrum necessitates consideration of growth responses and the costs to purchase and operate the lighting fixtures (Kusuma et al., 2020, 2022).

Modification of the PFD and/or light spectrum during indoor plant production can regulate growth and morphology, but some greenhouse studies suggest that the SL spectrum has less effect when the solar daily light integral (DLI) is high. For example, providing various ornamental seedlings with SL in a greenhouse increased seedling dry mass (DM), height, and leaf number, but the SL spectrum had little or no effect on these metrics (Poel and Runkle, 2017). Similarly, pre-harvest SL provided to greenhouse-grown lettuce plants increased leaf thickness, leaf greenness, and phytochemical concentrations, but there were few effects of SL quality on plant growth and quality, such as shoot FM and total phenolic content (Hooks et al., 2022). Therefore, SL can increase crop growth and quality, but its spectrum could have little effect when added to a broad-waveband spectrum and/or high DLI. It is less clear whether these trends apply to leafy greens produced indoors under sole-source lighting. The objectives of this study were to determine: 1) how different PFDs of supplemental narrow-waveband light affect phytochemical concentrations and leaf coloration when applied to a white-light background and 2) if the same percentage of SL applied to white light at different PFDs elicits similar responses. We hypothesized that: 1) the effects of the light spectrum will be attenuated by a high PFD; 2) supplemental B light will increase phenolic and anthocyanin concentrations more than other supplemental wavebands but will also decrease leaf expansion and biomass accumulation and 3) supplemental FR light will decrease phytochemical concentrations but increase leaf expansion and biomass accumulation.

Materials and methods

Plant materials and propagation

We sowed red-leaf lettuce 'Rouxai' and green-leaf lettuce 'Rex' (Johnny's Selected Seeds, Winslow, ME, USA) seeds in a temperature-controlled growth room (the Controlled Environment Lighting Laboratory) at Michigan State University. The seeds were sown in 200-cell (2.5 cm × 2.5 cm) rockwool plugs (AO 25/40 Starter Plugs; Grodan, Milton, ON, Canada) that were presoaked in deionized water with a pH of 4.5 that was adjusted using 10% sulfuric acid (H_2SO_4). After seed sow, the lettuce seeds were grown at 23 °C under a 24 $h \cdot d^{-1}$ photoperiod and a total PFD (TPFD; 300-750 nm) of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from warm-white (peak = 639 nm, correlated color temperature = 2700 K) LEDs. On day 3, the photoperiod was shortened to 20 $h \cdot d^{-1}$ for the rest of the production cycle. To increase humidity during germination and early seedling growth, the seedling trays were covered with clear plastic domes from day 0 to 5. From day 0 until day 8, before the seedlings were transplanted, we hand-irrigated the seedlings with deionized water supplemented with magnesium sulfate (Epsom salt; Pennington Seed, Inc., Madison, GA) and a water-soluble fertilizer (12N–4P2O5–16K2O RO Hydro FeED; JR Peters, Inc., Allentown, PA, USA) with the following nutrients (in mg·L–1): 125 N, 42 P, 167 K, 73 Ca, 49 Mg, 39 S, 1.7 Fe, 0.52 Mn, 0.56 Zn, 0.13 B, 0.47 Cu, and 0.13 Mo. The pH and electrical conductivity (EC) were monitored daily using a pH/EC meter (HI9814; Hanna Instruments, Woonsocket, RI, USA) and were adjusted as needed to maintain a pH of 5.6 and an EC of 1.6 $mS \cdot cm^{-1}$.

Environmental conditions and lighting treatments

The growth room consisted of four vertical hydroponic growing racks that had three canopies on each rack with recirculating nutrient solutions. On day 8, we randomly separated the

seedlings and transplanted 30 of them into each of 12 floating 36-cell rafts (Beaver Plastics, Ltd., Acheson, AB, Canada) with 2.5-cm-wide holes spaced 20 × 15 cm apart. We left the three holes on each end of the raft empty. Plants were provided the same nutrient solution as previously described but at a 20% higher concentration (i.e., 150 mg $N \cdot L^{-1}$). We measured the nutrient solution daily and adjusted it using additional fertilizer, potassium bicarbonate, and H_2SO_4 to maintain a pH and EC of 5.7 and 1.9 $mS \cdot cm^{-1}$, respectively. The air temperature was a constant 23 °C. We measured and calculated the mean (± SD) canopy temperature (Rep. 1 = 24.8 ± 0.9 °C; Rep. 2 = 25.0 ± 0.8 °C), relative humidity (Rep. 1 = 47.7 ± 5.6%; Rep. 2 = 45.2 ± 9.0%), and CO_2 concentration (Rep. 1 and 2 = 411 ± 21 $\mu mol \cdot mol^{-1}$) during each replication. Additional information about equipment, experimental conditions, and sensors can be found in detail in Kelly et al. (2020).

We delivered 12 lighting treatments from day 8 until the plants were harvested on day 28 (Table III-1 and Table III-2). Each lighting treatment consisted of a base spectrum of warm-white (WW) light at a TPFD of 90 or 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. We added 40 or 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of narrowband SL using blue (B; peak = 449 nm), green (G; peak = 526 nm), red (R; peak = 664 nm), or far-red (FR; peak = 733 nm) LEDs to the low or high TPFDs, respectively. We also created two treatments that consisted of an additional 40 or 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of WW light. This enabled the comparison of narrow-band SL on lettuce growth and quality attributes at the same percentage of the TPFD at a low (130 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and high (260 $\mu mol \cdot m^{-2} \cdot s^{-1}$) TPFD. We measured the TPFD and light spectrum of each treatment at nine locations at the plant canopy level using a spectroradiometer (PS200; Apogee Instruments, Inc., Logan, UT, USA). Mean PFDs and percentages of each waveband range are reported in Table 1 and Table 2, respectively.

Total phenolic and anthocyanin analysis

Immediately after harvest on day 28, we collected fresh leaf tissue of 'Rouxai' to measure the total phenolic concentration (TPC) and total anthocyanin concentration (TAC). To measure TPC, we collected 0.5 g of direct light-exposed leaf tissue from three biological samples, immediately froze them in liquid nitrogen, and then stored them in a −80 °C freezer until analysis. We analyzed the samples using a spectrophotometer according to the protocol used by Kelly and Runkle (2023), which was developed with slight modifications from the Ainsworth and Gillespie (2007) protocol. TAC analysis was conducted similarly, except we collected 0.3 g of plant tissue and analyzed the samples using a modified version of the Lee et al. (2005) pH differential method (AOAC Official Method 2005.2) previously described in Kelly and Runkle (2023).

Data collection and analysis

On day 28, before destructive plant measurements, we measured the relative chlorophyll concentration of ten randomly selected 'Rouxai' and 'Rex' plants using a SPAD meter (SPAD-502; Konica Minolta Sensing, Inc., Osaka, Japan) by measuring and averaging three spots on one fully expanded leaf exposed to direct light. Additionally, for the red-leaf cultivar 'Rouxai' we took overhead pictures of three randomly selected plants to measure leaf coloration using an R code developed to determine the lightness (black: $L^* = 0$; white: $L^* = 100$), redness (green: $a^* = -128$; red: $a^* = 127$), and blueness (blue: $b^* = -128$; yellow: $b^* = 127$) of each pixel of an imported TIFF picture. The L^*, a^*, and b^* values of each pixel were generated and averaged to quantify the average coloration of an entire plant from overhead.

After harvest, we randomly selected ten plants from each treatment and cultivar for morphological data collection. These plants were not used for biochemical analysis. We cut and

harvested all plant tissue and weighed each using an analytical balance (AG245; Mettler Toledo, Columbus, OH). Additionally, we measured the plant diameter (cm), leaf length (cm) and width (cm) of the fifth fully expanded leaf, and counted the number of leaves longer than 2 cm. Finally, we packed all plant tissue into paper bags and dried them in a drying oven (Blue M, Blue Island, IL) for 7 days at 60 °C before weighing them with the same balance.

This experiment was arranged as a randomized complete block design with two replications in time (September 23, 2020–October 22, 2020; October 21, 2020–November 11, 2020). We performed statistical analysis using R statistical analysis software (R Core Team, 2014; version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria). We analyzed the data by conducting an analysis of variance (ANOVA) and Tukey's honestly significant difference test ($\alpha = 0.5$) using the R package's 'dplyr' (Wickham et al., 2022) and 'agricolae' (Mendiburu, 2021).

Results

Fresh mass and dry mass

The WW PFD and SL waveband had a significant effect on lettuce 'Rouxai' and 'Rex' fresh mass (FM) and dry mass (DM) (Figure III-1; Table III-3). Adding 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light ($+B_{40}$) to 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of WW light (WW_{90}) did not affect the FM of either cultivar. In contrast, 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of additional G ($+G_{40}$), R ($+R_{40}$), FR ($+FR_{40}$), or WW ($+WW_{40}$) light increased 'Rouxai' FM, compared to no SL, by up to 71%, while only $+R_{40}$ increased 'Rex' FM. At a WW PFD of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (WW_{180}), the addition of 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B ($+B_{80}$) light decreased 'Rouxai' FM by 19% but had no effect on 'Rex' FM. Similar to WW_{90}, lettuce 'Rouxai' grown with an additional 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of G ($+G_{80}$), R ($+R_{80}$), FR ($+FR_{80}$), or WW ($+WW_{80}$) light had up to 42% greater FM than those grown without SL. Only the addition of R

and WW light increased ‘Rex’ FM (by 41%). Doubling the WW PFD and SL PFD increased FM of ‘Rouxai’ by 62 to 93% and ‘Rex’ by 79 to 154%. Not surprisingly, all plants grown under a TPFD of 260 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had a greater FM than plants grown under treatment with a TPFD of 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with the exception of ‘Rouxai’ grown under $WW_{180}B_{80}$.

In general, SL with the same waveband but at two PFDs had a somewhat similar effect on lettuce FM, although on a percentage basis, SL increased growth more at the low WW PFD than the higher one. For example, lettuce ‘Rouxai’ that was grown under $WW_{90}R_{40}$ or $WW_{180}R_{80}$ had 71% and 40% more FM, respectively, than the WW treatments without SL. In addition, as the yield PFD (YPFD) of the light spectrum increased, so did FM of both cultivars, except when SL B light was applied. For example, $WW_{180}B_{80}$ had a similar YPFD to $WW_{180}G_{80}$ and WW_{260}, but lettuce plants had up to 43% less FM. Additionally, lettuce ‘Rouxai’ grown under $WW_{180}FR_{80}$ had 62% more FM than plants grown under $WW_{180}B_{80}$, despite the YPFD being less.

Lettuce DM of both cultivars followed similar trends to FM (Figure III-1; Table III-3). At WW_{90}, adding G, R, FR, or WW light increased ‘Rouxai’ DM by up to 63%, but only additional G and R light increased ‘Rex’ DM. B light added to either cultivar had no effect on DM. At WW_{180}, all SL wavebands increased ‘Rouxai’ and ‘Rex’ DM except $+B_{80}$ for ‘Rouxai’. Similar to FM, SL at a low or high PFD had similar effects on FM, although the magnitude of the increase was usually greater under WW_{90} than WW_{180}. For instance, adding the same percentage of G light to the low or high WW PFD increased DM of ‘Rex’ by 57% and 26%, respectively.

Plant morphology and leaf number

The light spectrum and the TPFD influenced plant and leaf morphology (Figure III-2; Table III-3; Table III-4). The addition of 40 or 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light to WW_{90} or WW_{180}

decreased the leaf length of lettuce 'Rouxai' and 'Rex' by up to 21%. In contrast, at both WW PFDs, adding 40 or 80 μmol·m^{-2}·s^{-1} of FR light increased the leaf length of both cultivars by 23% to 30%. None of the other SL wavebands had an effect. Adding G, R, FR, or WW light to WW_{90} similarly increased the leaf width of 'Rex', by up to 27%, but additional B light to 'Rex' had no effect. There were similar spectral effect trends on leaf width of lettuce 'Rouxai', except that FR SL increased leaf width slightly more than additional WW light. At WW_{180}, only $+R_{80}$ and $+FR_{80}$ increased 'Rex' leaf width; the other SL treatments had no effect. 'Rouxai' leaf width, on the other hand, decreased by 11% when $+B_{80}$ light was added to WW_{180} and increased by 22% and 9% under the $+FR_{80}$ or $+WW_{80}$ treatments. Similar to leaf length, the plant diameter of both cultivars increased when FR light was added to both WW PFDs and decreased under additional B light (Table III-4). At WW_{90}, $+FR_{40}$ increased the plant diameter of 'Rouxai' to a greater extent than twice the PFD of FR light applied at twice the PFD of WW light. Finally, adding 40 or 80 μmol·m^{-2}·s^{-1} of WW light to the low or high WW PFD did not influence 'Rouxai' plant diameter, but at WW_{180}, $+WW_{80}$ decreased 'Rex' plant diameter.

The number of leaves greater than 2 cm of both cultivars was primarily affected by the TPFD, although supplemental light did influence leaf number as well. Lettuce grown under the higher TPFD had more leaves than those grown under the lower TPFD. For example, lettuce grown under WW_{180} had three to four more leaves than lettuce grown under WW_{90}. Additionally, lettuce grown under a higher WW PFD plus FR ($WW_{180}FR_{80}$) had fewer leaves than plants grown under WW_{180} without FR DL (Table III-4). In contrast, adding G or R light to WW_{90} increased the leaf number of both cultivars by up to 26%. At WW_{180}, the addition of SL did not increase lettuce leaf number of either cultivar except for R_{80} added to 'Rouxai', which increased leaf number by 12%.

Total phenolic and anthocyanin concentration

Supplemental B light was the most effective waveband at increasing both the TPC and TAC of lettuce 'Rouxai', while doubling the PFD of any light spectrum had less of an effect (Figure III-3; Table III-3). At WW_{90}, $+B_{40}$ increased TPC and TAC by 65% and 182%, respectively. No other supplemental waveband influenced TPC or TAC, although plants under $+R_{40}$ and $+FR_{40}$ had a similar TAC as $+B_{40}$ and the WW_{90} control. At WW_{180}, $+B_{80}$ increased lettuce TPC and TAC by 105% and 430%, respectively, compared to no SL. Additionally, 'Rouxai' grown under the higher TPFD had greater TPC and TAC than under the low TPFD. For example, lettuce grown under $WW_{180}B_{80}$ had 106% and 423% greater TPC and TAC, respectively, than plants grown under the same light spectrum but half the TPFD. At the high TPFD, $+R_{80}$ light and $+WW_{80}$ increased TPC, but the same SL wavebands had no effect on TPC at WW_{90}.

Leaf coloration and SPAD

We quantified 'Rouxai' leaf coloration using the $L^*a^*b^*$ color space. At WW_{90}, $+B_{40}$ increased leaf redness (more positive a^* value) by 39% and $+FR_{40}$ increased leaf redness by 15% (Figure III-4)). Furthermore, $+B_{40}$ slightly increased the blueness (lower b^* value) and darkness (lower L^* value) of lettuce leaves. At WW_{180}, $+B_{80}$ was the only treatment that increased leaf redness, blueness, and darkness; they were 93% redder than those grown without SL and 90% redder than plants grown under the same light spectrum but at a TPFD of 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Doubling the WW PFD without SL increased the SPAD of lettuce 'Rouxai' and 'Rex' by 34% and 20%, respectively (Table III-4). Additional B light increased the SPAD of both cultivars regardless of the WW PFD, while FR light decreased the SPAD of both cultivars only when added to WW_{180}.

Discussion

Supplemental red and far-red light enhance lettuce leaf expansion and growth

Phytochrome (phy) is the primary plant photoreceptor that senses R and FR light to initiate signal cascades leading to morphological and growth adaptations (Franklin and Whitelam, 2005). The increase in leaf expansion of lettuce grown under supplemental FR light can be attributed to a decrease in the R:FR of the light spectrum (Franklin, 2008; Franklin and Whitelam, 2005). The additional FR light decreases the R:FR and the phytochrome photoequilibrium (PPE), which is the ratio of the biologically active form of phytochrome (Pfr) relative to the total phytochrome pool, Pfr plus the inactive form of phytochrome (Pr) (Sager et al., 1988). The internal PPE (iPPE) considers the spectral distortion that occurs as light travels through the leaf (Kusuma and Bugbee, 2021). The iPPE of our various treatments were somewhat similar to each other (0.66 - 0.77) when the light spectrum consisted of only WW light or WW light plus supplemental B, G, or R light (Table I-1). When FR light was added to the light spectrum, the iPPE decreased to 0.31, indicating a strong shift in phytochrome forms. As FR light constitutes more of the light spectrum, phyB is converted to the Pr form, decreasing the PPE or iPPE. As the phyB pool is converted to more of the Pr form of phyB, it dissociates from phytochrome-interacting factor (PIF) 4 and PIF5, permitting them to move to and accumulate in the nucleus to promote the expression of shade-avoidance genes (Casal, 2012; de Lucas et al., 2008; Franklin, 2008; Tao et al., 2008). Furthermore, PIFs interact with the CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) and SUPRESSOR OF PHYA (SPA) enzyme complexes, which target, ubiquitinate, and degrade phyB as well as photomorphogenesis transcription factors like ELONGATED HYPOCOTYL 5 (HY5) that are involved in stem and leaf elongation (Casal, 2013; Jang et al., 2010; Rolauffs et al., 2012; Seo et al., 2004). Removing phyB from the

nucleus, either by dissociation from PIF4 and PIF5 or degradation mediated by COP1/SPA, allows for the increased expression of shade-avoidance genes that increase cell elongation and subsequently greater leaf surface area.

An increase in leaf expansion leads to greater light capture, which can increase whole-plant photosynthesis and sequent growth. Therefore, an increase in leaf area or canopy diameter often correlates with an increase in biomass accumulation (Legendre and van Iersel, 2021; Zhen and Bugbee, 2020). However, the addition of FR light to a white spectrum led to plants with fewer but larger leaves. For example, the addition of FR light to an R+B spectrum increased individual leaf area, but lettuce plants had fewer leaves leading to a similar total leaf area among lighting treatments (Kong and Nemali, 2021). FR light also can directly stimulate photosynthesis, especially when paired with R light, by preferentially stimulating photosystem I (Emerson and Rabinowitch, 1960; Emerson et al., 1957; Zhen et al., 2021). Thus, incorporating FR light into a light spectrum that contains R light increases plant leaf expansion and growth both indirectly and directly (Park and Runkle, 2017). Furthermore, supplemental B light inhibited FM accumulation of lettuce 'Rouxai', but only at the higher TPFD ($+B_{80}$) while extension growth (leaf length) of both cultivars was inhibited at both TPFDs. Adding R or WW light to the light spectrum also increased FM of both cultivars by increasing PAR with a higher quantum yield than B light (Mccree, 1972), while supplemental B light ($+B_{80}$) inhibited biomass accumulation of lettuce 'Rouxai' despite having a similar YPFD to light treatments containing supplemental R, G, or WW light.

In the current study, increasing the FR PFD ($+FR_{40}$ or $+FR_{80}$) increased the leaf length and width of both cultivars, regardless of the TPFD. FM of lettuce 'Rouxai' grown under additional FR light also increased relative to no SL, but was similar to plants grown under only

WW light at an increased TPFD or under WW+R light. In contrast, FM of 'Rex' was similar regardless of whether FR light was included in the light spectrum. Other studies have also reported that FR SL increased leaf expansion and growth. For example, lettuce grown under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of R+B light plus 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light delivered during the day or at the end of the day increased leaf area by nearly 50% (Zou et al., 2019). The FR SL also increased FM, especially when delivered during the entire day. In another study, adding ≈160 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR to a white light background increased leaf length, width, and stem length, as well as FM and DM (Li and Kubota, 2009). Additionally, when the TPFD remained constant but the FR light percentage increased, lettuce FM increased and was correlated with an increase in leaf area (Meng et al., 2019). Therefore, FR light, either when added or substituted for another waveband in a light spectrum, increases leaf expansion, light interception, and subsequent growth.

Supplemental light increased lettuce phytochemical concentrations

Phenolic compounds are a broad group of secondary metabolites including flavonoids and specific color-causing antioxidants such as anthocyanins. Increasing the B PFD greatly increased both TPC in leafy greens as well as anthocyanins (Kelly and Runkle, 2023; Lee, 2010; Li and Kubota, 2009). Cryptochrome 1 (cry1) is the primary photoreceptor that controls phenolic compound biosynthesis in response to high-energy, short-waveband light such as B (Brelsford et al., 2019). *CHALCONE SYNTHASE (CHS)* is the gene that encodes the enzyme involved in the first committed step of the flavonoid biosynthesis pathway, and is expressed under ultraviolet or B light in plants with a functional cry1 (Jenkins et al., 2001; Wade et al., 2001). Its expression is associated with increased concentrations of phenolic compounds (Park et al., 2007).

Increasing the PFD of ultraviolet, B, or total light generally increases phytochemical concentrations (Hooks et al., 2022; Kelly and Runkle, 2023). In this study, at the higher PFD

(WW_{180}), $+R_{80}$ and $+WW_{80}$ light increased lettuce 'Rouxai' TPC but not TAC. In another study, the TPC of two lettuce cultivars was not modified by R SL, but concentrations of individual phenolic compounds like chicoric acid, rutin, and kaempferol were greater in lettuce 'New Red Fire' than white light without SL or with B SL (Lee et al., 2019). In contrast, increasing the percentage of R light in a broad-waveband spectrum increased lettuce TPC (Li and Kubota, 2009). Increasing the PFD from 400 to 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at ambient CO_2 concentration increased the TPC of both red- and green-leaf lettuce (Pérez-López et al., 2018). Additionally, high light (800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for at least one day increased lettuce TPC (Oh et al., 2009). Therefore, lettuce TPC, and some specific phenolic compounds, are sensitive to specific wavebands of light, such as B light, and concentrations can also be increased by increasing the PFD of broad-waveband light. At least in lettuce and when applied at the same PFD, the results here and those of Kelly and Runkle (2023) indicate that B light is the most effective waveband at increasing TPC and anthocyanins, which are important for red-leaf lettuce pigment accumulation.

The PFD interacts with supplemental light

The specific waveband of SL and the WW PFD interacted to influence various plant traits such as 'Rouxai' FM, TPC, and TAC, and 'Rex' plant diameter and leaf length. For example, 'Rouxai' FM decreased when supplemental B light was applied, but only at the higher PFD. Additionally, +R and +WW light increased 'Rouxai' TPC only at the higher TPFD, while TAC increased under +B light, but to a greater extent under the higher TPFD. Few studies have investigated the effects of narrow-waveband SL applied to broad-waveband light at different TPFDs with the same spectral distribution. In this study, SL similarly affected a given plant trait at both WW PFDs, but the magnitude depended on the WW PFD even though the SL percentage remained the same. For example, +B light increased leaf redness, but to a greater extent at the

higher TPFD. Furthermore, FR light increased leaf length more when added to the lower WW PFD than twice the FR PFD at twice the WW PFD. Further research should investigate if these trends persist at even higher TPFDs (e.g., 500-800 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Increasing the PFD by increasing the intensity of the same light spectrum (additional WW light) and incorporating SL of a specific waveband both increase electrical costs, but is one more effective at increasing biomass accumulation or improving plant quality than the other? Our study indicates that doubling the WW PFD from 90 to 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was more effective at promoting biomass accumulation than adding any SL waveband to 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of WW light. There were exceptions with leaf expansion in which supplemental FR light applied to a low WW PFD increased leaf length more than doubling the WW PFD, although it did not lead to an increase in FM. When considering supplementing a white light with narrow-waveband light, it is important to consider the efficacy of the LEDs. For example, if supplemental G or FR light added to a white background similarly increase FM, FR LEDs may be preferred because of their higher photon efficacies (Kusuma et al., 2022). In our study, supplemental R light and an additional 40 or 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of WW light led to the greatest biomass accumulation. Assuming R LEDs have an average photon efficacy of 3.6 $\mu mol \cdot J^{-1}$ and WW LEDs have a photon efficacy of about 2.7 $\mu mol \cdot J^{-1}$, supplemental R light increased lettuce FM more per unit of energy input.

Conclusion

Both the light spectrum and photon flux density regulate lettuce growth, morphology, and coloration. It is important to identify the specific plant traits desired when supplementing a light spectrum with narrow-waveband light, or whether increasing the PFD of the same light spectrum (here, WW) is sufficient to elicit those attributes, which would alleviate the need to incorporate

additional LED types into a lighting system. In this study, doubling the TPFD of WW light had the most pronounced effect on FM, DM, and leaf number, while leaf expansion (plant diameter and leaf length) increased with supplemental FR light. Although supplementing WW light with FR light increased leaf expansion, additional R light was the most effective at increasing FM. Furthermore, B light increased TPC, TAC, and leaf coloration, but suppressed leaf expansion and FM compared to WW light with additional R or WW light. Finally, one should consider the efficacies and costs of individual LED types as well as electricity costs to increase the PFD to incrementally increase biomass accumulation.

Acknowledgements

This work was supported by the Specialty Crops Research Initiative (grant no. 2019-51181-30017) and Hatch project 192266 from the USDA National Institute of Food and Agriculture. We thank Ryan Warner for the use of his laboratory and Mckenna Merkel and Viktorija Vaštakaitė-Kairienė for experimental assistance. We also thank Chieri Kubota and Ryan Warner for their critical review of this manuscript.

LITERATURE CITED

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc. 24, 875–877. https://doi.org/10.1038/nprot.2007.102

Brelsford, C.C., Morales, L.O., Nezval, J., Kotilainen, T.K., Hartikainen, S.M., Aphalo, P.J., Robson, T.M., 2019. Do UV-A radiation and blue light during growth prime leaves to cope with acute high light in photoreceptor mutants of *Arabidopsis thaliana*? Physiol. Plant. 165, 537–554. https://doi.org/10.1111/PPL.12749

Casal, J.J., 2013. Photoreceptor signaling networks in plant responses to shade. Annu. Rev. Plant Biol. 64, 403–427. https://doi.org/10.1146/annurev-arplant-050312-120221

Casal, J.J., 2012. Shade Avoidance. Arab. Book Am. Soc. Plant Biol. 10, e0157. https://doi.org/10.1199/tab.0157

de Lucas, M., Davière, J.-M., Rodríguez-Falcón, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S., Fankhauser, C., Blázquez, M.A., Titarenko, E., Prat, S., 2008. A molecular framework for light and gibberellin control of cell elongation. Nature 451, 480–484. https://doi.org/10.1038/nature06520

Emerson, R., Chalmers, R., Cederstrand, C., 1957. Some factors influencing the long-wave limit of photosynthesis. Proc. Natl. Acad. Sci. U. S. A. 43, 133–133. https://doi.org/10.1073/PNAS.43.1.133

Emerson, R., Rabinowitch, E., 1960. Red drop and the role of auxiliary pigments in photosynthesis. Plant Physiol. 35, 477–485. https://doi.org/10.1104%2Fpp.35.4.477

Franklin, K.A., 2008. Shade avoidance. New Phytol. 179, 930–944. New Phytol. 179, 930–944. https://doi.org/10.1111/j.1469-8137.2008.02507.x

Franklin, K.A., Whitelam, G.C., 2005. Phytochromes and shade-avoidance responses in plants. Ann. Bot. 96, 169–169. https://doi.org/10.1093/AOB/MCI165

Fu, W., Li, P., Wu, Y., 2012. Effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce. Sci. Hortic. 135, 45–51. https://doi.org/10.1016/j.scienta.2011.12.004

Hooks, T., Sun, L., Kong, Y., Masabni, J., Niu, G., 2022. Short-term pre-harvest supplemental lighting with different light emitting diodes improves greenhouse lettuce quality. Horticulturae 8, 435. https://doi.org/10.3390/horticulturae8050435

Jang, I.C., Henriques, R., Seo, H.S., Nagatani, A., Chua, N.H., 2010. *Arabidopsis* PHYTOCHROME INTERACTING FACTOR proteins promote phytochrome B

polyubiquitination by COP1 E3 ligase in the nucleus. Plant Cell 22, 2370–2383. https://doi.org/10.1105/TPC.109.072520

Jenkins, G.I., Long, J.C., Wade, H.K., Shenton, M.R., Bibikova, T.N., 2001. UV and blue light signalling: pathways regulating chalcone synthase gene expression in *Arabidopsis*. New Phytol. 151, 121–131. https://doi.org/10.1046/J.1469-8137.2001.00151.X

Kelly, N., Choe, D., Meng, Q., Runkle, E.S., 2020. Promotion of lettuce growth under an increasing daily light integral depends on the combination of the photosynthetic photon flux density and photoperiod. Sci. Hortic. 272, 109565–109565. https://doi.org/10.1016/j.scienta.2020.109565

Kelly, N., Runkle, E.S., 2023. End-of-production ultraviolet a and blue light similarly increase lettuce coloration and phytochemical concentrations. HortScience 58, 525–531. https://doi.org/10.21273/HORTSCI17108-23

Kong, Y., Nemali, K., 2021. Blue and far-red light affect area and number of individual leaves to influence vegetative growth and pigment synthesis in lettuce. Front. Plant Sci. 12. https://doi.org/10.3389/fpls.2021.667407

Kusuma, P., Bugbee, B., 2021. Improving the predictive value of phytochrome photoequilibrium: consideration of spectral distortion within a leaf. Front. Plant Sci. 12. https://doi.org/10.3389/fpls.2021.596943

Kusuma, P., Pattison, P.M., Bugbee, B., 2022. Photon efficacy in horticulture: turning LED packages into LED luminaires. Plant Fact. Basics Appl. Adv. 115–128. https://doi.org/10.1016/B978-0-323-85152-7.00006-9

Kusuma, P., Pattison, P.M., Bugbee, B., 2020. From physics to fixtures to food: current and potential LED efficacy. Hortic. Res. 7, 56–56. https://doi.org/10.1038/s41438-020-0283-7

Lee, J., Durst, R.W., Wrolstad, R.E., Barnes, K.W., Eisele, ; T, Giusti, ; M M, Haché, ; J, Hofsommer, ; H, Koswig, ; S, Krueger, D.A., Kupina, ; S, Martin, ; S K, Martinsen, ; B K, Miller, T.C., Paquette, ; F, Ryabkova, ; A, Skrede, ; G, Trenn, ; U, Wightman, J.D., 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J. AOAC Int. 88, 1269–1278. https://doi.org/10.1093/JAOAC/88.5.1269

Lee, J.G., Oh, S.S., Cha, S.H., Jang, Y.A., 2010. Effects of red/blue light ratio and short-term light quality conversion on growth and anthocyanin contents of baby leaf lettuce. J. Bio-Environ. Control 19, 351–359.

Lee, M., Xu, J., Wang, W., Rajashekar, C.B., 2019. The effect of supplemental blue, red and far-red light on the growth and the nutritional quality of red and green leaf lettuce. Am. J. Plant Sci. 10, 2219–2235. https://doi.org/10.4236/ajps.2019.1012157

Legendre, R., van Iersel, M.W., 2021. Supplemental far-red light stimulates lettuce growth: disentangling morphological and physiological effects. Plants, 10, 166. https://doi.org/10.3390/PLANTS10010166

Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67, 59–64. https://doi.org/10.1016/j.envexpbot.2009.06.011

Marcelis, L.F.M., Broekhuijsen, A.G.M., Meinen, E., Nijs, E.M.F.M., Raaphorst, M.G.M., 2006. Quantification of the growth response to light quantity of greenhouse grown crops. Acta Horticulturae. 711, 97–103. https://doi.org/10.17660/ActaHortic.2006.711.9

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence-a practical guide. J. Exp. Bot. 51, 659–668. https://doi.org/10.1093/jexbot/51.345.659

Mccree, K.J., 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9, 191–216.

Mendiburu F. 2021. Agricolae: statistical procedures for agricultural research. https://CRAN.R-project.org/package=agricolae.

Meng, Q., Kelly, N., Runkle, E.S., 2019. Substituting green or far-red radiation for blue radiation induces shade avoidance and promotes growth in lettuce and kale. Environ. Exp. Bot. 162, 383–391. https://doi.org/10.1016/J.ENVEXPBOT.2019.03.016

Oh, M.-M., Carey, E.E., Rajashekar, C.B., 2009. Environmental stresses induce health-promoting phytochemicals in lettuce. Plant Physiol. Biochem. 47, 578–583. https://doi.org/10.1016/j.plaphy.2009.02.008

Park, J.-S., Choung, M.-G., Kim, J.-B.J.-B., Hahn, B.-S., Kim, J.-B.J.-B., Bae, S.-C., Roh, K.-H., Kim, Y.-H., Cheon, C.-I., Sung, M.-K., Cho, K.-J., 2007. Genes up-regulated during red coloration in UV-B irradiated lettuce leaves. Plant Cell Rep. 26, 507–516. https://doi.org/10.1007/s00299-006-0255-x

Park, Y., Runkle, E.S., 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Exp. Bot. 136, 41–49. https://doi.org/10.1016/J.ENVEXPBOT.2016.12.013

Pérez-López, U., Sgherri, C., Miranda-Apodaca, J., Micaelli, F., Lacuesta, M., Mena-Petite, A., Quartacci, M.F., Muñoz-Rueda, A., 2018. Concentration of phenolic compounds is increased in lettuce grown under high light intensity and elevated CO_2. Plant Physiol. Biochem. 123, 233–241. https://doi.org/10.1016/j.plaphy.2017.12.010

Poel, B.R., Runkle, E.S., 2017. Spectral effects of supplemental greenhouse radiation on growth and flowering of annual bedding plants and vegetable transplants. HortScience 52, 1221–1228. https://doi.org/10.21273/HORTSCI12135-17

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rolauffs, S., Fackendahl, P., Sahm, J., Fiene, G., Hoecker, U., 2012. *Arabidopsis* cop1 and spa genes are essential for plant elongation but not for acceleration of flowering time in response to a low red light to far-red light ratio. Plant Physiol. 160, 2015–2027. https://doi.org/10.1104/pp.112.207233

Sager, J.C., Smith, W.O., Edwards, J.L., Cyr, K.L., 1988. Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. Trans. ASAE 31, 1882–1889. https://doi.org/10.13031/2013.30952

Seo, H.S., Watanabe, E., Tokutomi, S., Nagatani, A., Chua, N.-H., 2004. Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. Genes Dev. 18, 617–622. https://doi.org/10.1101/gad.1187804

Son, K.-H., Oh, M.-M., 2013. Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. HortScience 48, 988–995. https://doi.org/10.21273/HORTSCI.48.8.988

Tao, Y., Ferrer, J.L., Ljung, K., Pojer, F., Hong, F., Long, J.A., Li, L., Moreno, J.E., Bowman, M.E., Ivans, L.J., Cheng, Y., Lim, J., Zhao, Y., Ballaré, C.L., Sandberg, G., Noel, J.P., Chory, J., 2008. Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. Cell 133, 164–176. https://doi.org/10.1016/J.CELL.2008.01.049

van Iersel, M.W., 2017. Optimizing led lighting in controlled environment agriculture, in: Light Emitting Diodes for Agriculture: Smart Lighting. pp. 59–80. https://doi.org/10.1007/978-981-10-5807-3_4

Vaštakaitė-Kairienė, V., Kelly, N., Runkle, E.S., 2021. Regulation of the photon spectrum on growth and nutritional attributes of baby-leaf lettuce at harvest and during postharvest storage. Plants, 10, 549. https://doi.org/10.3390/PLANTS10030549

Wade, H.K., Bibikova, T.N., Valentine, W.J., Jenkins, G.I., 2001. Interactions within a network of phytochrome, cryptochrome and UV-B phototransduction pathways regulate chalcone synthase gene expression in *Arabidopsis* leaf tissue. Plant J. 25, 675–685. https://doi.org/10.1046/J.1365-313X.2001.01001.X

Wickham, H., François, R., Henry, L., Müller, K., RStudio, 2022. dplyr: A Grammar of Data Manipulation.

Zhen, S., Bugbee, B., 2020. Far-red photons have equivalent efficiency to traditional photosynthetic photons: implications for redefining photosynthetically active radiation. Plant Cell Environ. 43, 1259–1272. https://doi.org/10.1111/pce.13730

Zhen, S., Haidekker, M., van Iersel, M.W., 2019. Far-red light enhances photochemical efficiency in a wavelength-dependent manner. Physiol. Plant. 167, 21–33. https://doi.org/10.1111/ppl.12834

Zhen, S., van Iersel, M., 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, 1158–1158. https://doi.org/10.3389/fpls.2021.693445

Zhen, S., van Iersel, M.W., 2017. Far-red light is needed for efficient photochemistry and photosynthesis. J. Plant Physiol. 209, 115–122.

Zou, J., Zhang, Yating, Zhang, Yuqi, Bian, Z., Fanourakis, D., Yang, Q., Li, T., 2019. Morphological and physiological properties of indoor cultivated lettuce in response to additional far-red light. Sci. Hortic. 257, 108725. https://doi.org/10.1016/j.scienta.2019.108725

APPENDIX

Table III-1. Total photon flux densities (300-750 nm), yield photon flux densities, and individual waveband photon flux densities for each lighting treatment. Treatments consisted of warm-white (WW) light delivered at 90 (WW_{90}) or 180 (WW_{180}) $\mu mol \cdot m^{-2} \cdot s^{-1}$ plus 40 or 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of blue (B, 400-499 nm), green (G, 500-599 nm), red (R, 600-699 nm), or far-red (FR, 700-750 nm) light. Subscripted values denote individual photon flux densities in $\mu mol \cdot m^{-2} \cdot s^{-1}$.

	Photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$)					
Treatment	Total	Yield	Blue	Green	Red	Far red
WW_{90}	87.1	74.1	5.7	26.9	48.4	6.1
+ B_{40}	131.4	106.6	48.2	27.6	48.9	6.8
+ G_{40}	132.8	109.3	8.3	68.1	48.8	7.4
+ R_{40}	134.4	116.8	6.9	26.8	93.1	7.6
+ FR_{40}	137.3	88.1	5.7	28.0	54.5	49.2
+ WW_{40}	128.3	107.7	9.7	38.8	69.3	10.3
WW_{180}	179.4	152.4	12.0	55.5	99.1	12.8
+ B_{80}	267.2	217.4	98.3	56.1	99.7	13.0
+ G_{80}	265.5	218.0	17.4	137.8	95.9	14.2
+ R_{80}	264.3	230.4	11.9	54.9	183.4	13.0
+ FR_{80}	269.4	172.5	11.9	54.5	106.5	96.6
+ WW_{80}	258.6	219.2	17.9	79.5	142.2	19.0

Table III-2. Individual waveband percentages for each lighting treatment. Treatments consisted of warm-white (WW) light delivered at a photon flux density (PFD) of 90 (WW_{90}) or 180 (WW_{180}) $\mu mol \cdot m^{-2} \cdot s^{-1}$ plus 40 or 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of supplemental blue (B; 400-499 nm), green (G; 500-599 nm), red (R; 600-699 nm), or far-red (FR; 700-750 nm) light. Treatment PFDs were determined by measuring and averaging nine representative locations at plant canopy height. Subscripted values denote individual PFDs.

Treatment	WW PFD	% Blue	% Green	% Red	% Far red
WW_{90}	90	6.6	29.2	53.9	10.1
+ B_{40}		35.4	20.2	37.3	7.0
+ G_{40}		4.6	51.0	37.3	7.0
+ R_{40}		4.6	29.2	68.1	7.0
+ FR_{40}		4.6	26.3	37.3	37.8
+ WW_{40}		6.6	29.2	53.9	10.1
WW_{180}	180	6.6	29.2	53.9	10.1
+ B_{80}		35.4	20.2	37.3	7.0
+ G_{80}		4.6	51.0	37.3	7.0
+ R_{80}		4.6	29.2	68.1	7.0
+ FR_{80}		4.6	26.3	37.3	37.8
+ WW_{80}		6.6	29.2	53.9	10.1

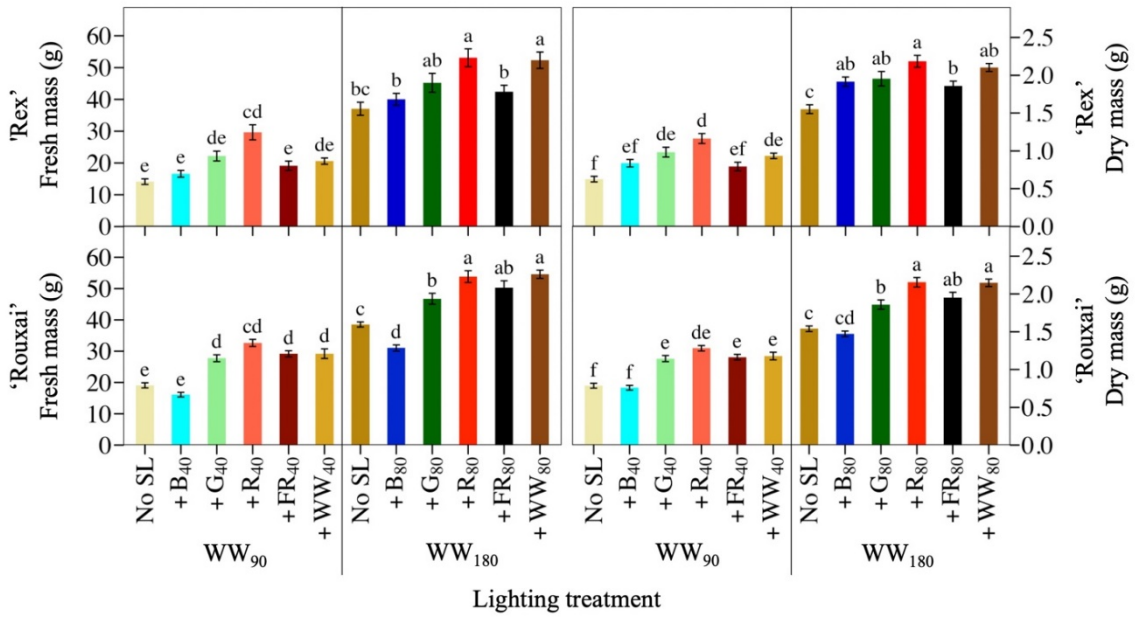

Figure III-1. Mean shoot fresh mass and dry mass of lettuce 'Rex' and 'Rouxai' grown under a warm-white (WW) photon flux density of 90 or 180 µmol·m^{-2}·s^{-1} plus supplemental blue (B; 400-499 nm), green (G; 500-599 nm), red (R; 600-699 nm), far-red (FR; 700-750 nm) or WW light. Subscript values indicate the photon flux density of each waveband, in µmol·m^{-2}·s^{-1}. Each bar represents the mean of two replications with ten samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars indicate the standard error of each treatment.

Table III-3. Results of two-factor analysis of variance for lettuce 'Rouxai' and 'Rex'. *P* values indicate the main effects of the supplemental light (SL) waveband, warm-white (WW) photon flux density (PFD), or their interaction on lettuce growth, morphology, relative chlorophyll concentration (SPAD), total phenolic concentration (TPC), total anthocyanin concentration (TAC), and leaf coloration ($L^*a^*b^*$). nd = not determined.

	'Rouxai'			'Rex'		
Factor	SL waveband	WW PFD	SL waveband × WW PFD	SL waveband	WW PFD	SL waveband × WW PFD
Fresh mass	<0.001	<0.001	0.009	<0.001	<0.001	0.163
Dry mass	<0.001	<0.001	0.048	<0.001	<0.001	0.627
Plant diameter	<0.001	0.005	<0.001	<0.001	<0.001	<0.001
Leaf length	<0.001	<0.001	0.027	<0.001	<0.001	<0.001
Leaf width	<0.001	<0.001	0.363	<0.001	<0.001	0.376
Leaf number	<0.001	<0.001	0.064	<0.001	<0.001	0.081
SPAD	<0.001	<0.001	0.062	<0.001	<0.001	<0.001
TPC	<0.001	<0.001	0.008	nd	nd	nd
TAC	<0.001	<0.001	<0.001	nd	nd	nd
L^*	<0.001	<0.001	<0.001	nd	nd	nd
a^*	<0.001	<0.001	<0.001	nd	nd	nd
b^*	<0.001	<0.001	<0.001	nd	nd	nd

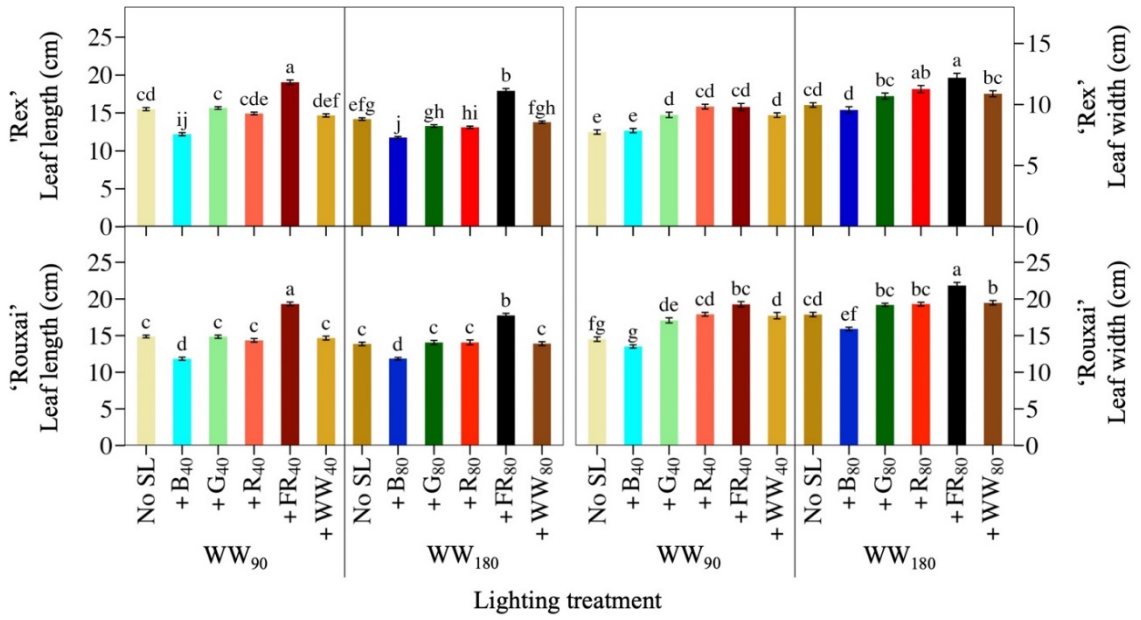

Figure III-2. Mean leaf length and leaf width of the fifth fully expanded leaf of lettuce 'Rex' and 'Rouxai' grown under warm-white (WW) light without or with supplemental light (SL). See Figure 1 for treatment information. Subscript values indicate the photon flux density of each waveband, in $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each bar represents the mean of two replications with ten biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars indicate the standard error of each treatment.

Table III-4. Mean plant diameter, leaf number, and relative chlorophyll concentration (SPAD) of lettuce 'Rouxai' and 'Rex' grown under a warm-white (WW) photon flux density (PFD) of 90 or 180 µmol·m^{-2}·s^{-1} without or with supplemental blue (B; 400-499 nm), green (G; 500-599 nm), red (R; 600-699 nm), far-red (FR; 700-750 nm) or WW light. Each value represents the mean of two replications with ten biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$).

Cultivar	WW PFD	Treatment	Plant diameter (cm)	Leaf number	SPAD
'Rouxai'	90	WW_{90}	24.2 c	11.3 e	12.8 d
		$+B_{40}$	20.1 d	10.7 e	16.1 c
		$+G_{40}$	25.6 c	13.1 d	13.5 d
		$+R_{40}$	24.7 c	13.8 cd	15.5 c
		$+FR_{40}$	33.0 a	11.4 e	12.4 d
		$+WW_{40}$	25.0 c	13.0 d	13.8 d
	180	WW_{180}	24.2 c	14.8 bc	17.1 bc
		$+B_{80}$	21.4 d	13.2 d	19.2 a
		$+G_{80}$	24.3 c	15.2 ab	17.9 ab
		$+R_{80}$	24.5 c	16.1 a	18.5 ab
		$+FR_{80}$	29.3 b	13.3 d	15.6 c
		$+WW_{80}$	24.5 c	16.4 a	18.4 ab
'Rex'	90	WW_{90}	30.2 b	13.5 ef	17.5 fg
		$+B_{40}$	23.4 g	14.4 de	21.6 cd
		$+G_{40}$	30.0 b	15.5 cd	18.0 f
		$+R_{40}$	28.4 bc	16.9 bc	20.4 de
		$+FR_{40}$	35.3 a	12.2 f	16.0 g
		$+WW_{40}$	28.7 bc	15.0 de	18.6 ef
	180	WW_{180}	27.2 cd	18.1 ab	21.0 d
		$+B_{80}$	21.4 fg	17.9 ab	25.1 a
		$+G_{80}$	24.9 ef	19.0 a	20.0 de
		$+R_{80}$	24.8 ef	19.2 a	23.1 bc
		$+FR_{80}$	33.7 a	15.7 cd	17.7 fg
		$+WW_{80}$	25.5 de	19.5 a	24.2 ab

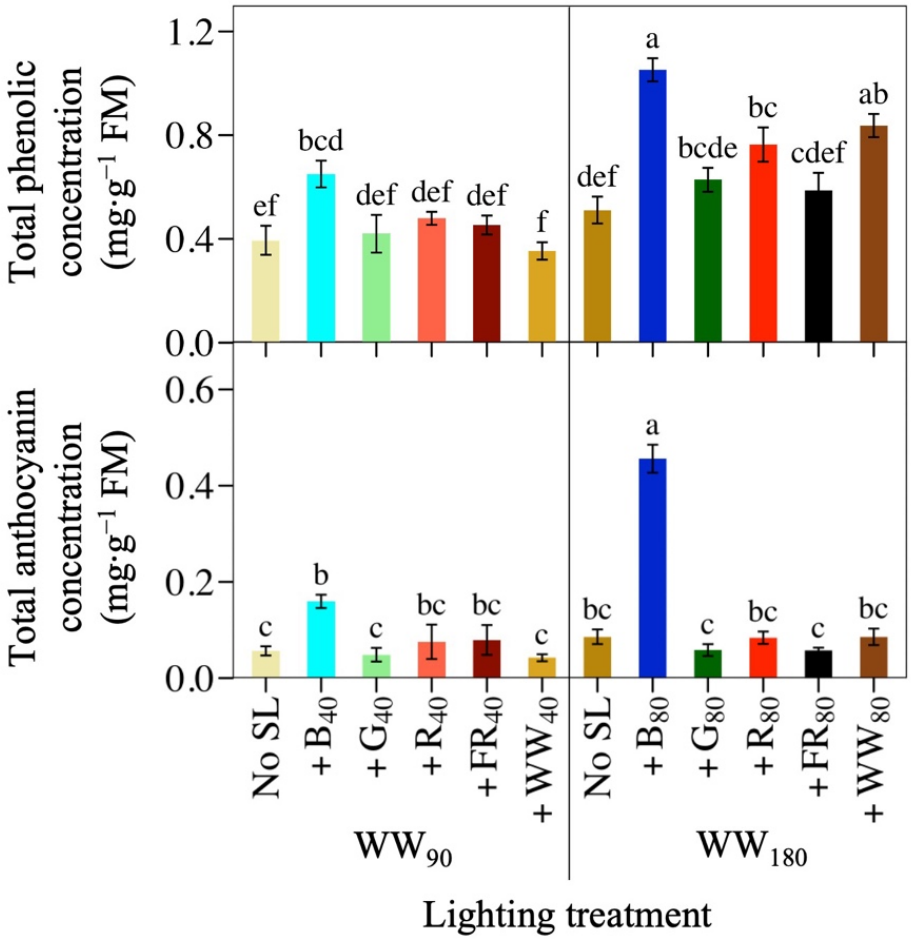

Figure III-3. Mean total phenolic concentration and total anthocyanin concentration of lettuce 'Rouxai' grown under warm-white (WW) light without or with supplemental light (SL). See Figure 1 for additional treatment information. Subscript values indicate the photon flux density of each waveband, in $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each bar represents the mean of two replications with three biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars indicate the standard error of each treatment.

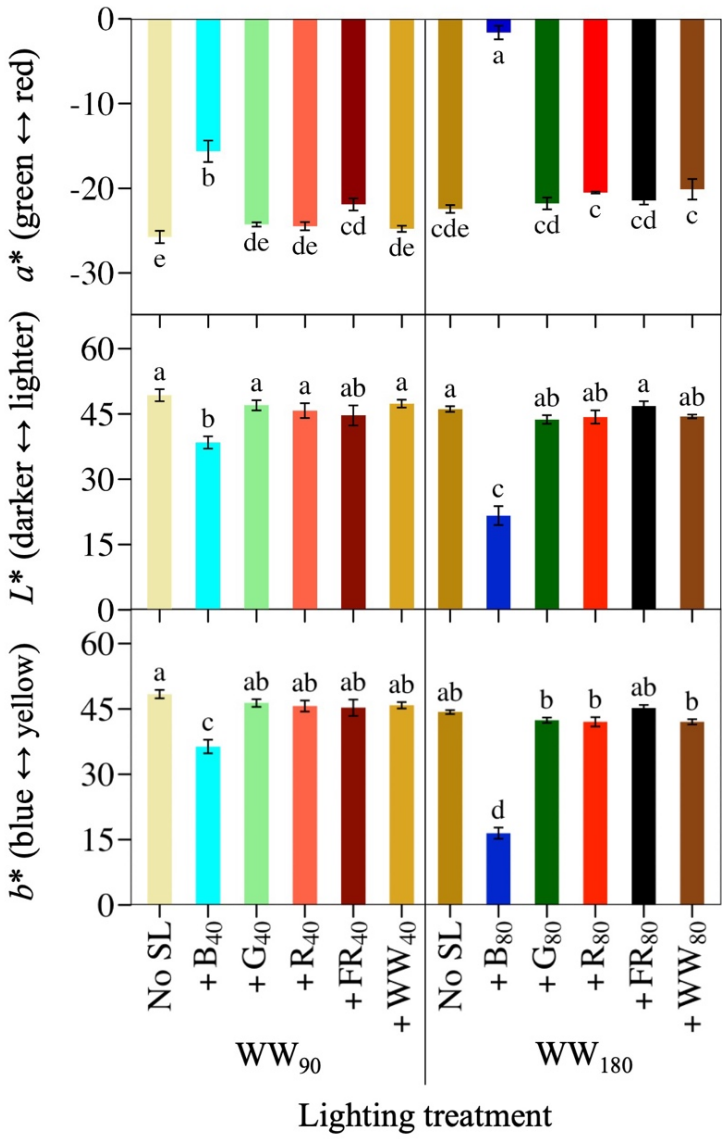

Figure III-4. Mean leaf pigmentation indicated by $L^*a^*b^*$ coloration index values of lettuce 'Rouxai' grown under warm-white (WW) light without or with supplemental light. See Figure 1 for additional treatment information. Subscript values indicate the photon flux density of each waveband, in $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each bar represents the mean of two replications with three biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars indicate the standard error of each treatment.

SECTION IV
DEPENDENCE OF FAR-RED LIGHT ON RED AND GREEN LIGHT AT INCREASING GROWTH OF LETTUCE

Dependence of far-red light on other red and green light at increasing growth of lettuce

Nathan H. Kelly and Erik S. Runkle*

Controlled-Environment Lighting Laboratory, Department of Horticulture, 1066 Bogue Street, Michigan State University, East Lansing, MI 48824

*Corresponding author. Tel.: +1 517 353 0350; fax: +1 517 353 0890. E-mail address: runkleer@msu.edu (E.S. Runkle)

Abstract

Light within the photosynthetically active radiation (PAR) waveband (400-700 nm) powers photosynthesis and regulates plant morphology and quality traits of horticultural crops. In addition, far-red (FR; 700-750 nm) light, especially when paired with red (R; 600-699 nm) light, can increase photosynthetic rates despite being outside of the traditionally defined PAR waveband. Furthermore, FR light induces shade-avoidance responses, increasing leaf expansion and light interception and thus, whole-plant growth. However, it is unclear how the efficacy of FR light depends on PAR wavebands and specifically if the substitution of R light with green (G; 500-599 nm) light would influence the efficacy of FR light on increasing plant growth. To determine this, we grew lettuce 'Rouxai' and 'Rex' at 23 °C under sole-source lighting at a total photon flux density (PFD) of 176 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with R, blue (B; 400-499 nm), G, and FR light-emitting diodes. Each lighting treatment consisted of a constant B PFD, three different G PFDs, and various ratios of R to FR light at each G PFD. Substitution of R with FR light increased the leaf area of both cultivars but did not continuously increase fresh mass. Under the greatest FR PFD, fresh mass was similar to lettuce grown without FR light. G light had less of an effect on leaf expansion and biomass than FR light, and lettuce plant diameter and leaf area were the greatest when G light fully replaced R light at the highest FR PFD. We conclude that fully replacing R with G light in the presence of FR light increased leaf expansion, and that at least some R light was required to maximize the promotion of FR light on biomass accumulation.

Introduction

Most indoor (vertical) farms are entirely reliant on electric lighting to produce high-value horticultural crops including propagative materials and leafy green vegetables. Light-emitting diodes (LEDs) are commonplace in indoor farms because of their high photosynthetic photon

efficacies, long operating lifetime, low heat emission, and possibility to fine-tune the light spectrum (Kusuma et al., 2020). The potential of customizing the light spectrum enables growers to regulate the growth, morphology, and quality attributes of their crops. For example, a relatively high fraction of blue (B; 400-499 nm) light produces more compact plants with higher concentrations of phenolic compounds compared to a spectrum with less B light where plants typically have greater biomass and leaf area but lower phenolic concentrations (Son and Oh, 2013; Wang et al., 2016). Red (R; 600-699 nm) and B LEDs efficiently drive plant photosynthesis and convert electricity to emitted photons, which is quantified by the micromoles of photons output per joule ($\mu mol \cdot J^{-1}$) of input power (Kusuma et al., 2020). White LEDs are commonly incorporated into LED fixtures because of their low cost and broad spectrum, including a trace amount of far-red (FR; 700-750) light. FR light is outside of the traditionally defined photosynthetically active radiation (PAR) waveband of 400−700 nm but increases leaf expansion, radiation capture, and plant photosynthesis (Park and Runkle, 2017; Zhen and van Iersel, 2017; Zhen et al., 2021). In addition, some FR LEDs are even more electrically effective than many R LEDs (Kusuma et al., 2020). Thus, greater adoption of FR LEDs in horticultural lighting fixtures can potentially increase growth per unit of electricity consumed.

Plants perceive light through various photoreceptors, which are proteins that detect specific wavebands of light and stimulate developmental and physiological responses affecting both metabolic regulation and whole-plant physiology (Möglich et al., 2010). Photoreceptors respond to light as stimuli and initiate a signal transduction cascade involving secondary messenger molecules and phosphorylation events, which leads to a physiological response caused by changes in gene expression (Kreslavski et al., 2009). Phytochrome (phy) is a group of photoreceptors that primarily absorbs R and FR light. Each type of phytochrome exists in two

forms (Pr and Pfr) that interconvert, depending on the incident light spectrum. Phytochrome B (phyB) is converted to its inactive form (Pr) when it is exposed to FR light (a low R:FR) for a prolonged period. The Pr form of phyB then dissociates from phytochrome-interacting factors 4 and 5 (PIF4, PIF5) to promote the expression of genes involved in shade-avoidance responses, such as cell elongation, due to increased PIF activity, increased DELLA protein degradation, and gibberellin (GA) biosynthesis (Franklin, 2008). In addition, under a low R:FR, increased PIF activity promotes the expression of auxin biosynthesis genes, which promotes shade-avoidance responses like the promotion of hypocotyl and petiole growth (Iglesias et al., 2018; Tao et al., 2008). Various mutants exhibit impaired shade-avoidance responses when they lack genes involved in auxin biosynthesis, conjugation, transport, perception, or signaling as well as PIF mutants (Iglesias et al., 2018). On the other hand, under R light (a high R:FR), the Pfr form of phyB binds to PIFs, inhibiting the expression of shade-avoidance response genes (Franklin, 2008; Li et al., 2011), and growth is typically more compact.

FR light also enhances plant photochemistry and photosynthesis when combined with PAR wavebands, such as R light, by preferentially exciting photosystem I (PSI) (McCree, 1972; Zhen and van Iersel, 2017). This phenomenon was first described as the Emerson enhancement effect, which states that photosynthetic rates are greater when light of 670-680 nm and >680 nm are applied together compared with the sum of the two wavebands applied separately (Emerson and Rabinowitch, 1960; Emerson et al., 1957). More recently, Zhen et al. (2019) demonstrated that adding R and FR light at wavebands from 686 or 688 to 703 nm to R+B light, or a simulated solar spectrum, progressively increased the quantum yield of photosystem II (PSII) and photosynthetic rates by exciting PSI and restoring excitation balance between the two photosystems. Additionally, FR light of 721 to 731 nm similarly increased the quantum yield of

PSII as 703-nm light (Zhen et al., 2019). FR light can also directly increase the activity of photosystem II (PSII) by increasing oxygen evolution (Pettai et al., 2005). These studies have shown that specific wavebands of FR light can independently drive plant photosynthesis or synergistically when added to PAR wavebands.

Although FR light can increase plant photosynthesis and biomass accumulation, the R:FR of a light spectrum regulates plant morphology and quality attributes such as leaf coloration, leaf thickness (texture), and nutritional quality. For example, adding FR light to a broad-waveband (white light) spectrum decreased anthocyanin concentration and increased shoot fresh mass (FM), stem length, leaf length, and leaf width of lettuce (*Lactuca* sativa) compared to the white light control treatment (Li and Kubota, 2009). Additionally, adding FR light to R+B light increased plant height, leaf area, and dry mass of ornamental seedlings (Park and Runkle, 2016, 2017). Moreover, FR light added to R+B light increased total plant phenolic (Lee et al., 2016) and soluble sugar content, but decreased chlorophyll content as the leaf area of lettuce increased (Zou et al., 2019). The magnitude of FR light responses can be influenced by other wavebands or the background photon flux density (PFD; $\mu mol \cdot m^{-2} \cdot s^{-1}$). For example, adding FR light to an R+B spectrum increased lettuce shoot FM and leaf length, but the effect was more pronounced under a higher B:R or lower PFD than a lower B:R or higher PFD, respectively (Meng and Runkle, 2019). Finally, substituting B light with FR light increased leaf expansion and FM of lettuce (Meng et al., 2019).

While the general effects of FR light on plant growth and morphology are clear, and R and FR light can synergistically increase photosynthesis, most FR light studies delivered a constant R PFD with incremental additions of FR light to decrease the R:FR. It has not yet been established how FR light operates in an environment that has diminishing PFDs of PAR

wavebands, such as R light. We grew red- and green-leaf lettuce under lighting conditions where R light was substituted with green (G, 500-599 nm) light, FR light, or both. Our objectives were to determine: 1) if FR light is as effective as R and/or G light at increasing shoot biomass and 2) how the promotion of growth from FR light depends on other light wavebands. We hypothesized: 1) that the inclusion of FR in the light spectrum would increase biomass accumulation by increasing leaf area and photosynthetic efficiency of R and G light and 2) substitution of R light with G light would progressively decrease the promotion of FR light on plant biomass accumulation.

Materials and methods

Plant materials and propagation

On Feb 18, 2022 (replication 1) and March 18, 2022 (replication 2), we sowed 300 seeds of red-leaf 'Rouxai' lettuce and green-leaf 'Rex' lettuce (Rijk Zwaan USA; Salinas, CA, USA) in a temperature-controlled growth room (Controlled Environment Lighting Laboratory) at Michigan State University (East Lansing, MI, USA). The seeds were sown in 200-cell (2.5 cm × 2.5 cm) rockwool plugs (AO 25/40 Starter Plugs; Grodan, Milton, ON, Canada) that were presoaked in deionized water adjusted to a pH of 4.5 using 10% diluted sulfuric acid (H_2SO_4). Lettuce seeds were grown at 23 °C under a 24 $h \cdot d^{-1}$ photoperiod at a total photon flux density (TPFD; 300-750 nm) of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from warm-white (peak = 639 nm, correlated color temperature = 2700 K) LEDs until day 3 when the photoperiod was shortened to 20 $h \cdot d^{-1}$. Seedling trays were covered with clear plastic domes from day 0 to 6 to increase humidity. We hand-irrigated seedlings from day 0 to 10 with deionized water supplemented with a water-soluble fertilizer (12N–$4P_2O_5$–$16K_2O$ RO Hydro FeED; JR Peters, Inc., Allentown, PA, USA) and magnesium sulfate (Epsom salt; Pennington Seed, Inc., Madison, GA) with the following

nutrients (in $mg \cdot L^{-1}$): 125 N, 42 P, 167 K, 73 Ca, 49 Mg, 39 S, 1.7 Fe, 0.52 Mn, 0.56 Zn, 0.13 B, 0.47 Cu, and 0.13 Mo. The pH and the electrical conductivity (EC) were periodically measured by a pH/EC meter (HI9814; Hanna Instruments, Woonsocket, RI, USA) and were 5.6 and 1.6 $mS \cdot cm^{-1}$, respectively.

Growth conditions and lighting treatments

The controlled-environment room consisted of four vertical hydroponic growing racks with three canopies on each rack, allowing us to create twelve independent lighting treatments. On day 10, we separated the seedlings and transplanted them into floating 36-cell rafts (Beaver Plastics, Ltd., Acheson, AB, Canada) with 2.5-cm-wide holes that were spaced 20 × 15 cm apart. Plants were provided with the same nutrient solution as previously described but at a 20% higher concentration (i.e., 150 mg $N \cdot L^{-1}$). We measured the nutrient solution each day and adjusted the pH and EC using potassium bicarbonate and H_2SO_4 to maintain an average of 5.7 and 1.9 $mS \cdot cm^{-1}$, respectively. During the growth period, we set the air temperature to 23 °C during the day and night, but the actual air temperature averaged 22.8 °C during both replications. Plant canopy temperature (24.5 ± 0.5 °C), relative humidity (39 ± 10%), and CO_2 concentration (427 ± 22 $\mu mol \cdot mol^{-1}$) were also continually measured and were similar during each replication. Additional information about experimental conditions, equipment, and environmental sensors can be found in Kelly et al. (2020).

We delivered twelve lighting treatments from day 10 until harvest on days 27 (‘Rex’) and 28 (‘Rouxai’) (Table IV-1). Each lighting treatment delivered a TPFD of approximately 176 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a constant B (peak = 449 nm) PFD of 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The remaining 154 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was delivered by different proportions of light by narrowband G (peak = 526 nm), R (peak = 664 nm), and FR (peak = 733 nm) LEDs. Three different groups of lighting treatments

that delivered G PFDs of 0, 44, or 88 $\mu mol \cdot m^{-2} \cdot s^{-1}$ replaced R light, which were designed to determine the effects of an increasing FR PFD in a decreasing R light environment. The TPFD and spectrum of each lighting treatment were measured at nine locations at plant canopy level using a portable spectroradiometer (PS200; Apogee Instruments, Inc., Logan, UT, USA), and means per treatment are reported.

Data collection and analysis

Before destructive plant measurements, we measured the relative chlorophyll concentration (SPAD) of ten randomly selected plants by measuring three spots on one fully expanded leaf exposed to direct light and averaged them using a SPAD meter (MC-100; Apogee Instruments, Inc, Logan, UT.). We measured the leaf coloration of lettuce 'Rouxai' by taking overhead pictures of three representative plants from each treatment and analyzing them using an R code developed to determine the lightness (black: $L^* = 0$; white: $L^* = 100$), redness (green: $a^* = -128$; red: $a^* = 127$), and blueness (blue: $b^* = -128$; yellow: $b^* = 127$) of each pixel of an imported TIFF image. The L^*, a^*, and b^* values of each pixel were generated and averaged to quantify the average coloration of an entire plant from overhead.

On day 27 or 28, we collected destructive morphological data from ten randomly selected plants from each lighting treatment. We measured lettuce shoot mass using an analytical balance (AG245; Mettler Toledo, Columbus, OH, USA) and separated the fifth fully expanded leaf. We measured plant diameter (cm), leaf number (> 2 cm in length), and leaf area of the fifth fully expanded leaf (cm^2). Lettuce shoots and the fifth fully expanded leaf were then put into separate paper bags to be dried for six days in a drying oven (Blue M, Blue Island, IL, USA). After the lettuce shoots and separate leaves were sufficiently dried, the dry mass (DM) of the fifth fully

expanded leaf was measured as well as all dry shoot tissue. Finally, we measure the specific leaf area of the fifth fully expanded leaf ($cm^2 \cdot g^{-1}$).

We arranged the experiment as a randomized complete block design with two replications in time (February 18, 2022–March 18, 2022; March 18, 2022–April 15, 2022) and performed statistical analysis using R statistical analysis software (R Core Team, 2014; version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria). We conducted multiple linear regression analysis and performed an analysis of variance (ANOVA) as well as Tukey's honestly significant difference test ($\alpha = 0.5$) using 'dplyr' (Wickham et al., 2022) and 'agricolae' (Mendiburu, 2021) to determine significant differences between individual treatments.

Results and discussion

Leaf morphology and plant diameter

We plotted data as a function of G light to highlight the effects of the FR PFD at different G PFDs (Figure IV-1; Figure IV-2). Table IV-2 shows the main effects of the G PFD, FR PFD, and their interaction on various plant growth and morphological metrics. The FR PFD generally had greater effects on lettuce growth attributes than the G PFD, although since the TPFD was kept constant, increasing the PFD of one waveband meant another was decreased. Substituting R with FR light increased leaf area and plant diameter of both cultivars, while substituting R with G light slightly increased leaf area and plant diameter (Table IV-2). For example, at a G PFD of 88 $\mu mol \cdot m^{-2} \cdot s^{-1}$, increasing the FR PFD from 0 to 66 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (with a corresponding decrease in the R PFD) increased leaf area of 'Rouxai' and 'Rex' by 66 and 47%, respectively. When G light was almost completely replaced by R light, increasing the PFD of FR light from 0 to 66 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased 'Rouxai' and 'Rex' leaf area by 48 and 53%, respectively. At the same FR PFD, G light did not affect leaf area of either cultivar, except for a slight increase in

'Rex' leaf area at an FR PFD of 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when the G PFD increased from 0 to 44 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The plant diameter of both cultivars followed similar trends to leaf area (Table IV-3). Increasing the FR PFD continuously increased both area metrics, while the G PFD had minor effects. As the plant diameter of both cultivars increased due to FR light, leaf number decreased (Table IV-3). Finally, lettuce that was grown under a higher FR light percentage had a higher specific leaf area ($cm^2 \cdot g^{-1}$), indicating that there was more leaf surface area for every gram of biomass (Table IV-3). Therefore, the leaves became thinner and fragile as the FR light replaced R light.

Many sole-source lighting studies investigated the addition of FR to a light spectrum, which increased the TPFD. For example, adding FR light to an R+B light spectrum increased leaf area, regardless of the R:FR, compared to a fluorescent or R+B light control (Lee et al., 2016). Furthermore, the addition of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light to an R, B, or R+B light spectrum increased lettuce leaf length, but the effect of FR light was more pronounced under a higher B:R than a lower one (Meng and Runkle, 2019). In the same study, adding up to 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light to 180 or 360 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B+R (B:R = 1:1) light increased lettuce leaf length, but to a greater extent when added to the low B+R PFD (Meng and Runkle, 2019). We observed similar increases in leaf expansion when R light was substituted by the same PFD of FR light, which maintained a constant TPFD.

The increase in leaf area caused by an increasing FR PFD is a shade-avoidance response that is controlled by a lower R:FR that shifts phytochrome from the Pfr to Pr form (Franklin, 2008; Franklin and Whitelam, 2005; Ruberti et al., 2012). A shift in phytochrome form changes the phytochrome photoequilibrium (PPE), which is the ratio of Pfr to Pr+Pfr. Shade-avoidance responses, such as leaf expansion and stem elongation, are induced by a low PPE (Casal, 2013).

When a low R:FR shifts the phyB pool from Pfr to Pr, phyB dissociates from PIF4 and PIF5, which allows PIFs to accumulate in the nucleus and promote the expression of shade-avoidance genes such as auxin biosynthesis, transport, and signaling genes as well as gibberellin synthesis genes (Casal, 2012; de Lucas et al., 2008; Franklin, 2008; Tao et al., 2008). Additionally, PIFs interact with enzyme complexes such as CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) and SUPRESSOR OF PHYA (SPA), which targets, ubiquitinates, and degrades phyB as well as photomorphogenesis transcription factors like ELONGATED HYPOCOTYL 5 (HY5) (Casal, 2013; Jang et al., 2010; Rolauffs et al., 2012; Seo et al., 2004). The removal of phyB from the nucleus, either by dissociation from PIF4 and PIF5 or degradation mediated by COP1/SPA, allows for the increased expression of shade-avoidance genes that can lead to an increase in cell elongation and greater leaf surface area formation.

We also investigated how the yield photon flux density (YPFD; $\mu mol \cdot m^{-2} \cdot s^{-1}$) and metrics that estimate phytochrome status influenced leaf area (Figure IV-1) and FM (Figure IV-2) of both cultivars. YPFD is the product of the TPFD and the relative quantum efficiency of each waveband (Mccree, 1972; Sager et al., 1988). The internal PPE (iPPE) is calculated based on the ratio of Pfr to Pr+Pfr but considers the spectral distortion that occurs as light travels through the leaf (Kusuma and Bugbee, 2021a). Finally, the FR fraction (FR/(R+FR)) is the ratio of FR light to the total amount of FR and R light (Kusuma and Bugbee, 2021b) and has an inverse relationship with iPPE. In the current study, the leaf area of both cultivars decreased as the YPFD increased. Leaf area also increased as the iPPE decreased and as the FR fraction of the light treatment increased. There was a strong quadratic relationship between YPFD, iPPE, or FR fraction and ‘Rex’ leaf area, while the relationship was more linear for ‘Rouxai’ leaf area. All

three models considered R and FR light, or the entire light spectrum, but were similarly effective at predicting leaf area and thus are likely to be better predictors than the FR PFD alone.

Biomass accumulation

Increasing the FR PFD while proportionately decreasing the R PFD increased lettuce shoot FM and DM, but only until 22 to 44 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light (Figure IV-2). For example, FM of both cultivars increased when 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR replaced R light, a 44 $\mu mol \cdot m^{-2} \cdot s^{-1}$ substitution had less of an effect, and replacing 66 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of R with FR light had a negative effect on FM. Furthermore, without G light, lettuce grown under an FR PFD of 0 or 66 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had a similar FM. However, at a G PFD of 88 $\mu mol \cdot m^{-2} \cdot s^{-1}$, increasing the FR PFD from 0 to 44 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased FM of 'Rouxai' by 33%. At the same PFD, plants grown under the greatest substitution of R light with FR light had a lower FM under than 44 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light, and FM was similar to that of plants grown without FR light. Lettuce 'Rex' followed similar and slightly attenuated trends. Without G light, lettuce 'Rex' had a similar FM under all FR PFDs. Lettuce grown under a G PFD of 44 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and either 0 or 66 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light had a similar FM but had less FM than lettuce grown under 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light. Finally, at a G PFD of 88 $\mu mol \cdot m^{-2} \cdot s^{-1}$, increasing the FR PFD from 0 to 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased lettuce 'Rex' FM by 35% while further increasing the FR PFD to 88 $\mu mol \cdot m^{-2} \cdot s^{-1}$ led to similar lettuce FM as the 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ FR treatment.

Our results are consistent with other studies that showed increasing the FR PFD (or FR fraction) or lowering the R:FR increased biomass accumulation. For example, decreasing the R:FR increased lettuce shoot FM and DM at 12 and 24 days compared to fluorescent lamp and R+B LED control treatments, although the TPFD also increased as the R:FR decreased since FR light was supplemented into the spectrum (Lee et al., 2016). In another study, adding 30

μmol·m^{-2}·s^{-1} of FR light to 90 μmol·m^{-2}·s^{-1} of B plus 90 μmol·m^{-2}·s^{-1} of R light increased FM and DM of lettuce 'Rex' and 'Cherokee', but the effect of FR light was less pronounced when the background B+R consisted of 30 μmol·m^{-2}·s^{-1} of B light and 150 μmol·m^{-2}·s^{-1} of R light (Meng and Runkle, 2019). However, the addition of FR light to a spectrum does not necessarily increase FM; adding 30 μmol·m^{-2}·s^{-1} of FR light to 180 μmol·m^{-2}·s^{-1} of B light led to the lowest FM and DM of both lettuce cultivars studied (Meng and Runkle, 2019). Therefore, FR responses are likely dependent on the TPFD, spectral distribution (e.g., B PFD), and specifically whether R light is in the light spectrum.

The increase in leaf area is, in part, responsible for the increase in biomass accumulation that occurred under higher FR light fractions. This is due to increased light interception and canopy photosynthesis (Zhen and Bugbee, 2020). Additionally, delivery of FR with R light preferentially excites PSI and increases photosynthetic rates compared to R or FR light alone (Emerson and Rabinowitch, 1960; Emerson et al., 1957; Zhen and van Iersel, 2017), increasing biomass accumulation. However, despite that an increasing FR PFD continually increased leaf area, FM was similar under 0 or 66 μmol·m^{-2}·s^{-1} of FR light (and 66 or 0 μmol·m^{-2}·s^{-1} of R light, respectively). The decrease in FM relative to leaf area could be explained by a decrease in photosynthetic rates caused by a decrease in R light. Furthermore, the increased photosynthetic rates of plants grown under R+FR light likely explains why FM was greater in lettuce grown under a moderate YPFD compared to a high YPFD where FR light was removed from the spectrum. Thus, removing most of or all of the R or FR light from the light spectrum can be disadvantageous and lead to lower yields.

Similar to leaf area, there were relationships between YPFD, iPPE, or the FR fraction and FM, except all of the relationships were more parabolic. FM of both cultivars increased as the

YPFD or iPPE of the light treatment increased, but then decreased in the near absence of FR light. Similarly, FM of both cultivars increased as the FR fraction increased, until its vertex (approximately 0.4-0.6), at which point FM began to decrease as FR light comprised a greater portion of the light spectrum.

Leaf coloration

Lettuce 'Rouxai' leaf coloration was influenced by the FR and G PFD, although there was an interactive effect on leaf redness (*a**) (Table IV-2). Generally, an increasing substitution of R light with FR light increased leaf lightness (*L**), increased yellowness (*b**), and decreased redness (*a**), especially at the low to moderate G PFDs (Figure IV-3). For example, at a G PFD of 44 $\mu mol \cdot m^{-2} \cdot s^{-1}$, replacing R with FR light from 0 to 22, 44, or 66 $\mu mol \cdot m^{-2} \cdot s^{-1}$ decreased leaf redness by up to 85%. In addition, in the near absence of FR light, increasing the G PFD from 0 or 44 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 88 $\mu mol \cdot m^{-2} \cdot s^{-1}$ decreased 'Rouxai' leaf redness by about 70%. As lettuce plants became redder, they became bluer (lower *b**) and darker (lower L*). Since the B PFD was constant and leaf redness decreased when the G or FR PFD increased, the change in leaf coloration can be attributed to the reduction of R light and the increase in leaf expansion caused by G and especially FR light. R light increased lettuce leaf redness when 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was delivered alone or with B light for 3 to 14 days at the end of the production cycle (Owen and Lopez, 2015). Compared to a broad-waveband white LED, increasing the FR PFD and decreasing the R:FR from 11.5 to 0.5 increased leaf expansion, but also decreased anthocyanin content and presumably leaf redness of lettuce (Li and Kubota, 2009). Finally, the addition of FR light to a B+R spectrum decreased leaf redness (Meng and Runkle, 2019).

Lettuce leaf redness is strongly associated with anthocyanin content (Park et al., 2008). An increase in anthocyanin content can be attributed to cryptochrome responses stimulated by

ultraviolet (UV) and B light (Brelsford et al., 2019). These high-energy wavebands cause plants to accumulate reactive oxygen species, which signals the activation of phenylalanine ammonia-lyase (Rabelo et al., 2020; Surjadinata et al., 2017), an enzyme that catalyzes the first committed step of the phenylpropanoid biosynthesis pathway (Bate et al., 1994; Vogt, 2010). The phenylpropanoid biosynthesis pathway is responsible for phenolic compound production including flavonoid compounds like anthocyanins (Vogt, 2010). Furthermore, FR light can induce anthocyanin biosynthesis mediated by phyA (Li et al., 2014). FR light is perceived by phyA, which inhibits COP1 activity and allows MYELOBLASTOSIS (MYB) transcription factors to accumulate (Maier et al., 2013). MYB accumulation leads to the transcription of anthocyanin biosynthesis genes (Maier et al., 2013). Increased phyA activity from FR light collaboratively regulates anthocyanin biosynthesis by positively regulating PHYTOCHROME-INTERACTING FACTOR 3 (PIF3) that binds to and expresses anthocyanin biosynthetic genes (Li et al., 2014; Maier et al., 2013; Shin et al., 2007).

Although FR light can induce anthocyanin biosynthesis mediated by phyA, incorporating FR light into the light spectrum often decreases anthocyanin concentration due to the simultaneous increase in leaf area. As leaves expand at a faster rate due to FR light, anthocyanin concentrations become more diluted (Oren-Shamir, 2009). For example, adding FR light to white light, at the same TPFD, decreased total anthocyanin concentrations compared to white light alone or white plus B light (Li et al., 2021). Furthermore, as the FR PFD in a white-light background increased, leaf area of lettuce ‘Cherokee’ increased while anthocyanin content decreased (Liu and van Iersel, 2022). A decrease in leaf redness could also be associated with the decrease in the R:FR and PPFD because both R light and a higher PPFD can induce anthocyanin biosynthesis (Kang et al., 2013; Lee et al., 2016; Li and Kubota, 2009; Zhang et al., 2018). In the

present study, it is likely that 22 μmol·m^{-2}·s^{-1} of B light in all treatments led to similar anthocyanin biosynthesis between treatments and that the inclusion of FR light in the spectrum increased leaf area, thus diluting anthocyanin concentrations and leaf pigmentation.

Conclusion

FR light increases whole-plant photosynthesis indirectly by increasing leaf area and directly by increasing the quantum yield of PSII (Legendre and van Iersel, 2021). In the current study, replacing PAR with FR light continually increased leaf expansion of lettuce 'Rouxai' and 'Rex', but decreased plant quality metrics such as leaf coloration, relative chlorophyll concentration, SLA, and overall visual appearance. Additionally, when FR replaced R light, regardless of the G PFD, lettuce FM was similar to a spectrum lacking FR light or both R and FR light. This indicates that a continual increase in leaf expansion did not correlate with greater FM accumulation when the spectrum was deficient in R light. Therefore, the inclusion of FR in a light spectrum can increase lettuce FM and potentially lower energy consumption, but R light is necessary to maximize growth because G light was not as effective.

iPPE and FR fraction are phytochrome metrics that can be used to predict plant responses to R and FR light. Both metrics were good predictors of leaf area, although the relationship was linear for 'Rouxai' and quadratic for 'Rex'. There was less clear of an effect of these metrics on FM, but FM was similar under a spectrum without FR (and high R light) light or high FR (and low red light). This suggests that an intermediate iPPE or FR fraction (similar PFDs of R and FR light) elicits the greatest lettuce biomass, at least when the B PFD is relatively low. More research is needed to determine if and how these responses are influenced by the B PFD and TPFD.

Acknowledgements

This work was supported by the Specialty Crops Research Initiative (grant no. 2019-51181-30017) and Hatch project 192266 from the USDA National Institute of Food and Agriculture. We thank Ryan Warner for the use of his laboratory and Mckenna Merkel and Viktorija Vaštakaitė-Kairienė for experimental assistance. We also thank Chieri Kubota and Ryan Warner for their critical review of this manuscript.

LITERATURE CITED

Bate, N.J., Orr, J., Ni, W., Meromi, A., Nadler-Hasssar, T., Doerner, P.W., Dixon, R.A., Lamb, C.J., Elkind, Y., 1994. Quantitative relationship between phenylalanine ammonia-lyase levels and phenylpropanoid accumulation in transgenic tobacco identifies a rate-determining step in natural product synthesis. Proc. Natl. Acad. Sci. 91, 7608–7612. https://doi.org/10.1073/pnas.91.16.7608

Brelsford, C.C., Morales, L.O., Nezval, J., Kotilainen, T.K., Hartikainen, S.M., Aphalo, P.J., Robson, T.M., 2019. Do UV-A radiation and blue light during growth prime leaves to cope with acute high light in photoreceptor mutants of *Arabidopsis thaliana*? Physiol. Plant. 165, 537–554. https://doi.org/10.1111/PPL.12749

Casal, J.J., 2013. Photoreceptor signaling networks in plant responses to shade. Annu. Rev. Plant Biol. 64, 403–427. https://doi.org/10.1146/annurev-arplant-050312-120221

Casal, J.J., 2012. Shade avoidance. Arab. Book Am. Soc. Plant Biol. 10, e0157. https://doi.org/10.1199/tab.0157

de Lucas, M., Davière, J.-M., Rodríguez-Falcón, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S., Fankhauser, C., Blázquez, M.A., Titarenko, E., Prat, S., 2008. A molecular framework for light and gibberellin control of cell elongation. Nature 451, 480–484. https://doi.org/10.1038/nature06520

Emerson, R., Chalmers, R., Cederstrand, C., 1957. Some factors influencing the long-wave limit of photosynthesis. Proc. Natl. Acad. Sci. U. S. A. 43, 133–133. https://doi.org/10.1073/PNAS.43.1.133

Emerson, R., Rabinowitch, E., 1960. Red drop and the role of auxiliary pigments in photosynthesis. Plant Physiol. 35, 477–485. https://doi.org/10.1104%2Fpp.35.4.477

Franklin, K.A., 2008. Shade avoidance. New Phytol. 179, 930–944. https://doi.org/10.1111/j.1469-8137.2008.02507.x

Franklin, K.A., Whitelam, G.C., 2005. Phytochromes and Shade-avoidance Responses in Plants. Ann. Bot. 96, 169–169. https://doi.org/10.1093/AOB/MCI165

Iglesias, M.J., Sellaro, R., Zurbriggen, M.D., Casal, J.J., 2018. Multiple links between shade avoidance and auxin networks. J. Exp. Bot. 69, 213–228. https://doi.org/10.1093/jxb/erx295

Jang, I.C., Henriques, R., Seo, H.S., Nagatani, A., Chua, N.H., 2010. *Arabidopsis* PHYTOCHROME INTERACTING FACTOR proteins promote phytochrome B polyubiquitination by COP1 E3 ligase in the nucleus. Plant Cell 22, 2370–2383. https://doi.org/10.1105/TPC.109.072520

Kang, J.H., KrishnaKumar, S., Atulba, S.L.S., Jeong, B.R., Hwang, S.J., 2013. Light intensity and photoperiod influence the growth and development of hydroponically grown leaf lettuce in a closed-type plant factory system. Hortic. Environ. Biotechnol. 54, 501–509. https://doi.org/10.1007/s13580-013-0109-8

Kelly, N., Choe, D., Meng, Q., Runkle, E.S., 2020. Promotion of lettuce growth under an increasing daily light integral depends on the combination of the photosynthetic photon flux density and photoperiod. Sci. Hortic. 272, 109565–109565. https://doi.org/10.1016/j.scienta.2020.109565

Kreslavski, V.D., Carpentier, R., Klimov, V.V., Allakhverdiev, S.I., 2009. Transduction mechanisms of photoreceptor signals in plant cells. J. Photochem. Photobiol. C Photochem. Rev. 10, 63–80. https://doi.org/10.1016/j.jphotochemrev.2009.04.001

Kusuma, P., Bugbee, B., 2021a. Improving the predictive value of phytochrome photoequilibrium: consideration of spectral distortion within a leaf. Front. Plant Sci. 12. https://doi.org/10.3389/fpls.2021.596943

Kusuma, P., Bugbee, B., 2021b. Far-red fraction: an improved metric for characterizing phytochrome effects on morphology. J. Am. Soc. Hortic. Sci. 146, 3–13. https://doi.org/10.21273/JASHS05002-20

Kusuma, P., Pattison, P.M., Bugbee, B., 2020. From physics to fixtures to food: current and potential LED efficacy. Hortic. Res. 7, 56–56. https://doi.org/10.1038/s41438-020-0283-7

Lee, M.-J., Son, K.-H., Oh, M.-M., 2016. Increase in biomass and bioactive compounds in lettuce under various ratios of red to far-red LED light supplemented with blue LED light. Hortic. Environ. Biotechnol. 57, 139–147. https://doi.org/10.1007/s13580-016-0133-6

Legendre, R., van Iersel, M.W., 2021. Supplemental far-red light stimulates lettuce growth: disentangling morphological and physiological effects. Plants 10, 166–166. https://doi.org/10.3390/PLANTS10010166

Li, J., Li, G., Wang, H., Wang Deng, X., Deng, X.W., 2011. Phytochrome signaling mechanisms. Arab. Book 9, e0148–e0148. https://doi.org/10.1199/tab.0148

Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67, 59–64. https://doi.org/10.1016/j.envexpbot.2009.06.011

Li, T., Jia, K.-P., Lian, H.-L., Yang, X., Li, L., Yang, H.-Q., 2014. Jasmonic acid enhancement of anthocyanin accumulation is dependent on phytochrome A signaling pathway under far-red light in *Arabidopsis*. Biochem. Biophys. Res. Commun. 454, 78–83. https://doi.org/10.1016/J.BBRC.2014.10.059

Li, Y., Wu, L., Jiang, H., He, R., Song, S., Su, W., Liu, H., 2021. Supplementary far-red and blue lights influence the biomass and phytochemical profiles of two lettuce cultivars in plant factory. Mol. 26, 7405–7405. https://doi.org/10.3390/MOLECULES26237405

Liu, J., van Iersel, M.W., 2022. Far-red light effects on lettuce growth and morphology in indoor production are cultivar specific. Plants 11, 2714. https://doi.org/10.3390/plants11202714

Maier, A., Schrader, A., Kokkelink, L., Falke, C., Welter, B., Iniesto, E., Rubio, V., Uhrig, J.F., Hülskamp, M., Hoecker, U., 2013. Light and the E3 ubiquitin ligase COP1/SPA control the protein stability of the MYB transcription factors PAP1 and PAP2 involved in anthocyanin accumulation in *Arabidopsis*. Plant J. 74, 638–651. https://doi.org/10.1111/tpj.12153

McCree, K.J., 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9, 191–216. https://doi.org/10.1016/0002-1571%2871%2990022-7

Mendiburu F. 2021. Agricolae: statistical procedures for agricultural research. https://CRAN.R-project.org/package=agricolae.

Meng, Q., Kelly, N., Runkle, E.S., 2019. Substituting green or far-red radiation for blue radiation induces shade avoidance and promotes growth in lettuce and kale. Environ. Exp. Bot. 162, 383–391. https://doi.org/10.1016/J.ENVEXPBOT.2019.03.016

Meng, Q., Runkle, E.S., 2019. Far-red radiation interacts with relative and absolute blue and red photon flux densities to regulate growth, morphology, and pigmentation of lettuce and basil seedlings. Sci. Hortic. 255, 269–280. https://doi.org/10.1016/J.SCIENTA.2019.05.030

Möglich, A., Yang, X., Ayers, R.A., Moffat, K., 2010. Structure and function of plant photoreceptors. Annu. Rev. Plant Biol. 61, 21–47. https://doi.org/10.1146/annurev-arplant-042809-112259

Oren-Shamir, M., 2009. Does anthocyanin degradation play a significant role in determining pigment concentration in plants? Plant Sci. 177, 310–316. https://doi.org/10.1016/j.plantsci.2009.06.015

Owen, W.G., Lopez, R.G., 2015. End-of-production supplemental lighting with red and blue light-emitting diodes (LEDs) influences red pigmentation of four lettuce varieties, HortScience 50, 674–684. https://doi.org/10.21273/HORTSCI.50.5.676

Park, J.-S.S., Kim, J.-B.B., Cho, K.-J.J., Cheon, C.-I.I., Sung, M.-K.K., Choung, M.-G.G., Roh, K.-H.H., 2008. *Arabidopsis* R2R3-MYB transcription factor AtMYB60 functions as a transcriptional repressor of anthocyanin biosynthesis in lettuce (*Lactuca sativa*). Plant Cell Rep. 27, 985–994. https://doi.org/10.1007/s00299-008-0521-1

Park, Y., Runkle, E.S., 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Exp. Bot. 136, 41–49. https://doi.org/10.1016/J.ENVEXPBOT.2016.12.013

Park, Y., Runkle, E.S., 2016. Investigating the merit of including far-red radiation in the production of ornamental seedlings grown under sole-source lighting. Acta Hortic. 259–266. https://doi.org/10.17660/ActaHortic.2016.1134.35

Pettai, H., Oja, V., Freiberg, A., Laisk, A., 2005. Photosynthetic activity of far-red light in green plants. Biochim. Biophys. Acta - Bioenerg. 1708, 311–321. https://doi.org/10.1016/j.bbabio.2005.05.005

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rabelo, M.C., Bang, W.Y., Nair, V., Alves, R.E., Jacobo-Velázquez, D.A., Sreedharan, S., de Miranda, M.R.A., Cisneros-Zevallos, L., 2020. UVC light modulates vitamin C and phenolic biosynthesis in acerola fruit: role of increased mitochondria activity and ROS production. Sci. Rep. 10, 21972. https://doi.org/10.1038/s41598-020-78948-1

Rolauffs, S., Fackendahl, P., Sahm, J., Fiene, G., Hoecker, U., 2012. *Arabidopsis* COP1 and SPA genes are essential for plant elongation but not for acceleration of flowering time in response to a low red light to far-red light ratio. Plant Physiol. 160, 2015–2027. https://doi.org/10.1104/pp.112.207233

Ruberti, I., Sessa, G., Ciolfi, A., Possenti, M., Carabelli, M., Morelli, G., 2012. Plant adaptation to dynamically changing environment: The shade avoidance response. Biotechnol. Adv. 30, 1047–1058. https://doi.org/10.1016/j.biotechadv.2011.08.014

Sager, J.C., Smith, W.O., Edwards, J.L., Cyr, K.L., 1988. Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. Trans. ASAE 31, 1882–1889. https://doi.org/10.13031/2013.30952

Seo, H.S., Watanabe, E., Tokutomi, S., Nagatani, A., Chua, N.-H., 2004. Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. Genes Dev. 18, 617–622. https://doi.org/10.1101/gad.1187804

Shin, J., Park, E., Choi, G., 2007. PIF3 regulates anthocyanin biosynthesis in an HY5-dependent manner with both factors directly binding anthocyanin biosynthetic gene promoters in *Arabidopsis*. Plant J. 49, 981–994. https://doi.org/10.1111/j.1365-313X.2006.03021.x

Son, K.-H., Oh, M.-M., 2013. Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. HortScience 48, 988–995. https://doi.org/10.21273/HORTSCI.48.8.988

Surjadinata, B.B., Jacobo-Velázquez, D.A., Cisneros-Zevallos, L., 2017. UVA, UVB and UVC light enhances the biosynthesis of phenolic antioxidants in fresh-cut carrot through a synergistic effect with wounding. Mol. 22, 668. https://doi.org/10.3390/molecules22040668

Tao, Y., Ferrer, J.L., Ljung, K., Pojer, F., Hong, F., Long, J.A., Li, L., Moreno, J.E., Bowman, M.E., Ivans, L.J., Cheng, Y., Lim, J., Zhao, Y., Ballaré, C.L., Sandberg, G., Noel, J.P., Chory, J., 2008. Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. Cell 133, 164–176. https://doi.org/10.1016/J.CELL.2008.01.049

Vogt, T., 2010. Phenylpropanoid Biosynthesis. Mol. Plant 3, 2–20. https://doi.org/10.1093/mp/ssp106

Wang, J., Lu, W., Tong, Y., Yang, Q., 2016. Leaf morphology, photosynthetic performance, chlorophyll fluorescence, stomatal development of lettuce (*Lactuca sativa* L.) exposed to different ratios of red light to blue light. Front. Plant Sci. 7. https://doi.org/10.3389/fpls.2016.00250

Wickham, H., François, R., Henry, L., Müller, K., RStudio, 2022. dplyr: A Grammar of Data Manipulation.

Zhang, Y., Xu, S., Cheng, Y., Peng, Z., Han, J., 2018. Transcriptome profiling of anthocyanin-related genes reveals effects of light intensity on anthocyanin biosynthesis in red leaf lettuce. PeerJ 6, e4607–e4607. https://doi.org/10.7717/peerj.4607

Zhen, S., Bugbee, B., 2020. Far-red photons have equivalent efficiency to traditional photosynthetic photons: Implications for redefining photosynthetically active radiation. Plant Cell Environ. 43, 1259–1272. https://doi.org/10.1111/pce.13730

Zhen, S., Haidekker, M., van Iersel, M.W., 2019. Far-red light enhances photochemical efficiency in a wavelength-dependent manner. Physiol. Plant. 167, 21–33. https://doi.org/10.1111/ppl.12834

Zhen, S., van Iersel, M., 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, 1158–1158.

Zhen, S., van Iersel, M.W., 2017. Far-red light is needed for efficient photochemistry and photosynthesis. J. Plant Physiol. 209, 115–122. https://doi.org/10.1016/J.JPLPH.2016.12.004

Zou, J., Zhang, Yating, Zhang, Yuqi, Bian, Z., Fanourakis, D., Yang, Q., Li, T., 2019. Morphological and physiological properties of indoor cultivated lettuce in response to additional far-red light. Sci. Hortic. 257, 108725. https://doi.org/10.1016/j.scienta.2019.108725

APPENDIX A: TABLES AND FIGURES

Table IV-1. Individual waveband and total photon flux densities (300-750 nm) delivered in each lighting treatment. Treatments consisted of different combinations of blue (B; 400-499 nm), green (G; 500-599 nm), red (R; 600-699 nm), and far-red (FR; 700-750 nm) light to achieve a target total of 176 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Subscripted values denote waveband photon flux densities in $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Lighting treatment	Photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$)				
	Blue	Green	Red	Far-red	Total
$B_{22}G_{0}R_{154}FR_{0}$	21.7	1.0	154.1	1.9	178.7
$B_{22}G_{0}R_{132}FR_{22}$	21.7	0.9	132.1	22.2	177.2
$B_{22}G_{0}R_{110}FR_{44}$	22.0	0.7	113.8	45.2	181.8
$B_{22}G_{0}R_{88}FR_{66}$	20.2	0.6	87.6	66.2	174.7
$B_{22}G_{44}R_{110}FR_{0}$	22.7	45.7	110.2	1.4	180.3
$B_{22}G_{44}R_{88}FR_{22}$	22.7	46.1	87.3	24.6	180.6
$B_{22}G_{44}R_{66}FR_{44}$	23.2	44.2	67.5	45.0	180.1
$B_{22}G_{44}R_{44}FR_{66}$	21.2	41.6	43.5	68.8	175.3
$B_{22}G_{88}R_{66}FR_{0}$	21.3	86.1	67.8	1.0	176.5
$B_{22}G_{88}R_{44}FR_{22}$	22.8	90.3	47.7	21.1	182.2
$B_{22}G_{88}R_{22}FR_{44}$	20.2	88.7	22.7	44.7	176.6
$B_{22}G_{88}R_{0}FR_{66}$	21.3	86.7	6.9	61.8	177.0

Table IV-2. Results of two-factor analysis of variance for lettuce ‘Rouxai’ and ‘Rex’. *P* values indicate the main effects of the green (G) photon flux density, far-red (FR) photon flux density, or their interaction on lettuce growth and coloration. n = 20 except for *L**, *a**, *b** (n = 6). nd = not determined.

	‘Rouxai’			‘Rex’		
Factor	G	FR	G × FR	G	FR	G × FR
Fresh mass	0.050	<0.001	0.420	0.052	<0.001	0.600
Dry mass	0.151	0.014	0.018	0.024	<0.001	0.187
Leaf area	0.006	<0.001	0.196	0.001	<0.001	0.003
Specific leaf area	<0.001	<0.001	0.705	0.335	<0.007	0.014
Plant diameter	<0.001	<0.001	0.031	<0.001	<0.001	<0.001
Leaf number	0.004	<0.001	<0.001	0.133	<0.001	0.013
SPAD	<0.001	<0.001	0.019	<0.001	<0.001	0.013
*L**	0.052	<0.001	0.583	nd	nd	nd
*a**	<0.001	<0.001	0.038	nd	nd	nd
*b**	<0.001	0.006	0.413	nd	nd	nd

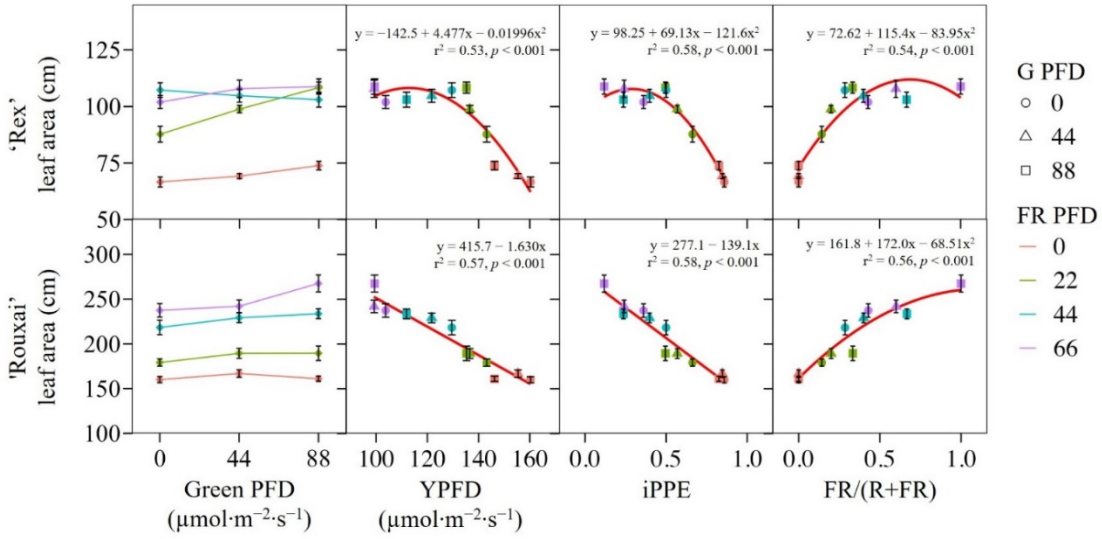

Figure IV-1. Mean leaf area of the fifth fully expanded leaf of lettuce 'Rex' and 'Rouxai'. Each line or symbol color represents a different far-red (FR) photon flux density (PFD) and each shape represents a different green (G) PFD. Each symbol represents the mean of two replications with ten biological samples per treatment and replication (n = 20). Error bars indicate the standard error of each treatment. Regression lines, equations, and r^2 and *p* values were calculated based on linear or quadratic relationships between yield PFD (YPFD), estimated internal phytochrome photoequilibria (iPPE), or FR fraction (FR/(red + FR), and raw leaf area data (n = 240).

Table IV-3. Mean shoot dry mass, specific leaf area (SLA) of the fifth fully expanded leaf, plant diameter, and leaf number of lettuce 'Rouxai' and 'Rex'. Treatment subscripts indicate the photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$) of blue (B; 400-499 nm), green (G; 500-599 nm), red (R; 600-699 nm), and far-red (FR; 700-750 nm) light. Each value represents the mean of two replications with ten biological samples per treatment and replication. Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$).

Cultivar	Treatment	Dry mass (g)	SLA ($cm^2 \cdot g^{-1}$)	Plant diameter (cm)	Leaf number
'Rouxai'	$B_{22}G_0R_{154}FR_0$	1.14 b	734.2 cd	23.1 f	13.6 ab
	$B_{22}G_0R_{132}FR_{22}$	1.94 a	710.5 d	27.0 de	14.7 a
	$B_{22}G_0R_{110}FR_{44}$	1.34 ab	897.0 abcd	29.3 cd	13.7 ab
	$B_{22}G_0R_{88}FR_{66}$	1.18 b	899.1 abc	29.3 cd	11.9 c
	$B_{22}G_{44}R_{110}FR_0$	1.21 b	718.9 cd	23.0 f	13.2 bc
	$B_{22}G_{44}R_{88}FR_{22}$	1.28 ab	864.3 abcd	28.8 cd	14.0 ab
	$B_{22}G_{44}R_{66}FR_{44}$	1.21 b	895.5 abcd	30.4 bc	12.8 bc
	$B_{22}G_{44}R_{44}FR_{66}$	1.21 b	955.7 ab	32.4 ab	12.1 c
	$B_{22}G_{88}R_{66}FR_0$	1.09 b	802.5 bcd	24.5 ef	14.0 ab
	$B_{22}G_{88}R_{44}FR_{22}$	1.17 b	997.4 a	30.0 bc	13.9 ab
	$B_{22}G_{88}R_{22}FR_{44}$	1.34 ab	990.7 a	31.4 bc	13.2 bc
	$B_{22}G_{88}R_0FR_{66}$	1.27 b	959.2 ab	34.5 a	10.4 d
'Rex'	$B_{22}G_0R_{154}FR_0$	1.49 bcd	656.1 b	22.8 f	14.7 abc
	$B_{22}G_0R_{132}FR_{22}$	1.67 abc	705.4 ab	26.6 d	14.9 ab
	$B_{22}G_0R_{110}FR_{44}$	1.77 ab	721.0 ab	30.1 c	13.7 bc
	$B_{22}G_0R_{88}FR_{66}$	1.44 cd	886.0 a	31.5 bc	13.6 bc
	$B_{22}G_{44}R_{110}FR_0$	1.45 cd	745.3 ab	23.9 ef	14.8 abc
	$B_{22}G_{44}R_{88}FR_{22}$	1.82 a	870.2 ab	29.9 c	15.3 a
	$B_{22}G_{44}R_{66}FR_{44}$	1.62 abc	708.0 ab	32.3 ab	13.8 abc
	$B_{22}G_{44}R_{44}FR_{66}$	1.44 cd	780.8 ab	32.8 ab	13.3 c
	$B_{22}G_{88}R_{66}FR_0$	1.31 d	772.7 ab	25.4 de	14.8 abc
	$B_{22}G_{88}R_{44}FR_{22}$	1.59 abcd	913.2 a	32.5 ab	15.1 ab
	$B_{22}G_{88}R_{22}FR_{44}$	1.71 abc	709.6 ab	33.4 a	13.9 abc
	$B_{22}G_{88}R_0FR_{66}$	1.30 d	753.0 ab	33.4 a	11.6 d

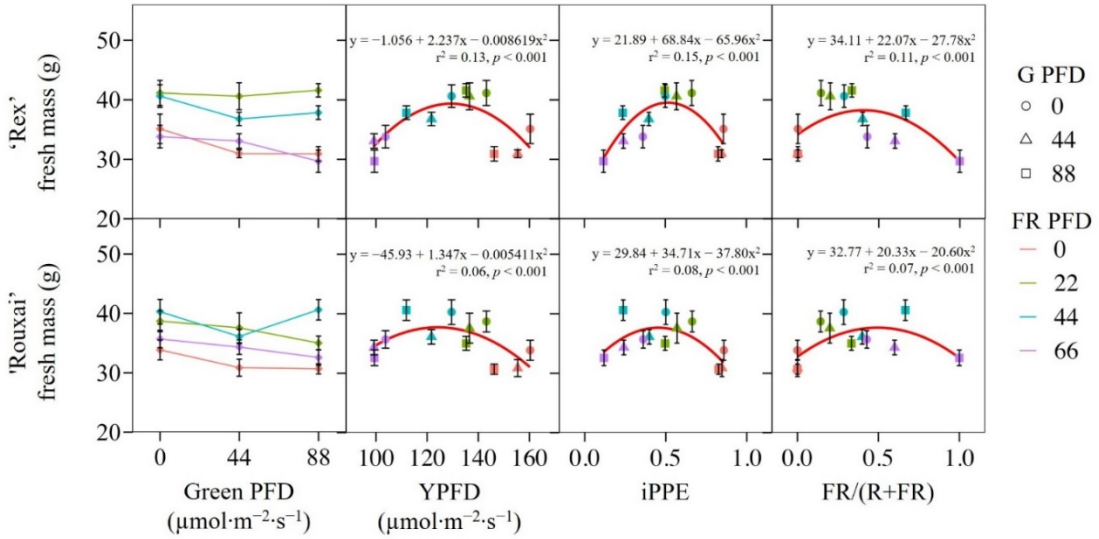

Figure IV-2. Mean shoot fresh mass (g) of lettuce 'Rex' and 'Rouxai'. Each line or symbol color represents a different far-red (FR; 700-750) photon flux density (PFD; µmol·m^{-2}·s^{-1}) and each shape represents a different green (G; 500-599) PFD. Each point represents the mean of two replications with ten biological samples per treatment and replication (n = 20). Error bars indicate the standard error of each treatment. Regression lines, equations, r^2, and *p* values are calculated based on the linear or quadratic relationship between yield PFD (YPFD), internal phytochrome photoequilibria (iPPE), or FR fraction (FR/(red + FR), and raw leaf area data (n = 240).

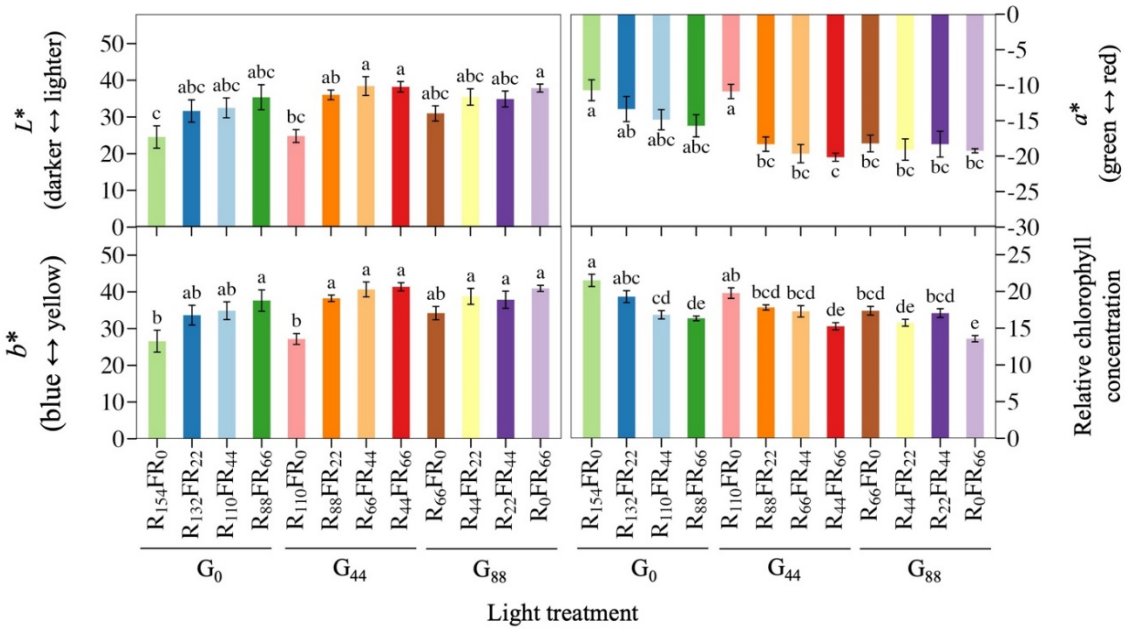

Figure IV-3. Mean leaf pigmentation indicated by *L*a*b** coloration index values and mean relative chlorophyll concentration (SPAD) of lettuce 'Rouxai'. Subscript values indicate the photon flux density of each waveband, in $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each bar represents the mean of two replications with three biological samples per treatment and replication, except for SPAD (n = 20). Means with different letters are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars indicate the standard error of each treatment.

APPENDIX B: LEAF COLORATION ANALYSIS

Lettuce plant coloration methodology and analysis

Lettuce leaf coloration was quantified by using an R script that measured the Lab Color Space of each pixel in an image. Each photo was taken by an iPhone 11 Pro or 12 Pro using the Adobe Lightroom camera app with no filters, pre-processing, or post-processing settings. The phone was fixed 18 inches above the photo-taking surface on a tripod facing down towards the plant. The photos were taken under lighting fixtures where the color-rendering index was adjusted to 90 to maintain high color accuracy. Each photo was then imported into Adobe Photoshop so the background could be removed. After the background of each photo was removed, the photos were exported as a .tif/.tiff file format and imported in R statistical analysis software and the script below was executed to quantify leaf coloration. The R script measured the Lab Color Space (L^*, a^*, b^*) of every pixel of the imported image. The coloration of every pixel was then averaged to provide an average leaf coloration of the plant viewed from overhead. Three photos per treatment and replication (n = 6) were taken to generate the mean leaf coloration of plants grown under each lighting treatment.

The R script below was the template used for the measurement of a single photograph of lettuce.

Please note that, as of publication of this dissertation, the R package ‘rtiff’ is no longer available.

```
# Nathan H. Kelly
# Michigan State University
# 8/22/22

# General script for picture coloration analysis
# This script is for one "treatment" with one picture
# Repeat steps for each additional picture or treatment

#####

# Install packages
install.packages(‘rtif’f) # rtiff is no longer available on R
install.packages(‘matrixStats’)
install.packages(‘dplyr’)
install.packages(‘tidyverse’)

# Load packages
library(rtiff)
library(matrixStats)
library(dplyr)
library(tidyverse)

# Import pictures in .tif/.tiff format
pic.tif_1 <- readTiff('trt1.tif') # Use own file name or path here
```

```
# Convert tiff to RBG format
pic.rgb_1 <- data.frame(red=c(pic.tif_1@red), green=c(pic.tif_1@green),
blue=c(pic.tif_1@blue))

# Convert RBG to Lab color space format
pic.Lab_1 <- convertColor(pic.rgb_1, from = "sRGB", to = "Lab", clip = NA)

# Write data to table
write.table(pic.lab, 'pic_lab_1.txt', sep = '\t')

# Convert matix to data frame
pic_Lab_data_1 <- data.frame(pic.Lab_1)

# Optional
# Add L* filter to filter out overly light or dark pixels in the image caused by reflections and
shadows
pic_Lab_data_filtered_1 <- filter(pic_Lab_data_1, L > 1, L < 90)

# Generate mean and standard deviation of L*, a*, b*
trt1_summary <- summarize(pic_Lab_data_filtered_1, L_mean=mean(L, na.rm = TRUE),
L_sd=sd(L, na.rm = TRUE), a_mean=mean(a, na.rm = TRUE),
a_sd=sd(a, na.rm = TRUE), b_mean=mean(b, na.rm = TRUE), b_sd=sd(b, na.rm = TRUE))

# Export table as .txt file
write.table(trt1_summary,"trt1_summary.txt", sep = "\t")

#####

# Data table contains L*a*b* measurements for every pixel in the image
# Generate mean of L*, a*, b* columns to get average coloration of the image
# Conduct preferred statistical analysis
```